New Trends in Health Sciences

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Assoc. Prof. Dilek Atik, MD,

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Mesotherapy in the Treatment of Musculoskeletal Pain

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Mesotherapy (or local intradermal therapy) is a treatment of intra- or subcutaneous injections containing liquid mixture of compounds (pharmaceutical and homeopathic medications, plant extracts, vitamins, and other ingredients) to treat local medical and cosmetic conditions (Sarkar ,2011)

Michel Pistor was the first to use the term "mesotherapy",and Sergio Maggiori, emphasized that superficial inoculation allowed to reach the clinical effect with a lower dose of drug and named the technique''local intradermal therapy.'' (LIT) (Mammucari and Maggiori, 2020)

There are several clinical studies examinining the effects of mesotherapy in acute and chronic musculoskeletal pain. Mesotherapy can be integrated into the treatment plan of for each patient . and be helpfull for the treatment of certain types of localized pain. When the systemic route of a drug is not recommended and the painful symptoms is localized in musculoskeletal conditions mesotherapy can be considered as the first choice to reduce the systemic impact of drugs. (Mammucari and Massimo & Vellucci ,2014)

There are different kinds of drugs used for intradermal therapy; and each of them has different pharmacokinetic properties(Mammucari and Gatti, 2012).Local intradermal injected anti-inflammatory ,anesthetic and antibiotic drugs remains in the tissue for longer time than intramuscularly administered drugs and reduces the amount or frequency of systemic drug use (Binaglia, 1981 1:15-28; L. Binaglia,1981,1:85-91; Pitzurra,1982; Mammucari and Gatti,2011)

The most common medications used to treat musculoskeletal pain in mesotherapy are nonsteroid antiinflammatory drugs (NSAIDs), muscle relaxants, lidocaine/procaine, sterile water solution, isotonic saline solution.. Corticosteroids are not suggested in mesotherapy (Mammucari and Gatti, 2012,2011;Mammucari and Maggiori ,2016) With LIT the "first pass effect" is avoided and therefore using a prodrug is not appropriate. (Mammucari and Gatti, 2011) Interestingly there are studies using only sterile water or saline solution for intradermal injections in low back pain,cervicogenic headache, myofascial pain syndrome (Koyucu, 2018; Lindhal,1961) The use of a mixture of drugs can increase the risk of interaction but lidocaine and NSAID mixture has been reported to be safe (Mammucari and Gatti ,2011,Saggini ,2015) In painful conditions originated from muscle myorelaxants are a rational and safe choice (Cereser, 1985; Narvarte, 2011)

Musculoskeletal originated pain can be nociceptive pain due to inflammation of peripheral tissues or neuropathic pain due to a lesion in the nerve pathways (Freynhagen, 2006) NSAİDs used for musculoskeletal pain treatment act via COX inhibition and the reduction in the levels of prostaglandin and other inflammatory mediators (Gatzche, 2002) An important effect of NSAIDs isto

activate neuronal nitricoxide synthase. (Romero, 2011) Nitric oxide is a molecule that has important local antiinflammatory and antinociceptive effects.(Romero, 2011; Wahl,2003) High drug concentrations in the subcutaneous tissue can negatively affect near inflammatory cells, sensory fibers, and vascular mediators and lead to reduction of inflammation and pain (Mammucari and Gatti, 2011; Xiao, 2008)

Lidocaine used in LIT blocks the sodium channels, generates ectopic activity in afferent neurons and decreases ongoing and evoked neuropathic pain. The suppressive effects of lidocaine are greater in A-fibers than in C-fibers in muscle cutaneous afferents. (Bach, 1990; Boas, 1982)

Unless there is documented evidence on the tolerability and efficacy a single drug is recommended in the same syringe. (Mammucari and Massimo & Vellucci ,2014) The practice of using different syringes (and injecting different drugs in separate locations) remains the safest technique. (Mammucari and Massimo & Vellucci, 2014)

The effects of LIT is not only a pharmocological effect ; there are different peripheral neuronal mechanisms.Although is not obviously described keratinocytes and T lymphocytes have an important role in peripheral nociception (Khodorova,2003; Verma-Gandhu,2006) It is also identified that the glial cells have network in the dermis and the direct connection with sensory neurons for pain control (Abdo, 2019) In studies mesotherapy with lidocaine versus dry mesotherapy on trigger points showed a reduction of pain even though anesthetic mesotherapy on trigger points was more effective (Paolucci,2016) This effect is due to reflex activation of nerve fibres.(Mammucari and Gatti, 2011; Koyucu, 2018;Tringali, 2017; Crenna, 1981;Cui, 2016; Lee ,2011; Derry, 2012; Byrn, 1993; Mammucari and Maggiori,2019) Also the endogenous opioid system is involved in pain reduction at the level of some trigger point(Fine 1988)

Mechanical distension of skin layers after the drug inoculation activates the cutaneous and subcutaneous receptors and they mediate the production of endorphins and other molecules for the analgesic effect (Mammucari and Gatti, 2012) Increased tissue pressure results with activation of afferent nerve fibers (A-delta and C fibers) and of gate control. Studies using Sterile water injection explaine the effect by mechanical distension by osmotic irritation and activation of reflex pain mechanism. (Koyucu, 2018; Lindhal ,1961)

In mesotherapy technique, the original approach is the inoculation of a drug by simultaneous injections 2–3 cm apart using the needles 4-mm, 27 gauge or 13-mm, 30/31 gauge, positioned at 30–45 degrees with respect to the skin surface. Only 0.10–0.20 mL of drug is injected at each point. Two mesotherapy infiltration techniques are recognized:'' Profound intradermal injection'' and

‘’superficial intradermic injection’’ for which the injection is made at depths of 2–4 mm and 1–2 mm (Paolucci,2019). According to some studies, the depth of intradermal injection could be 1 to 1.5mm (Laurent,2007;Van Mulder, 2017).

The treatment protocol (drug, number of sessions, patient management and follow-up) varies depending on the nature, severity, site of the condition,pharmacological and clinical parameters (Maggiori, 2004**)** Before applying the mesotherapy the type, location and the intensity of pain must be well understood. In acute musculoskeletal pain a single session reported succesful. (Cui 2016;Yang 2018) In chronic musculoskeletal pain 3 or 5 to a maximum of 9 mesotherapy sessions are advised(Mammucari and Gatti, 2012;Cereser,1985) The best result is obtained in acute pain compared to chronic pain.

The technique requires medical and pharmacological knowledge and rules of disinfection must be obeyed. Cutaneous infections are reported in the literature after mesotherapy for aesthetic purposes (Jabbour, 2019)

NSAIDs, myorelaxants, EDTA, calcitonin, or vasorelaxants alone or in combination with an anesthetic were used in the studies for pain management and no serious adverse events were reported (Mammucari and Gatti,2011) Local effects and reversible adverse reactions (allergic reactions, ecchymosis, and urticaria), discomfort, and irritation have been reported and these adverse effects are thought to be due to the type of drug (Maggiori, 2010,2004)

Encouraging results were reported in different musculoskeletal pathologies with mesotherapy in combination with physical therapy (Maggiori,2004; Monticone,2004**)** in sports injuries (Cereser, 1985)in the management of neuropathic pain **(**Currò,1985) as adjuvant therapy in the treatment of osteoporosis pain (Piantoni,1985) and calcified tendinitis of the shoulder (Cacchio, 2009; Soncini, 1998; Gazzi,1984) Intradermal therapy can synergize with physical therapy modalities like ultrasound, or antalgic electrotherapy (Cacchio, 2009; Florio, 1999; Palermo, 1991) In patients undergoing rehabilitation programs for musculoskeletal disease (Paolucci,2019) or after sports trauma (Mammucari and Gatti, 2012) mesotherapy has a remarkable success.

Mesotherapy showes good results in reducing pain and improving function in musculoskeletal pain disorders,has advantages and few or no side effects. It is an effective and well tolerated treatment for patients with comorbidities and NSAID allergy.(Mason2004; Derry 207;Katz ,2009; Sostres ,2009) Mesotherapy can be recommended in the treatment of musculoskeletal pain alone or in combination with rehabilitation treatment.

References

1. Abdo H., Calvo-Enrique L., Lopez J. M. et al., "Specialized cutaneous Schwann cells initiate pain sensation," Science, vol. 365, no. 6454, pp. 695–699, 2019.
2. Bach FW, Jensen TS, Kastrup J, Stigsby B, Dejgard A. The effect of intravenous lidocaine on nociceptive processing in diabetic neuropathy. Pain. 1990;40:29–34.
3. Binaglia L., P. Maconi, and M. Pitzurra, "Absorption of Na ketoprofen administered intradermally," Journal of Mesotherapy, vol. 1, pp. 85–91, 1981
4. Binaglia L., P. Maconi, and M. Pitzurra, "The diffusion of intradermally administered procaine," Journal of Mesotherapy, vol. 1, pp. 15–28, 1981.
5. Boas RA, Covino BG, Shahnarian A. Analgesic responses to i.v. lignocaine. Br J Anaesth. 1982;54:501–505. doi:10.1093/bja/54.5.501
6. Byrn C, Lindh M, Hösterey U, Fogelberg M, Linder LE, Bunketorp O. Subcutaneous sterile water injections for chronic neck and shoulder pain following whiplash injuries. Lancet. 1993;341:449–452. doi:10.1016/0140-6736(93)90204-T
7. Cacchio A, E.DeBlasis,P.Desiati,G.Spacca,W.Santilli,and F. De Paulis, "Effectiveness of the treatment of calcific tendinitis of the shoulder by disodium EDTA," Arthritis & Rheumatology, vol. 61, no. 1, pp. 84–91, 2009.
8. Cereser C, Ganzit Gp, Gribaudo C. Injuries affecting the locomotory system during the game of rugby. Reports of 133 cases treated with mesotherapy. J Mesother 1985; 5: 9-19.
9. Crenna P, Mancia P. Reflex actions in mesotherapy. J Mesother. 1981;1:29–40
10. Cui JZ, Geng ZS, Zhang YH, Feng JY, Zhu P, Zhang XB. Effects of intracutaneous injections of sterile water in patients with acute low back pain: a randomized, controlled, clinical trial. Braz J Med Biol Res. 2016;49(3). doi:10.1590/1414-431X20155092
11. Currò F, B Earzatto A. Mesotherapy in the treatment of post-zoster neuritis. J Mesother 1985; 5: 37-43.
12. Derry S, Straube S, Moore RA, Hancock H, Collins SL. Intracutaneous or subcutaneous sterile water injection compared with blinded controls for pain management in labour. Cochrane Database Syst Rev. 2012;1:CD009107. doi:10.1002/14651858. CD009107
13. Derry S, Wiffen PJ, Kalso EA, et al. Topical analgesics for acute and chronic pain in adults an overview of Cochrane Reviews. Cochrane

Database Syst Rev. 2017;12(5):CD008609. doi:10.1002/14651858. CD008609.pub2 Review.
14. Fine P.G., R. Milano, and B. D. Hare, “The effects of myofascial trigger point injections are naloxone reversible,” Pain, vol. 32, no. 1, pp. 15–20, 1988.
15. Florio R., CerutiG. Guazzini-Visconti S,and Cisari C. “The sequelae of cervical whiplash injury Static posturography for evaluating disability and the efficacy of rehabilitation (mesotherapyversusphysiotherapy),”EuropeanJournalofMedical Physics, vol. 35, pp. 171–176, 1999.
16. Freynhagen R, Baron R, Tölle T, Stemmler E, Gockel U, Stevens M. Screening of neuropathic pain components in patients with chronic back pain associated with nerve root compression: a prospective observational pilot study (MIPORT). Curr Med Res Opin. 2006;22 (3):529–537. doi:10.1185/030079906X89874
17. Gazzi A, Ponzetti F, R Icci L, Cortese G, Buosı C. La mesoterapia con EDTA nella periartrite scapolo omerale calcifica (morbo di Duplay), risultati incoraggianti. Riabilitazione 1984; 17: 141-145
18. Gøtzsche P. Non-steroidal anti-inflammatory drugs. Clin Evid. 2002;8:1203–1211.
19. Jabbour S.F. ,A. E.Malek,E.G.Kechichian,R. R.Tomb, and M. W. Nasr, “Nontuberculous Mycobacterial Infections after cosmetic procedures: a systemic review and management algorithm,” Dermatologic Surgery, vol. 46, no.1, pp.116–121, 2019.
20. Katz JD, Shah T. Persistent pain in the older adult: what should we do now in light of the 2009 american geriatrics society clinical practice guideline? Pol Arch Med Wewn. 2009;119(12):795–800.
21. Khodorova A., B. Navarro, L. S. Jouaville et al., “Endothelin-B receptor activation triggers an endogenous analgesic cascade at sites of peripheral injury,” Nature Medicine, vol. 9, no. 8, pp. 1055–1061, 2003.
22. Koyucu R. G., N. Demirci, and A. E. Yumru, “Effects of intradermal sterile water injections in women with low back pain in labor:a randomized, controlled, clinical trial,”Balkan Medical Journal, vol. 35, pp. 148–154, 2018.
23. Laurent, F. Mistretta, D. Bottigioli et al., “Echographic measurement of skin thickness in adults by high frequency ultrasound to assess the appropriate microneedle length for intradermal delivery of vaccines,” Vaccine, vol. 25, no. 34, pp. 6423–6430, 2007.

24. Lee N, Coxeter P, Beckmann M, et al. A randomised non-inferiority controlled trial of a single versus a four intradermal sterile water injection technique for relief of continuous lower back pain during labour. BMC Pregnancy Childbirth. 2011;11:21. doi:10.1186/14712393-11-21
25. Lindahl O. , "Experimental skin pain induced by injection of water-soluble substances in humans," Acta Physiologica Scandinavica, vol. 179, pp. 1–89, 1961.
26. Maggiori E. , C. A. Bartoletti, S. Maggiori, F. Tomaselli, and D. Dorato, "Local intradermotherapy (ITD) with mesoglicano in PEFS and IVLC, retrospective study," Trends in Medicine, vol. 10, no. 1, pp. 73–78, 2010.
27. Maggiori S. Manuale di intradermoterapia distrettuale. La mesoterapia in Italia. Roma: EMSI Editore; 2004
28. Mammucari M, Maggiori E, Russo D, Giorgio C, Ronconi G, Ferrara PE, Canzona F, Antonaci L, Violo B, Vellucci R, Mediati DR, Migliore A, Massafra U, Bifarini B, Gori F, di Carlo M, Brauneis S, Paolucci T, Rocchi P, Cuguttu A, Di Marzo R, Bomprezzi A, Santini S, Giardini M, Catizzone AR, Troili F, Dorato D, Gallo A, Guglielmo C, Natoli S. Mesotherapy: From Historical Notes to Scientific Evidence and Future Prospects. ScientificWorldJournal. 2020 May 1;2020:3542848. doi: 10.1155/2020/3542848. PMID: 32577099; PMCID: PMC7305548.
29. Mammucari M., Maggiori E., Antonaci L., Fanelli R. Gallo A., Guglielmo C., Russo D., Canzona F., Dorato D., Di Marzo R., Natoli S. Rocchi P., Cuguttu A., Giardini M, Gallo R. and Bomprezzi A. Research & Investigations in Sports Medicine Volume5 Issue4 2019 november Rational for the Intradermal Therapy (Mesotherapy) in Sport Medicine: From Hypothesis to Clinical Practice
30. Mammucari M., Maggiori E., Lazzari M. , and Natoli S, "Should the general practitioner consider mesotherapy (intradermal therapy) to manage localized pain?" Pain and Therapy, vol. 5, no. 1, pp. 123–126, 2016.
31. Mammucari, M., Vellucci R., Mediati D., Migliore A., Cuomo A., Maggiori E., Natoli S., Lazzari M., Gafforio P.,Palumbo E., Tufaro G., Meo B., Dora-To D., Giardini M.,Giorgio C., Jacovitti S., Laurenza M., Salciccia P., Trocchi G., Dauri M., What is mesotherapy? Recommendations from an international consensus. Trends in Medicine. 2014. 1-10. 10.13140/2.1.3268.8640.
32. Mammucari M,Gatti A,Maggiori S,Sabato AF.Role of mesotherapy in musculoskeletal pain:opinions from the Italian Society of Mesotherapy. Evid Based Complement Altern Med. 2012;2012:436959. doi:10.1155/ 2012

33. Mammucari M, Gatti A, Maggiori S., Bartoletti C. A., and. Sabato A. F , "Mesotherapy, definition, rationale and clinical role: a consensus report from the Italian Society of Mesotherapy," European Review for Medical and Pharmacological Sciences, vol. 15, no. 6, pp. 682–694, 2011
34. .Mason L, Moore RA, Edwards JE,Derry S,McQuay HJ.Topical nsaids for chronic musculoskeletal pain: systematic review and meta-analysis. BMC Musculoskelet Disord. 2004;5:28. doi:10.1186/1471-2474-5-27
35. Montıcone M, Barbarıno A, Testı C, Arzano S, Moschı A, Negrını S. Symptomatic efficacy of stabilizing treatment versus laser therapy for subacute low back pain with positive tests for sacroiliac dysfunction: a randomised clinical controlled trial with 1 year follow-up. Eura Medicophys 2004; 40: 263-268
36. Narvarte D. A. and Rosset-Llobet J. , "Safety of subcutaneous microinjections (mesotherapy) in musicians," Medical Problems of Performing Artists, vol. 26, no. 2, pp. 79–83, 2011
37. Palermo S, Riello R, and Cammardella M. P. , "TENS+mesotherapy association in the therapy of cervicobrachialgia: preliminary data," Minerva Anestesiol, vol. 58, no. 10, pp. 1084-1085, 1991.
38. Paolucci T, Bellomo RG, Centra MA, Giannandrea N, Pezzi L, Saggini R. Mesotherapy in the treatment of musculoskeletal pain in rehabilitation: the state of the art. J Pain Res. 2019 Jul 30;12:2391-2401. doi: 10.2147/JPR.S209610. PMID: 31440078; PMCID: PMC6679691.
39. Paolucci T. , Piccinini G., Dan Trifan P., Zangrando F., and Saraceni V.M.,"Efficacy of trigger points mesotherapy for the treatment of chronic neck pain: a short term retrospective study," International Journal of Physical Therapy & Rehabilitation, vol. 2, p. 113, 2016.
40. Pıantonı D, Cotıchellıl E, Santıllı W. Use of calcitonin in regional osteoporosis. J Mesother 1985; 5: 21-23.
41. Pitzurra M., Cavallo R. , Farinelli S., Sposini T., Cipresso T, and Scaringi L., "The intradermal inoculation of antibiotics: some experimental data," Journal of Mesotherapy, vol. 1, pp. 9–14, 1982.
42. Romero TRL, Resende LC, Duarte IDG. The neuronal NO synthase participation in the peripheral antinociception mechanism induced by several analgesic drugs. Nitric Oxide. 2011;25(4):431–435. doi:10.1016/j.niox.2011.08.002
43. Saggini R, Di Stefano A, Dodaj I, Scarcello L, Bellomo RG. Pes anserine bursitis in symptomatic osteoarthritis patients: a mesotherapy treatment study. J Altern Compl Med. 2015;21 (8):480–484. doi:10.1089/acm.2015.0007

44. Sarkar R, Garg VK, Mysore V. Position paper on mesotherapy. Indian J Dermatol Venereol Leprol. 2011;77(2):232–237. doi:10.4103/03786323.77479.)
45. Soncını G, Costantıno C. The treatment of pathologic calcification of the shoulder tendons with EDTA bisodium salt by mesotherapy. Acta Biomed Ateneo Parmense 1998; 69: 133-138.
46. Sostres C, Gargallo CJ, Arroyo MT, Lanas A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2010;24(2):121–132. doi:10.1016/j.bpg.2009.11.005
47. Tringali G, Navarra P. Optimal solubility of diclofenac β-cyclodextrin in combination with local anaesthetics for mesotherapy applications. Evid Based Complement Alternat Med. 2017;2017:8321325. doi:10.1155/2017/8321325
48. Van Mulder T. J. S. , de Koeijer M, Theeten H. et al., "High frequency ultrasound to assess skin thickness in healthy adults," Vaccine, vol. 35, no. 14, pp. 1810–1815, 2017.
49. Verma-Gandhu M., Bercik P., Motomura Y. et al., "CD4+ Tcell modulation of visceral nociception in mice," Gastroenterology, vol. 130, no. 6, pp. 1721–1728, 2006.
50. S. M. Wahl, N. McCartney-Francis, J. Chan, R. Dionne, L. Ta, and J. M. Orenstein, "Nitric oxide in experimental joint İnflammation:benefit or detriment?"Cells Tissues Organs, vol. 174, no. 1-2, pp. 26–33, 2003
51. Xiao WH, Bennett GJ. C-fiber spontaneous discharge evoked by chronic inflammation is suppressed by a long-term infusion of lidocaine yielding nanogram per milliliter plasma levels. Pain. 2008;137 (1):218–228. doi:10.1016/j.pain.2008.02.018
52. Yang XN, Geng ZS, Zhang XL, et al. Single intracutaneous injection of local anesthetics and steroids alleviates acute nonspecific neck pain: A CONSORT-perspective, randomized, controlled clinical trial. Medicine (Baltimore).2018;97(28):e11285.doi:10.1097/MD.0000000000011285

Nursing/ Midwifery Management in Women With Vaginismus

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INTRODUCTION

Vaginismus, also known as sheath (vagina) contraction; It is an involuntary contraction of the muscles surrounding 1/3 of the vagina starting from the entrance part of the vagina during sexual intercourse and closing itself to sexual intercourse (Poroy, 2010; Öztürk and Uluşahin, 2016). This contraction causes the closure of the vaginal entrance and prevents sexual intercourse (Poroy, 2010). Vaginismus is a sexual dysfunction defined under the title of "Physiological Disorders and Behavioral Syndromes Associated with Physical Factors" in ICD-10 (Öztürk and Uluşahin, 2016). While this disorder is covered under the "Diseases with Sexual Pain" in the "Sexual Dysfunctions" headline in DSM-IV, it is defined as "pain in the genitals-pelvis/penetrating disorder" in the same sub-title in DSM-V. Various methods are used in the treatment of vaginismus, which is very common in our country. Vaginismus, which is a functional sexual disorder, responds well to these treatments. Vaginismus treatment; requires multidisciplinary work involving gynecologist, psychiatrist, sexual therapist and midwife-nurse (Köroğlu, 2018).

ETIOLOGY

Although the exact reason is not known completely, vaginismus in general is a situation in which social, cultural, psychological and physiological reasons complement and integrate each other (Dağ et al., 2012). According to its beginning, vaginismus is approached in two ways as primary and secondary (Aydemir, 2019). Primary vaginismus is defined as the inability to have sexual intercourse in any way since the sexual age begins (Aydemir, 2019). The fear of the pain related to the first sexual intercourse experience, psychological reasons, the upbringing style that causes women to identify sexuality with the feeling of getting dirty, shame and sin, and fears from childhood may cause this type of vaginismus (Poroy, 2010; Öztürk & Uluşahin, 2016; Sadock et al., 2016). Anxiety may come from the fear of pain and ‘’breaking up because of a penis’’ that the woman symbolically enlarged in her mind (Öztürk & Uluşahin, 2016), as well as the fear that the severe pain she experienced in childhood due to surgical or dental treatment will damage her body integrity. (Sadock et al., 2016). In addition, fears of becoming pregnant are also important (Öztürk & Uluşahin, 2016). In secondary vaginismus, the woman has a normal sexual life. It is the involuntary contractions experienced after a traumatic event at any stage of his life, although she can have sexual intercourse (Aydemir, 2019). This situation is usually caused by involuntary muscle contractions as a result of a traumatic event such as rape or painful sexual intercourse during periods such as menopause (Dağ et al., 2012). It can occur as a bodily reaction of the female body, especially

against any physical pain experienced in previous sexual intercourse (Poroy, 2010).

In general, it is thought that marital adjustment, the relationships between women's parents, the father-daughter relationship that affects the electra confusion, the bodily image of women, social norms regarding male-female roles, phobic characteristics in women and symptoms of anxiety have effects on the emergence of vaginismus (Tuğrul, 2016; Öztürk & Uluşahin). , 2016). There is an extensive literature on psychosocial factors related to vaginismus, but still all data are inconclusive. This situation leads to the conclusion that more research should be done on the etiology of vaginismus (Fadul et al., 2019).

EPIDEMIOLOGY

Although the data on the frequency of vaginismus is limited, it also varies (Aydemir, 2019). While there are sources stating that vaginismus is the most common sexual dysfunction in women (Crowley et al., 2006; Ter Kuile et al., 2007), there are also claims reporting that it is less common than orgasm disorder (Sadock et al., 2016). However, this situation is also associated with the fact that some of the women who suffer from the vaginismus diagnostic criteria do not apply to clinics for treatment due to embarrassment and shyness (Aydemir, 2019). Although vaginismus is the most common sexual dysfunction in couples applying to therapy in our country, its frequency analysis has been reported as 15.3% (Yılmaz et al., 2010; Aydemir, 2019). In another study, the results of the research conducted in primary care show the frequency of vaginismus as 41.7% (Doğan and Saraçoğlu, 2009). A study conducted with 54 women with sexual dysfunction who applied to the psychiatry clinic shows that more than three-quarters of women (75.9%) suffer from vaginismus for life long.(Doğan and Saraçoğlu, 2009). When we look at the countries in the world, Turkey has similar results. According to the data stated in the literature, the frequency analysis of vaginismus is 40% in India (Khajehei et al., 2009), 14-16% in Brazil (Junqueira et al., 2005; Bento de Lima et al., 2014), % in the USA. 43 (Lewis et al., 2004). Studies have revealed that it is most common in women with higher education and high socioeconomic status (Sadock et al., 2016). When the demans are made for the sexual treatment to the centers, it is examined and seen that the majority of the applicants with the diagnosis of vaginismus are young couples the marital relations of these women are generally good, and their husbands mostly have undestanding, dependent and passive personalities (Yıldırım Hacıoğlu, 2017).

DIAGNOSIS AND TREATMENT

It can be difficult to diagnose vaginismus because painful intercourse and vaginismus can be confused with each other. It is necessary to take an extensive anamnesis, including a detailed medical history, psychosocial relationship, and sexual traumatic experience events (Crowley et al., 2006). There are also cases that do not apply with vaginismus complaints but occur during gynecological examination. Involuntary contraction of the pelvic floor muscle may cause speculum used during the gynecological examination to not enter, and the patient may not take the shape of dorsal lithotomy position. In this case, vaginismus can be mentioned (Sadock et al., 2016). In this regard, the gynecological examination experiences of the patients are also a guide for sexual therapists. Gynecological examination can sometimes be the only way to reveal the underlying pathological problem of the patient's vaginismus, which may also have effects on his physical health. Therefore, it is necessary to develop a chain of pleasure on women's health (Yalom, 2012). Women with vaginismus can be distinguished by normal controls on the basis of pelvic floor muscle situation.The main difference in these women is that it causes physical pain during coitus (Ebert et al., 2013).

MIDWIFERY AND NURSING MANAGEMENT

Patients who describe their emotional and psychological problems with physical function and structural disorders need qualified medical care and midwifery/nursing care, and in this process, attention should be paid to the collection of subjective data as well as objective data (Kum, 2000). Although the evaluation of sexual life, which is one of these subjective data, and giving advisory support on this issue are among the duties of the midwife-nurse, most midwives-nurses do not fulfill these competencies (Dağ et al., 2012)

According to the subjective data stated by Townsend in 1988, situations such as low confidence, depression mood, denial of emotional problems, inability to fulfill role expectations, repressed or inappropriately expressed anger may develop in women in this process, and midwifery/nursing interventions should be planned for this purpose (Kum, 2000). . A detailed clinical history including sexual, physical, psychological and sociocultural evaluation of the woman should be taken and midwifery/nursing interventions should be planned within the framework of a holistic approach to women (Dağ et al., 2012). The behavioral approach chosen in treatment should also be used by nurses and physicians working with this female population (Rosenbaum, 2011). Women with vaginismus generally tend to show sexual disgust and anxiety. Patience and empathy are required for women to feel safe and for successful treatment (Rosenbaum, 2011; Ebert et al., 2013). Midwives/nurses can also take part in

dilator therapy, which includes relaxation techniques, homework, and partner participation (Rosenbaum, 2011). The purpose in this behavioral approach is to ensure that new and correct responses are learned instead of the responses of the woman which are learnt in an incorrect way. This approach includes gradually applying the vaginal finger entry or dilators, kegel and coitus exercises, starting with the exercises that allow the woman to recognize her own body (Dağ et al., 2012). The process may vary between one and six patient visits (Rosenbaum, 2011). The partner of the woman should be included in this therapy, and the midwife/nurse is responsible for comforting and educating both the woman and her partner about the use of dilators and exercises during this therapy (Pacik and Geletta, 2017). Necessary coping methods should be taught and the couple should be motivated to cope with the stress that therapy creates. (Yalom, 2012). It is also known that women with vaginismus who cooperate with a competent health professional can and do have a vaginal delivery with special intrapartum care, and this care minimizes the frequency of vaginal examinations during delivery. For this reason, not only psychiatrists and sexual therapists are involved in meeting the needs of women with vaginismus; nurses and midwives also play an important role (Deliktaş Demirci and Kabukçuoğlu, 2019). Nurses and midwives have an important role in revealing women's approaches to sexuality, raising awareness about sexuality, revealing unfinished husband-wife marriages, early diagnosis of the current problem, receiving the necessary medical and psychological support of women, and increasing their quality of life by providing counseling services to women.

CONCLUSION: Vaginismus, which is very common in women, responds well to treatments and by this way problem disappears. This treatment should be given by a multidisciplinary team member. Nurses and midwives, who are the health professionals who spend the most time with the patient, play a significant role in this team. Nurses and midwives should provide an effective and qualified service in the care they will give to patients with vaginismus.

REFERENCES

1. Aydemir, G. (2019). Determination of Sexual Attitude and Perfectionism Characteristics of Women with and Without Vaginismus Diagnosis. Department of Psychology, Master Thesis: Istanbul.
2. Bento de Lima, H. J. et al. (2014). Sexual dysfunction and associated factors reported in the postpartum period. Acta Paulista De Enfermagem, 27(6): 573.
3. Crowley, T., Richardson, D., Goldmeier, D. (2006). Recommendations for The Management of Vaginismus: BASHH Special Interest Group for Sexual Dysfunction International Journal of STD&AIDS, 17(1): 14-18.
4. Mountain, H., Dönmez, S., Kavlak, O. (2012). The Hidden Aspect of Female Sexuality: Vaginismus and the Role of the Nurse.
5. Deliktaş Demirci, A., Kabukçuoğlu, K. (2019). "Being a Woman" in the Shadow of Vaginismus: The Implications of Vaginismus for Women. Current Psychiatry Research and Reviews. 15: 1-6.
6. Doğan, S., Saraçoğlu, G. V. (2009) Evaluation of sexual knowledge, marital characteristics, sexual function and satisfaction in women with lifelong vaginismus. Trakya University Faculty of Medicine Department, Istanbul.
7. Ebert, M.H., Loosen, P.T., Nurcombe, B., Leckman, J.F. (Trans. Ed. Candansayar, S.) (2013). Current Psychiatry Diagnosis and Treatment. Istanbul: Güneş Medical Books.
8. Fadul, R. et al. (2019). Psychosocial Correlates of Vaginismus Diagnosis: A Case-Control Study, Journal of Sex & Marital Therapy, 45(1): 73-83.
9. Junqueira, F. R. R. et al. (2005). Setting up a sexuality outpatient clinic in a ginecology service of a university hospital: Result of one year report. Reproduction climaterial, 20: 13–16.
10. Khajehei, M., Ziyadlou, S., Kashefi, F. (2009). Los Problemas Sexuales son muy Frecuentes después del Parto, (Sexual problems are very commom after delivery). Indian Journal of Community Medicine. 34(2): 126–130.
11. Köroglu, E. (Ed.) (2018). DSM-V American Psychiatric Association diagnostic and statistical manual of mental disorders. 5th Edition. Ankara: Physicians Publication Association.
12. Sand, N. (Ed) (2000). Psychiatric Nursing Handbook. Istanbul: Vehbi Koç Publications.
13. Lewis, R.W. et al. (2004). Epidemiology/risk factors of sexual dysfunction. J Sex Med. 1:35–9.

14. Pacik, P.T., Geletta, S. (2017). Vaginismus Treatment: Clinical Trials Follow Up 241 Patients. Sexual Medicine, 5(2): 1-5.
15. Poroy, A. (2010). The Handbook of Sexuality. Special edition. Istanbul: Alfa Publications.
16. Rosenbaum, T. (2011). Addressing Anxiety In Vivo in Physiotherapy Treatment of Women with Severe Vaginismus: A Clinical Approach. Journal of Sex & Marital Therapy, 37(2): 89-93.
17. Sadock, B.J., Sadock, V.A., Ruiz P. (2016). Kaplan & Sadock Psychiatry. Eleventh Edition. İstanbul: Güneş Medical Bookstores.
18. Ter Kuile, M.M., Van Lankveld, J.J., Groot, E., Melles, R., Neffs, J., Zandbergen, M. (2007). Cognitive-behavioral therapy women with lifelong vaginismus: Process and prognostic factors. Behaviour Research and Therapy, 45(2): 359-73.
19. Tuğrul, C., Kabakçı, E. (2016) Predictive variables in vaginismus cases. Turkish Journal of Psychiatry, 7 (3): 201-207.
20. Yalom, I.D. (2012). Sex Therapy. Istanbul: Prestige Publications.
21. Yıldırım Hacıoğlu, M. (2017). Comparison of Vaginismus with Other Sexual Dysfunctions in terms of Sociodemographic and Clinical Characteristics. Clinical Psychiatry, 20: 37-44.
22. Yılmaz, E., Zeytinci, İ.E., Sarı, S., Karababa, İ.F., Çilli, A.S. & Kucur, R. (2010). Investigation of Sexual Problems in Married People Living in The Center of Konya. Turkish Journal of Psychiatry, 21(2): 126-134.

Exosomes; Cute Little Regulators in the Nervous System

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1. Introduction

Extracellular vesicles (EVs) are lipid-bound molecules that can be released from all kinds of cells and provide intercellular communication. First described by Wolf in plasma, EVs have a heterogeneous population of diverse origins (Wolf 1967). It contains various microvesicles that can transport molecules such as nucleic acids, proteins, and lipids to target cells. Although EVs were initially classified according to the source cell, size, or morphology, today they are divided into three main groups (Table 1); exosomes, apoptosomes, and microvesicles (Van Niel et al. 2018). Exosomes are nano-sized lipid bilayer extracellular vesicles released from various somatic cells that enable intercellular communication. Exosomes can be taken up by endocytosis, fuse with the cell membrane, and transfer their contents to the recipient cell, or they can provide cellular communication through receptor-ligand interaction. Exosomes can maintain intracellular balance by playing a role in intercellular communication through the various nucleic acids, proteins, and metabolites they contain. In this way, exosomes can play a role in the pathogenesis and treatment of many diseases, as well as affecting the activities of recipient cells by carrying various molecules in the intercellular space. Exosomes also play a role in signaling mechanisms and cell or tissue metabolism, and may also influence tissue responses to injury and disease (Phinney and Pittenger 2017).

Table 1. Overview of the main characteristics of extracellular vesicles

Vesicle Type	Origin	Size (nm)	Marker	Contents	Morphology
Apoptotic Bodies	Plasma membrane (outward overflowing of apoptotic cell membrane)	~1000-5000	Extensive amounts of phosphatidyl serine (PS)	Nuclear Fractions and organelles	Cup shaped
Microvesicles	Plasma membrane (outward overflowing of apoptotic cell membrane)	~50-1000	Integrins Selectins CD40 ligand	Cytoplasmic and membrane proteins RNAs	Cup shaped
Exosomes	Endosomal pathway	~30-120	Flotillin Alix TSG101 Tetraspanins PS etc.	Lipids RNAs (mRNA, miRNA, non-coding RNAs) Cytoplasmic and membrane proteins MHC molecules	Heterogeneous

(Van der Pol et al. 2012; Kawahara and Hanayama 2018)

2. Exosome Biogenesis

An exosome is a membrane vesicle released from cells as a result of the fusion of the multivesicular body with the plasma membrane (Hessvik and Llorente 2018). Exosomes are highly heterogeneous and reflect the contents of their cells of origin (Kalluri 2016). Consisting of a lipid bilayer, exosomes also contain extracellular receptors and matrix proteins (Yellon and Davidson 2014; Gurung et al. 2021). Exosomes generally contain nucleic acids, members of the tetraspanin family, endosomal-sorting complex required for transport (ESCRT) proteins involved in exosome biogenesis, integrins, growth factors, and cytokines, heat shock, cytoskeletal, membrane transport, and fusion proteins (Figure 1).

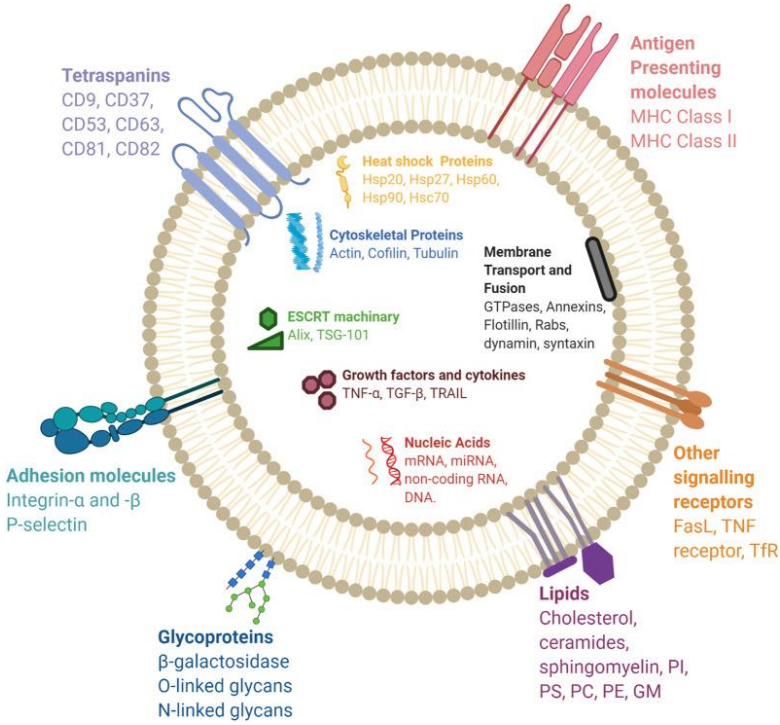

Figure 1. Biological Composition of Exosomes
(Gurung et al. 2021)

Nano-sized exosomes are formed within the endosomal network (Yáñez-Mó et al. 2015). The first stage of exosome biogenesis is the formation of early endosomes by the fusion of primary endocytic vesicles (Mashouri et al. 2019). Endocytic cargoes assemble early endosomes and later early endosomes transform into late endosome (LE)/multivesicular bodies (MVBs) (Mashouri et al., 2019). MVBs, which are known as late endosomes and formed by the union of early endosomes, contains intraluminal

vesicle (ILV) carrying specific lipid, protein, and cytosolic components (Alenquer and Amorim 2015; Gurung et al. 2021). MVBs transport to the plasma membrane and fuse with this membrane, releasing the ILVs, which are formed by the inward budding of the endosomal membranes, out of the cell. ILVs released from the cell membrane are now called exosomes (Alenquer and Amorim 2015; Gurung et al. 2021). In cases where early endosomes do not form LE/MVBs, endocytic cargoes are packaged as ILVs and released extracellularly by fusing directly with the plasma membrane.

Classification of cargo by ILVs can be an ESCRT-dependent as well as ESCRT-independent pathway. The ESCRT-dependent system starts with an available path for cargo transportation. All ESCRT subunits play a role in this system determined by the ubiquitin (ub) checkpoint. ESCRT-0 enables the recognition of mono-ubiquitinated proteins, while ESCRT-I and II join ESCRT-0 to form a high-affinity recognition site for ubiquitinated substrates at the endosomal membrane. Then, ESCRT-III joins the ESCRT-0/I/II complex, constricting the membrane and releasing the buds into the endosome. After this stage, ILVs carrying cargo whose ubiquitins are not removed by de-ubiquitinating enzymes (DUBs) are degraded by the lysosomes (Mashouri et al. 2019).

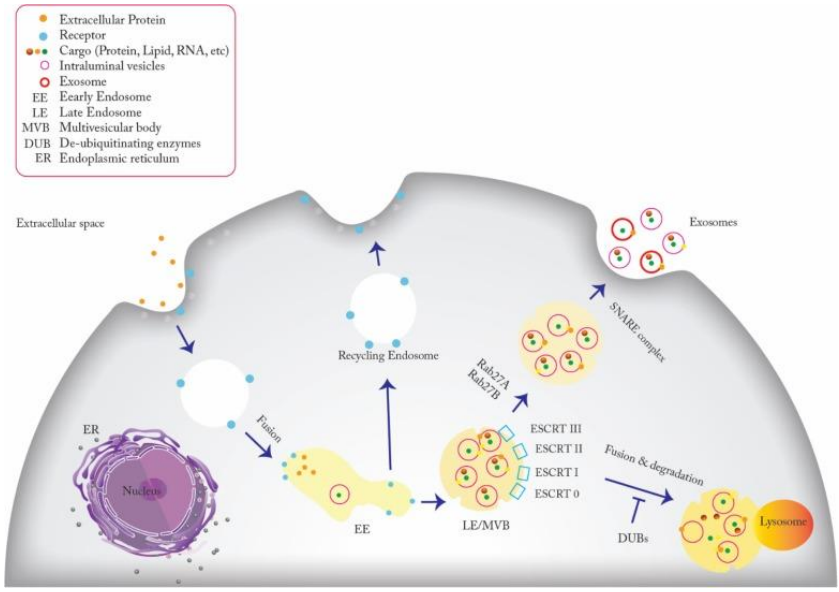

Figure 2. Exosome Biogenesis. *The process of exosome biogenesis begins within the endosomal system. In this system, endocytic cargos are either packaged as ILV and released from the plasma membrane, or early endosomes carrying endocytic cargos mature into LE/MVBs. After this process, MVBs can secrete their ILVs from the plasma membrane as exosomes or degrade by fusing with lysosomes (Mashouri et al. 2019).*

3. Role of Exosomes in the Nervous System

Exosomes can be secreted from neural stem cells (NSC), neuronal cells, and many glial cells such as astrocytes and oligodendrocytes in the nervous system. Exosomes transfer molecules belonging to the source cell to the receiving neuronal or glial cell and thus play a vital role in the cellular communication of the nervous system by providing an intercellular information transmission (Qing et al. 2018). Exosomes in the nervous system contribute to the survival of neuronal cells by playing a role in cellular communication between motor and sensory neurons, as well as between interneurons and glial cells, through molecules such as miRNA, mRNA, and protein (J. Y. Li, Li, and Sheng 2021). In addition, nervous system exosomes may also play a role in many neuronal processes such as myelin formation, regeneration of axons, neurite outgrowth, regulation of synapse function, and nerve cell survival (Doyle and Wang 2019).

3.1. Neural Communication

Exosome, a lipid bimolecular vesicle, is 30-120 nm in size and thus has the ability to cross the blood-brain barrier (BBB). Exosomes released from nervous system cells can penetrate the BBB and circulate in the central nervous system (CNS) and join the peripheral circulation (J. Y. Li, Li, and Sheng 2021). Exosomes, which are an important component of the inter-neuronal and neuronal-glial communication system, provide cellular communication by transporting the contents of the source cell and thus play a role in the regulation of nervous system cell functions.

Many glial cells, mainly astrocytes, Schwann cells (SCs), and oligodendrocytes (OLs), can communicate with neurons via secreted exosomes. Oligodendrocytes are a rich source of myelin-associated and stress-protective proteins. Therefore, OL-derived exosomes may also contribute to stabilizing the production of myelin proteins and lipids in the recipient neuronal cell (Krämer-Albers et al. 2007). Neuronal cells can improve cellular viability under stress conditions using the content of OL exosomes. Studies have shown that OL exosomes can provide survival of neurons under oxidative stress by transferring superoxide dismutase and catalase to neuronal cells (Fröhlich et al. 2014). SCs exosomes are taken up by axons in the peripheral nervous system (PNS) and contribute to cellular events such as axonal regeneration and neurite outgrowth. SCs exosomes are among the important factors that play a role in neural regeneration and protection of axons (Lopez-Verrilli and Court 2012). Besides the OL and SC, astrocytic exosomes may also be uptake by neurons and play a role in the survival of neuronal cells. Studies have shown that astrocytic exosomes contain synapsin I, which promotes neurite outgrowth and neuronal

survival when taken up by neurons (Wang et al. 2011). In addition to these cells, the uptake of microglial cell exosomes by neurons has not been proven yet, but there are reports in the literature that microvesicles released from these microglial cells, known as immune cells of the nervous system, rather than microglial exosomes, stimulate synaptic activity in neurons (Antonucci et al. 2012; Janas et al. 2016). Mesenchymal stem cells (MSCs), which have a role in tissue regeneration and can differentiate into a wide variety of cell lines, can also communicate with neurons via the exosome. MSC-derived exosomes can be internalized by neuronal cells and thus support neuronal recovery. Studies showed that MSC exosomes, which have a very heterogeneous structure, transfer molecules such as miR-133 to neurons, provide neurite outgrowth in damaged neuronal cells, and contribute to neuronal recovery functionally (Xin et al. 2013).

3.2. Neural Maintenance

Neurons and glial cells in both the CNS and the PNS can secrete exosomes. These exosomes provide intercellular communication, mediate the realization of various neuronal and glial activities, and play a role in protecting these cells. One of the main examples of this process is the participation of exosomes, which are involved in communication between presynaptic and postsynaptic cells, in the neuronal homeostasis (Korkut et al. 2013). Another is that neurons release exosomes that communicate with oligodendrocytes and myelin cells, which regulate myelin maintenance and synaptic function to maintain neuronal integrity (Domingues et al. 2020; J. Y. Li et al. 2021). In the nervous system, cells carry out this communication through molecules specific to the source cell carried by the exosomes they secrete (Table 2). For example, glial cell exosomes contribute to their protection by providing metabolic support to neurons through cytoplasmic and nuclear proteins such as heat shock protein 70, proteolipid protein (PLP), and myelin-associated glycoprotein (MAG) they carry (Potolicchio et al. 2005; Krämer-Albers et al. 2007; Frühbeis, Fröhlich, and Krämer-Albers 2012; Lopez-Verrilli and Court 2012). Besides such proteins, exosomes contribute to neural development and the protection of neurons, especially through the miRNAs that they contain. These miRNAs also play a role in the myelin sheathing of axons and the proliferation of myelination cells (He et al. 2012; Svaren 2014). In addition to neurons and glial cells, MSCs, blood cells, fibroblasts, bone marrow mesenchymal stem cells (BMSCs), and adipose-derived stem cells (ADSCs) are also sources that have an important role in neuronal protection through the exosomes they secrete (J. Y. Li et al. 2021). ADSCs exosomes reduce neuronal cell death through miRNAs such as miR126 (Geng et al. 2019). BMSCs can inhibit neuronal apoptosis through exosomal miR-134

(Xiao et al. 2019). In addition to all these nervous system exosomes and exosomal molecules, the removal of various cellular wastes by neurons through the exosomes they create can be considered a protective mechanism for neurons.

Table 2. List of exosomal molecules and their effects on neural maintenance

Source of Exosome	Cargo Component	Function	Reference
Microglia	miR-124	Reduces neuronal apoptosis (neuronal survival)	Song et al. 2019a
	miR-137	Neuroprotection	Zhang et al. 2021
	Nervous Growth/Differentiation Factor (Ngdf)	Nerve Regeneration	Raffo-Romero et al. 2018
Schwann Cells	$P75^{NTR}$	Neurite outgrowth and axonal regeneration	Lopez-Verrilli, Picou, and Court 2013
	miR-21	Axonal regeneration	López-Leal et al. 2020
Cortical Neurons	miR-124a	Regulates glutamate transporter-1 (GLT1) expression and modulates synaptic activation	Morel et al. 2013
Glia	Synapsin I	Axonal growth and neuronal survival	Wang et al. 2011
Neuron	miR-181c-3p	Inhibits neuroinflammation	Song et al. 2019b
$NG2^{+}$ Glia	Retinoic acid	Enhances axonal growth	Goncalves et al. 2018
Astrocyte	miR-92b-3p	Reduces axonal death (neuronal survival)	Xu et al. 2019
MSC	miR-133b	Increase neuroplasticity	Xin et al. 2013
	miR-233-3p	Inhibit neuroinflammation	Zhao et al. 2020a
Fibroblast	Wnt10b	Axonal regeneration	Tassew et al. 2017
Plasma	miR-126	Neuroprotection (by downregulation DNA methyltransferase)	Cui et al. 2020
	miR-451a	Neuronal survival	Li et al. 2021

3.3. Axonal Regeneration

After damage such as neuronal injury in the nervous system, glial cells play a role rather than neurons themselves in the process of regeneration of axons (Uyeda and Muramatsu 2020). OLs in the CNS and SCs in the PNS are responsible for the regeneration of axons and their myelin sheath. In addition to glial cells, exosomes secreted by these cells have been shown to support axons through various exosomal molecules (Krämer-Albers et al., 2007). In the nervous system, in conditions such as inflammation that causes degeneration of axons, OL and SC exosomes are internalized by neurons through the axon or soma and play a role in the regeneration of axons in neurons (Xia et al. 2022).

Exosomes contain several molecules that can modulate neurite outgrowth as well as support axons. SC and OL exosomes can regulate axonal physiology by causing changes in protein expression in axons through molecules such as miRNA and mRNA specific to their source cell (Lopez-Leal and Court 2016). Studies have shown that SC exosomes regulate some pathways in the growth cone that enable the growth of axons through these molecules. For example, these exosomes inhibit Rho, a GTPase that inhibits axonal elongation, or anti-regenerative programs (Lopez-Verrilli and Court 2012; Lopez-Leal and Court 2016). In addition, SC exosomes taken up by axons promote neurite outgrowth and regeneration of axons via a myelinating protein they carry, such as $p75^{NTR}$ (Lopez-Verrilli et al. 2013). In addition, it has been shown that SC exosomes cultured with dorsal root ganglia activate the growth cone and also elongate axons (Lopez-Verrilli and Court 2012). Also, astrocytic embryonic glial-restricted precursor cells can promote axonal growth and motor function recovery through the integrin-binding extracellular matrix protein Periostin they secrete (Shih et al. 2014). Synapsin I, another molecule found in exosomes released from glial cells, can be carried by exosomes of astrocyte-enriched primary cultures and may play a role in neurite outgrowth in neurons (Fassio et al. 2011). In addition to these molecules, a non-coding RNA such as miR-21 is responsible for the axonal high regeneration capacity of SCs exosomes in the neurons (López-Leal et al. 2020). Besides glial cells, exosomes originating from immune cells can also induce axonal regeneration through the inhibition of PTEN, a negative regulator of the neuronal regeneration (Hervera et al. 2018). Similarly, MSC exosomes have been shown to be involved in the regeneration and elongation of axons through a mechanism that may include the presence or miRNA-mediated regulation of multiple neurotrophic factors (Bucan et al. 2019; Zhao et al. 2020b).

3.4. Synaptic Function

Exosome release can be induced by neuronal activation and can occur from dendrites or the post-synaptic soma (Men et al. 2019). It is thought that exosomes released from neurons play a regulatory role in synapses through the synaptic protein, mRNA, and miRNA that they contain (Li et al. 2020). An example of these molecules is the activity-regulated cytoskeleton-associated protein (ARC), which is the main regulator of synaptic plasticity. The ARC protein travels between cells via exosomes and is transferred from the pre-synaptic terminals to the postsynaptic muscles, where it plays a role in synapse maturation and regulation of activity-dependent synaptic plasticity (Ashley et al. 2018; Xia et al. 2022). Moreover, exosomes released from pre-synaptic cells and neuronal exosomes containing the Ca^{2+} sensor synaptotagmin-4 regulate retrograde postsynaptic signaling, thereby enabling pre-synaptic growth and quantal neurotransmitter release at neuromuscular junctions (Korkut et al. 2013; Xia et al. 2022; Zhang et al. 2011). Neuronal exosomes also regulate synaptic activation through synaptic proteins such as glycosylphosphatidylinositol (GPI)-anchored prion protein and glutamate receptor subunit GluR2/3 (Fauré et al. 2006). Exosomes released by neurons can be taken up by glial cells, and functions modulating synaptic activity can be regulated. For example, exosomes taken up by astrocytes regulate the expression of GLT1, a glutamate transporter, thus protecting synapses from neurotoxicity by ensuring appropriate glutamate uptake by astrocytes (Huang et al. 2011; Sicot et al. 2017; Men et al. 2019; Xia et al. 2022). Additionally, exosomes released from glial cells such as astrocytes, oligodendrocytes, and microglia also play a role in synapse formation and synaptic activity. Found in glial cell exosomes, Synapsin I, as a synaptic vesicle protein, plays a role in synaptic vesicle turnover and modulation of the neurotransmitter release (Fassio et al. 2011). Astrocytic exosomes can activate some signaling pathways that lead to the formation of synapses in neurons through the molecules they carry on their surface (Patel and Weaver 2021). Microglial exosomes can regulate synaptic activity in glutamatergic neurons as well as synaptic remodeling.

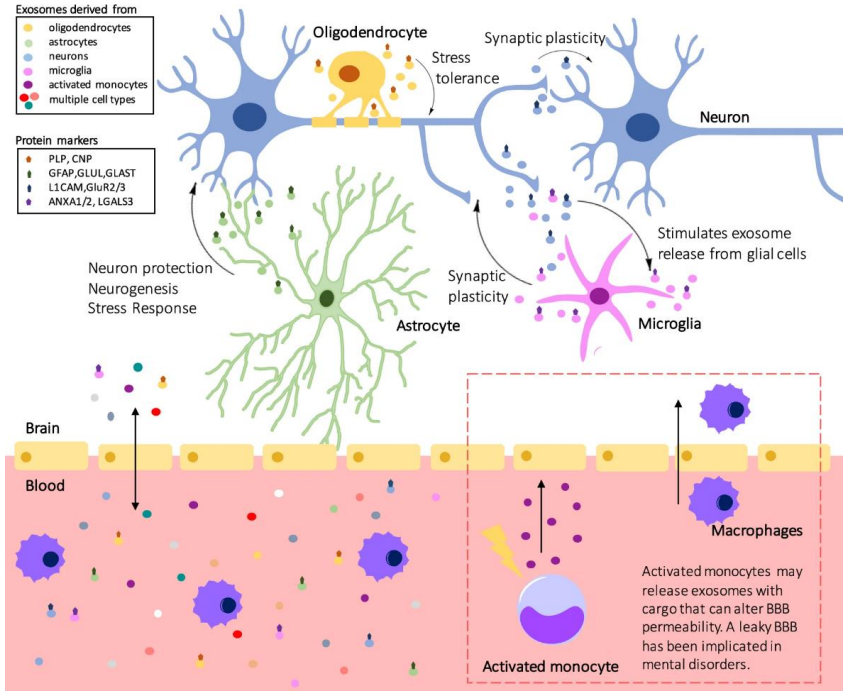

Figure 3. The role of exosomes in physiological brain processes (Saeedi et al. 2019)

3.5. Neuronal Inflammation

The inflammatory response that occurs after damage/injury in the nervous system is an important process involved in the nerve repair (Huang et al. 2011). MSCs, which play an important role in this process, provide suitable conditions for the repair of nerve cells through the exosomes they secrete (Liu et al. 2019). Studies have shown that bone marrow MSC exosomes inhibit the expression of proinflammatory cytokines such as TNF-a and interleukin (IL)-1β in traumatic brain injury (TBI) models. In addition, these exosomes have been shown to modulate microglia/macrophage polarization by reducing the expression of inducible nitric oxide synthase (iNOS), thereby inhibiting early neuroinflammation (Ni et al. 2019). Similarly, it has been shown that extracellular vesicles released from umbilical cord MSCs decrease the level of proinflammatory cytokines such as IL-6 and IL-1β and exhibit an immunosuppressive effect on peripheral nerve repair by increasing the level of an anti-inflammatory cytokine such as IL-10 (Ma et al. 2019). In addition to MSCs, SCs exosomes have also been shown to have anti-inflammatory effects. However, SC exosomes were found to exhibit regulatory effects in the inflammatory phase of nerve damage (Wei et al. 2019). A study with microglia,

known as immune cells of the nervous system, showed that microglia exosomes also have an anti-inflammatory effect. Huang et al. showed that miR-124-3p promotes anti-inflammatory M2 polarization in microglial cells, and also reduces neuronal inflammation through the microglial exosomes (Huang et al. 2018). Besides, it has been shown that microglial exosomal miR-124-3p affects the growth of neurites, decreases the expression of some neurodegenerative proteins and Rho, and thus has inhibitory effects on the neuroinflammation (Huang et al. 2018).

3.6. Therapeutic Role of Exosomes

The biogenesis of exosomes, which are lipid bilayers and nano-sized, is a very important factor for the exosomal treatment approach. Increasing evidence suggests that exosomes, which transport active biological molecules such as nucleic acids and proteins between cells, may play a role in the treatment of various diseases. Exosomes can thus create an environment unsuitable for the development of many diseases, including nervous system diseases (NSDs). Studies showed that exosomes have a reparative role in both CNS and PNS. It is known that exosomes originating from neurons, glial cells or MSCs contribute to neural protection by playing a role in various cellular pathways in the neuronal cell through the specific exosomal molecules. Exosomes have many effects such as neuronal survival, neural development, axonal growth and regeneration, synaptic regeneration, and anti-inflammatory. Considering these reparative properties of exosomes, it can be thought that synaptic dysfunction and neural degeneration caused by abnormal protein accumulation, which is one of the common features of NSDs, can be repaired through the exosomes.

In addition to their neuroprotective role, exosomes are seen as a suitable tool for cell-free therapies, as they can penetrate the BBB and do not cause an immunological reaction. In addition to these properties, exosomes can be used as a drug delivery system for small molecules in the treatment of many diseases, including NSDs (Janas et al. 2016). Exosomes can also be protected from various lysosomal degradations and destructive enzymes such as RNase and protease (Turturici et al. 2014; Ha, Yang, and Nadithe 2016). Additionally, exosomes are molecules amenable to modification. Thanks to this feature, targeted treatment approaches can be applied by making surface modifications in exosomes. These properties make exosomes a particularly suitable tool for the treatment of NSDs.

References

1. Alenquer, Marta, and Maria João Amorim. 2015. "Exosome Biogenesis, Regulation, and Function in Viral Infection." *Viruses* 7(9):5066–83. doi: 10.3390/v7092862.
2. Antonucci, Flavia, Elena Turola, Loredana Riganti, Matteo Caleo, Martina Gabrielli, Cristiana Perrotta, Luisa Novellino, Emilio Clementi, Paola Giussani, Paola Viani, Michela Matteoli, and Claudia Verderio. 2012. "Microvesicles Released from Microglia Stimulate Synaptic Activity via Enhanced Sphingolipid Metabolism." *EMBO Journal* 31(5):1231–40. doi: 10.1038/emboj.2011.489.
3. Ashley, James, Benjamin Cordy, Diandra Lucia, Lee G. Fradkin, Vivian Budnik, and Travis Thomson. 2018. "Retrovirus-like Gag Protein Arc1 Binds RNA and Traffics across Synaptic Boutons." *Cell* 172(1–2):262-274.e11. doi: 10.1016/j.cell.2017.12.022.
4. Bucan, Vesna, Desiree Vaslaitis, Claas Tido Peck, Sarah Strauß, Peter M. Vogt, and Christine Radtke. 2019. "Effect of Exosomes from Rat Adipose-Derived Mesenchymal Stem Cells on Neurite Outgrowth and Sciatic Nerve Regeneration After Crush Injury." *Molecular Neurobiology* 56(3):1812–24. doi: 10.1007/s12035-018-1172-z.
5. Cui, Junhe, Na Liu, Zhehan Chang, Yongsheng Gao, Mulan Bao, Yabin Xie, Wenqiang Xu, Xiaolei Liu, Shuyuan Jiang, You Liu, Rui Shi, Wei Xie, Xiaoe Jia, Jinghua Shi, Changhong Ren, Kerui Gong, Chunyang Zhang, Rengui Bade, Guo Shao, and Xunming Ji. 2020. "Exosomal MicroRNA-126 from RIPC Serum Is Involved in Hypoxia Tolerance in SH-SY5Y Cells by Downregulating DNMT3B." *Molecular Therapy - Nucleic Acids* 20(June):649–60. doi: 10.1016/j.omtn.2020.04.008.
6. Domingues, Helena S., Ana Mendanha Falcão, Inês Mendes-Pinto, António J. Salgado, and Fábio G. Teixeira. 2020. "Exosome Circuitry during (De)(Re)Myelination of the Central Nervous System." *Frontiers in Cell and Developmental Biology* 8(June):1–10. doi: 10.3389/fcell.2020.00483.
7. Doyle, Laura M., and Michael Zhuo Wang. 2019. "Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis." *Cells* 8(7):29–39. doi: 10.3390/cells8070727.
8. Fassio, Anna, Andrea Raimondi, Gabriele Lignani, Fabio Benfenati, and Pietro Baldelli. 2011. "Synapsins: From Synapse to Network Hyperexcitability and Epilepsy." *Seminars in Cell and Developmental Biology* 22(4):408–15. doi: 10.1016/j.semcdb.2011.07.005.

9. Fauré, J., G. Lachenal, M. Court, J. Hirrlinger, C. Chatellard-Causse, B. Blot, J. Grange, G. Schoehn, Y. Goldberg, V. Boyer, F. Kirchhoff, G. Raposo, J. Garin, and R. Sadoul. 2006. "Exosomes Are Released by Cultured Cortical Neurones." *Molecular and Cellular Neuroscience* 31(4):642–48. doi: 10.1016/j.mcn.2005.12.003.
10. Fröhlich, D., W. D. Kuo, C. Frühberis, J. J. Sun, C. M. Zehendner, H. J. Luhmann, S. Pinto, J. Toedling, J. Trotter, and E. M. Kramer-Albers. 2014. "Multifaceted Effects of Oligodendroglial Exosomes on Neurons." *Phil. Trans. R. Soc.* 369(1652).
11. Frühbeis, Carsten, Dominik Fröhlich, and Eva Maria Krämer-Albers. 2012. "Emerging Roles of Exosomes in Neuron-Glia Communication." *Frontiers in Physiology* 3 APR(April):1–7. doi: 10.3389/fphys.2012.00119.
12. Geng, Wujun, Hongli Tang, Shan Luo, Ya Lv, Dongdong Liang, Xianhui Kang, and Wandong Hong. 2019. "Exosomes from Mirna-126-Modified Adscs Promotes Functional Recovery after Stroke in Rats by Improving Neurogenesis and Suppressing Microglia Activation." *American Journal of Translational Research* 11(2):780–92.
13. Goncalves, Maria B., Yue Wu, Diogo Trigo, Earl Clarke, Tony Malmqvist, John Grist, Carl Hobbs, Thomas P. Carlstedt, and Jonathan P. T. Corcoran. 2018. "Retinoic Acid Synthesis by NG2 Expressing Cells Promotes a Permissive Environment for Axonal Outgrowth." *Neurobiology of Disease* 111(November 2017):70–79. doi: 10.1016/j.nbd.2017.12.016.
14. Gurung, Sonam, Dany Perocheau, Loukia Touramanidou, and Julien Baruteau. 2021. "The Exosome Journey: From Biogenesis to Uptake and Intracellular Signalling." *Cell Communication and Signaling* 19(1):1–19. doi: 10.1186/s12964-021-00730-1.
15. Ha, Dinh, Ningning Yang, and Venkatareddy Nadithe. 2016. "Exosomes as Therapeutic Drug Carriers and Delivery Vehicles across Biological Membranes: Current Perspectives and Future Challenges." *Acta Pharmaceutica Sinica B* 6(4):287–96. doi: 10.1016/j.apsb.2016.02.001.
16. He, Xuelian, Yang Yu, Rajeshwar Awatramani, and Q. Richard Lu. 2012. "Unwrapping Myelination by MicroRNAs." *Neuroscientist* 18(1):45–55. doi: 10.1177/1073858410392382.
17. Hervera, Arnau, Francesco De Virgiliis, Ilaria Palmisano, Luming Zhou, Elena Tantardini, Guiping Kong, Thomas Hutson, Matt C. Danzi, Rotem Ben Tov Perry, Celio X. C. Santos, Alexander N. Kapustin, Roland A. Fleck, José Antonio Del Río, Thomas Carroll, Vance Lemmon, John L. Bixby, Ajay M. Shah, Mike Fainzilber, and Simone Di Giovanni. 2018.

"Reactive Oxygen Species Regulate Axonal Regeneration through the Release of Exosomal NADPH Oxidase 2 Complexes into Injured Axons." *Nature Cell Biology* 20(3):307–19. doi: 10.1038/s41556-018-0039-x.

18. Hessvik, Nina Pettersen, and Alicia Llorente. 2018. "Current Knowledge on Exosome Biogenesis and Release." *Cellular and Molecular Life Sciences* 75(2):193–208. doi: 10.1007/s00018-017-2595-9.
19. Huang, Shan, Xintong Ge, Jinwen Yu, Zhaoli Han, Zhenyu Yin, Ying Li, Fanglian Chen, Haichen Wang, Jianning Zhang, and Ping Lei. 2018. "Increased MiR-124-3p in Microglial Exosomes Following Traumatic Brain Injury Inhibits Neuronal Inflammation and Contributes to Neurite Outgrowth via Their Transfer into Neurons." *FASEB Journal* 32(1):512–28. doi: 10.1096/fj.201700673R.
20. Huang, Yunlong, Lixia Zhao, Beibei Jia, Li Wu, Yuju Li, Norman Curthoys, and Jialin C. Zheng. 2011. "Glutaminase Dysregulation in HIV-1-Infected Human Microglia Mediates Neurotoxicity: Relevant to HIV-1-Associated Neurocognitive Disorders." *Journal of Neuroscience* 31(42):15195–204. doi: 10.1523/JNEUROSCI.2051-11.2011.
21. Janas, Anna M., Karolina Sapoń, Teresa Janas, Michael H. B. Stowell, and Tadeusz Janas. 2016. "Exosomes and Other Extracellular Vesicles in Neural Cells and Neurodegenerative Diseases." *Biochimica et Biophysica Acta - Biomembranes* 1858(6):1139–51. doi: 10.1016/j.bbamem.2016.02.011.
22. Kalluri, Raghu. 2016. "The Biology and Function of Exosomes in Cancer." *Journal of Clinical Investigation* 126(4):1208–15. doi: 10.1172/JCI81135.
23. Kawahara, Hironori, and Rikinari Hanayama. 2018. "The Role of Exosomes/Extracellular Vesicles in Neural Signal Transduction." *Biological and Pharmaceutical Bulletin* 41(8):1119–25. doi: 10.1248/bpb.b18-00167.
24. Korkut, Ceren, Yihang Li, Kate Koles, Cassandra Brewer, James Ashley, Motojiro Yoshihara, and Vivian Budnik. 2013. "Regulation of Postsynaptic Retrograde Signaling by Presynaptic Exosome Release." *Neuron* 77(6):1039–46. doi: 10.1016/j.neuron.2013.01.013.
25. Krämer-Albers, Eva Maria, Niko Bretz, Stefan Tenzer, Christine Winterstein, Wiebke Möbius, Hendrik Berger, Klaus Armin Nave, Hansjörg Schild, and Jacqueline Trotter. 2007. "Oligodendrocytes Secrete Exosomes Containing Major Myelin and Stress-Protective Proteins: Trophic Support for Axons?" *Proteomics - Clinical Applications* 1(11):1446–61. doi: 10.1002/prca.200700522.
26. Li, He, Yin Luo, Peng Liu, Pei Liu, Weilong Hua, Yongxin Zhang, Lei

Zhang, Zifu Li, Pengfei Xing, Yongwei Zhang, Bo Hong, Pengfei Yang, and Jianmin Liu. 2021. “Exosomes Containing MiR-451a Is Involved in the Protective Effect of Cerebral Ischemic Preconditioning against Cerebral Ischemia and Reperfusion Injury.” *CNS Neuroscience and Therapeutics* 27(5):564–76. doi: 10.1111/cns.13612.

27. Li, Jia Ying, Qi Qi Li, and Rui Sheng. 2021. “The Role and Therapeutic Potential of Exosomes in Ischemic Stroke.” *Neurochemistry International* 151(September). doi: 10.1016/j.neuint.2021.105194.
28. Li, Xiao Hui, Juan Zhang, Ding Feng Li, Wei Wu, Zhong Wen Xie, and Qiang Liu. 2020. “Physiological and Pathological Insights into Exosomes in the Brain.” *Zoological Research* 41(4):365–72. doi: 10.24272/j.issn.2095-8137.2020.043.
29. Liu, Wei, Yongxiang Wang, Fangyi Gong, Yuluo Rong, Yongjun Luo, Pengyu Tang, Zheng Zhou, Zhimin Zhou, Tao Xu, Tao Jiang, Siting Yang, Guoyong Yin, Jian Chen, Jin Fan, and Weihua Cai. 2019. “Exosomes Derived from Bone Mesenchymal Stem Cells Repair Traumatic Spinal Cord Injury by Suppressing the Activation of A1 Neurotoxic Reactive Astrocytes.” *Journal of Neurotrauma* 36(3):469–84. doi: 10.1089/neu.2018.5835.
30. Lopez-Leal, Rodrigo, and Felipe A. Court. 2016. “Schwann Cell Exosomes Mediate Neuron–Glia Communication and Enhance Axonal Regeneration.” *Cellular and Molecular Neurobiology* 36(3):429–36. doi: 10.1007/s10571-015-0314-3.
31. López-Leal, Rodrigo, Florencia Díaz-Viraqué, R. J. Catalan, C. Saquel, and Et Al. 2020. “Schwann Cell Reprogramming into Repair Cells Increases Exosome-Loaded MiRNA-21 Promoting Axonal Growth.” *Stem Cells* 133(12).
32. Lopez-Verrilli, M. Alejandra, and Felipe A. Court. 2012. “Transfer of Vesicles from Schwann Cells to Axons: A Novel Mechanism of Communication in the Peripheral Nervous System.” *Frontiers in Physiology* 3(205). doi: 10.3389/fphys.2012.00205.
33. Lopez-Verrilli, María Alejandra, Frederic Picou, and Felipe A. Court. 2013. “Schwann Cell-Derived Exosomes Enhance Axonal Regeneration in the Peripheral Nervous System.” *Glia* 61(11):1795–1806. doi: 10.1002/glia.22558.
34. Ma, Yongbin, Liyang Dong, Dan Zhou, Li Li, Wenzhe Zhang, Yu Zhen, Ting Wang, Jianhua Su, Deyu Chen, Chaoming Mao, and Xuefeng Wang. 2019. “Extracellular Vesicles from Human Umbilical Cord Mesenchymal Stem Cells Improve Nerve Regeneration after Sciatic Nerve Transection

in Rats." *Journal of Cellular and Molecular Medicine* 23(4):2822–35. doi: 10.1111/jcmm.14190.

35. Mashouri, Ladan, Hassan Yousefi, Amir Reza Aref, Ali Mohammad Ahadi, Fatemeh Molaei, and Suresh K. Alahari. 2019. "Exosomes: Composition, Biogenesis, and Mechanisms in Cancer Metastasis and Drug Resistance." *Molecular Cancer* 18(1):1–14. doi: 10.1186/s12943-019-0991-5.
36. Men, Yuqin, Julia Yelick, Shijie Jin, Yang Tian, Ming Sum R. Chiang, Haruki Higashimori, Eoin Brown, Rachel Jarvis, and Yongjie Yang. 2019. "Exosome Reporter Mice Reveal the Involvement of Exosomes in Mediating Neuron to Astroglia Communication in the CNS." *Nature Communications* 10(1). doi: 10.1038/s41467-019-11534-w.
37. Morel, Lydie, Melissa Regan, Haruki Higashimori, Seng Kah Ng, Christine Esau, Svetlana Vidensky, Jeffrey Rothstein, and Yongjie Yang. 2013. "Neuronal Exosomal Mirna-Dependent Translational Regulation of Astroglial Glutamate Transporter Glt1." *Journal of Biological Chemistry* 288(10):7105–16. doi: 10.1074/jbc.M112.410944.
38. Ni, Haoqi, Su Yang, Felix Siaw-Debrah, Jiangnan Hu, Ke Wu, Zibin He, Jianjing Yang, Sishi Pan, Xiao Lin, Haotuo Ye, Zhu Xu, Fan Wang, Kunlin Jin, Qichuan Zhuge, and Lijie Huang. 2019. "Exosomes Derived from Bone Mesenchymal Stem Cells Ameliorate Early Inflammatory Responses Following Traumatic Brain Injury." *Frontiers in Neuroscience* 13(JAN):1–10. doi: 10.3389/fnins.2019.00014.
39. Van Niel, Guillaume, Gisela D'Angelo, and Graça Raposo. 2018. "Shedding Light on the Cell Biology of Extracellular Vesicles." *Nature Reviews Molecular Cell Biology* 19(4):213–28. doi: 10.1038/nrm.2017.125.
40. Patel, Mikin R., and Alissa M. Weaver. 2021. "Astrocyte-Derived Small Extracellular Vesicles Promote Synapse Formation via Fibulin-2-Mediated TGF-β Signaling." *Cell Reports* 34(10):108829. doi: 10.1016/j.celrep.2021.108829.
41. Phinney, D. G., and Mark F. Pittenger. 2017. "Concise Review: MSC-Derived Exosomes for Cell-Free Therapy DONALD." *Stem Cells Express* 35(4):851–58. doi: 10.1002/stem.2575.
42. Van der Pol, Edwin, Anita N. Böing, Paul Harrison, Augueste Sturk, and Rienk Nieuwland. 2012. "Classification, Functions, and Clinical Relevance of Extracellular Vesicles." *Pharmacological Reviews* 64(3):676–705. doi: 10.1124/pr.112.005983.
43. Potolicchio, Ilaria, Gregory J. Carven, Xiaonan Xu, Christopher Stipp,

Richiard J. Riese, Lawrence J. Stern, and Laura Santambrogio. 2005. "Proteomic Analysis of Microglia-Derived Exosomes: Metabolic Role of the Aminopeptidase CD13 in Neuropeptide Catabolism." *The Journal of Immunology* 175(4):2237–43. doi: 10.4049/jimmunol.175.4.2237.

44. Qing, Liming, Huanwen Chen, Juyu Tang, and Xiaofeng Jia. 2018. "Exosomes and Their MicroRNA Cargo: New Players in Peripheral Nerve Regeneration." *Physiology & Behavior* 176(1):139–48. doi: 10.1177/1545968318798955.Exosomes.
45. Raffo-Romero, Antonella, Tanina Arab, Issa S. Al-Amri, Francoise Le Marrec-Croq, Christelle Van Camp, Quentin Lemaire, Michel Salzet, Jacopo Vizioli, Pierre Eric Sautiere, and Christophe Lefebvre. 2018. "Medicinal Leech CNS as a Model for Exosome Studies in the Crosstalk between Microglia and Neurons." *International Journal of Molecular Sciences* 19(12). doi: 10.3390/ijms19124124.
46. Saeedi, Saumeh, Sonia Israel, Corina Nagy, and Gustavo Turecki. 2019. "The Emerging Role of Exosomes in Mental Disorders." *Translational Psychiatry* 9(1). doi: 10.1038/s41398-019-0459-9.
47. Shih, Chung Hsuan, Michelle Lacagnina, Kelly Leuer-Bisciotti, and Christoph Pröschel. 2014. "Astroglial-Derived Periostin Promotes Axonal Regeneration after Spinal Cord Injury." *Journal of Neuroscience* 34(7):2438–43. doi: 10.1523/JNEUROSCI.2947-13.2014.
48. Sicot, Géraldine, Laurent Servais, Diana M. Dinca, Axelle Leroy, Cynthia Prigogine, Fadia Medja, Sandra O. Braz, Aline Huguet-Lachon, Cerina Chhuon, Annie Nicole, Noëmy Gueriba, Ruan Oliveira, Bernard Dan, Denis Furling, Maurice S. Swanson, Ida Chiara Guerrera, Guy Cheron, Geneviève Gourdon, and Mário Gomes-Pereira. 2017. "Downregulation of the Glial GLT1 Glutamate Transporter and Purkinje Cell Dysfunction in a Mouse Model of Myotonic Dystrophy." *Cell Reports* 19(13):2718–29. doi: 10.1016/j.celrep.2017.06.006.
49. Song, He, Xiangjian Zhang, Rong Chen, Jiangyong Miao, Lina Wang, Lili Cui, Hui Ji, and Ying Liu. 2019b. "Cortical Neuron-Derived Exosomal MicroRNA-181c-3p Inhibits Neuroinflammation by Downregulating CXCL1 in Astrocytes of a Rat Model with Ischemic Brain Injury." *NeuroImmunoModulation* 26(5):217–33. doi: 10.1159/000502694.
50. Song, Yaying, Zongwei Li, Tingting He, Meijie Qu, Lu Jiang, Wanlu Li, Xiaojing Shi, Jiaji Pan, Linyuan Zhang, Yongting Wang, Zhijun Zhang, Yaohui Tang, and Guo Yuan Yang. 2019a. "M2 Microglia-Derived Exosomes Protect the Mouse Brain from Ischemia-Reperfusion Injury via Exosomal MiR-124." *Theranostics* 9(10):2910–23. doi:

10.7150/thno.30879.
51. Svaren, John. 2014. “MicroRNA and Transcriptional Crosstalk in Myelinating Glia.” *Neurochemistry International* 77:50–57. doi: 10.1016/j.neuint.2014.06.010.
52. Tassew, Nardos G., Jason Charish, Alireza P. Shabanzadeh, Valbona Luga, Hidekiyo Harada, Nahal Farhani, Philippe D’Onofrio, Brian Choi, Ahmad Ellabban, Philip E. B. Nickerson, Valerie A. Wallace, Paulo D. Koeberle, Jeffrey L. Wrana, and Philippe P. Monnier. 2017. “Exosomes Mediate Mobilization of Autocrine Wnt10b to Promote Axonal Regeneration in the Injured CNS.” *Cell Reports* 20(1):99–111. doi: 10.1016/j.celrep.2017.06.009.
53. Turturici, Giuseppina, Rosaria Tinnirello, Gabriella Sconzo, and Fabiana Geraci. 2014. “Extracellular Membrane Vesicles as a Mechanism of Cell-to-Cell Communication: Advantages and Disadvantages.” *American Journal of Physiology - Cell Physiology* 306(7). doi: 10.1152/ajpcell.00228.2013.
54. Uyeda, Akiko, and Rieko Muramatsu. 2020. “Molecular Mechanisms of Central Nervous System Axonal Regeneration and Remyelination: A Review.” *International Journal of Molecular Sciences* 21(21):1–14. doi: 10.3390/ijms21218116.
55. Wang, Shiwei, Fabrizia Cesca, Gabriele Loers, Michaela Schweizer, Friedrich Buck, Fabio Benfenati, Melitta Schachner, and Ralf Kleene. 2011. “Synapsin I Is an Oligomannose-Carrying Glycoprotein, Acts as an Oligomannose-Binding Lectin, and Promotes Neurite Outgrowth and Neuronal Survival When Released via Glia-Derived Exosomes.” *Journal of Neuroscience* 31(20):7275–90. doi: 10.1523/JNEUROSCI.6476-10.2011.
56. Wei, Zhijian, Baoyou Fan, Han Ding, Yang Liu, Haoshuai Tang, Dayu Pan, Jiaxiao Shi, Pengyuan Zheng, Hongyu Shi, Heng Wu, Ang Li, and Shiqing Feng. 2019. “Proteomics Analysis of Schwann Cell-Derived Exosomes: A Novel Therapeutic Strategy for Central Nervous System Injury.” *Molecular and Cellular Biochemistry* 457(1–2):51–59. doi: 10.1007/s11010-019-03511-0.
57. Wolf, P. 1967. “The Nature and Significance of Platelet Products in Human Plasma.” *British Journal of Haematology* 13(3):269–88. doi: 10.1111/j.1365-2141.1967.tb08741.x.
58. Xia, Xiaohuan, Yi Wang, Ying Qin, Shu Zhao, and Jialin C. Zheng. 2022. “Exosome: A Novel Neurotransmission Modulator or Non-Canonical Neurotransmitter?” *Ageing Research Reviews* 74:101558. doi:

10.1016/j.arr.2021.101558.
59. Xiao, Yilei, Fengyang Geng, Guifang Wang, Xueyuan Li, Jianxin Zhu, and Weijie Zhu. 2019. "Bone Marrow–Derived Mesenchymal Stem Cells–Derived Exosomes Prevent Oligodendrocyte Apoptosis through Exosomal MiR-134 by Targeting Caspase-8." *Journal of Cellular Biochemistry* 120(2):2109–18. doi: 10.1002/jcb.27519.
60. Xin, Hongqi, Yi Li, Ben Buller, Mark Katakowski, Yi Zhang, Xinli Wang, Xia Shang, Z. G. Zhang, and M. Chopp. 2013. "Exosome-Mediated Transfer of MiR-133b from Multipotent Mesenchymal Stromal Cells to Neural Cells Contributes to Neurite Outgrowth." *Stem Cells* 30(7):1556–64. doi: 10.1002/stem.1129.Exosome-Mediated.
61. Xu, Lili, Hui Cao, Yi Xie, Yao Zhang, Mingyang Du, Xiaohui Xu, Ruidong Ye, and Xinfeng Liu. 2019. "Exosome-Shuttled MiR-92b-3p from Ischemic Preconditioned Astrocytes Protects Neurons against Oxygen and Glucose Deprivation." *Brain Research* 1717:66–73. doi: 10.1016/j.brainres.2019.04.009.
62. Yáñez-Mó, María, Pia R. M. Siljander, Zoraida Andreu, Apolonija Bedina Zavec, Francesc E. Borràs, Edit I. Buzas, Krisztina Buzas, Enriqueta Casal, Francesco Cappello, Joana Carvalho, Eva Colás, Anabela Cordeiro-Da Silva, Stefano Fais, Juan M. Falcon-Perez, Irene M. Ghobrial, Bernd Giebel, Mario Gimona, Michael Graner, Ihsan Gursel, Mayda Gursel, Niels H. H. Heegaard, An Hendrix, Peter Kierulf, Katsutoshi Kokubun, Maja Kosanovic, Veronika Kralj-Iglic, Eva Maria Krämer-Albers, Saara Laitinen, Cecilia Lässer, Thomas Lener, Erzsébet Ligeti, Aija Line, Georg Lipps, Alicia Llorente, Jan Lötvall, Mateja Manček-Keber, Antonio Marcilla, Maria Mittelbrunn, Irina Nazarenko, Esther N. M. Nolte-'t Hoen, Tuula A. Nyman, Lorraine O'Driscoll, Mireia Olivan, Carla Oliveira, Éva Pállinger, Hernando A. Del Portillo, Jaume Reventós, Marina Rigau, Eva Rohde, Marei Sammar, Francisco Sánchez-Madrid, N. Santarém, Katharina Schallmoser, Marie Stampe Ostenfeld, Willem Stoorvogel, Roman Stukelj, Susanne G. Van Der Grein, M. Helena Vasconcelos, Marca H. M. Wauben, and Olivier De Wever. 2015. "Biological Properties of Extracellular Vesicles and Their Physiological Functions." *Journal of Extracellular Vesicles* 4(2015):1–60. doi: 10.3402/jev.v4.27066.
63. Yellon, Derek M., and Sean M. Davidson. 2014. "Exosomes Nanoparticles Involved in Cardioprotection?" *Circulation Research* 114(2):325–32. doi: 10.1161/CIRCRESAHA.113.300636.
64. Zhang, Dianquan, Guoliang Cai, Kai Liu, Zhe Zhuang, Kunping Jia, Siying Pei, Xiuzhen Wang, Hong Wang, Shengnan Xu, Cheng Cui, Manchao Sun,

Sihui Guo, Wenli Song, and Guofeng Cai. 2021. “Microglia Exosomal MiRNA-137 Attenuates Ischemic Brain Injury through Targeting Notch1.” *Aging* 13(3):4079–95. doi: 10.18632/aging.202373.

65. Zhang, Guo, Hua Bai, Hai Zhang, Camin Dean, Qiang Wu, Juxue Li, Sara Guariglia, Qingyuan Meng, and Dongsheng Cai. 2011. “Neuropeptide Exocytosis Involving Synaptotagmin-4 and Oxytocin in Hypothalamic Programming of Body Weight and Energy Balance.” *Neuron* 69(3):523–35. doi: 10.1016/j.neuron.2010.12.036.
66. Zhao, Jiuhong, Yali Ding, Rui He, Kui Huang, Lu Liu, Chaona Jiang, Zhuozhou Liu, Yuanlan Wang, Xiaokai Yan, Fuyang Cao, Xueying Huang, Yanan Peng, Rui Ren, Yuebin He, Tianwei Cui, Quanpeng Zhang, Xianfang Zhang, Qibing Liu, Yunqing Li, Zhijian Ma, and Xinan Yi. 2020a. “Dose-Effect Relationship and Molecular Mechanism by Which BMSC-Derived Exosomes Promote Peripheral Nerve Regeneration after Crush Injury.” *Stem Cell Research and Therapy* 11(1):1–17. doi: 10.1186/s13287-020-01872-8.
67. Zhao, Yangmin, Yunxiao Gan, Gewei Xu, Kouzhen Hua, and Dandan Liu. 2020b. “Exosomes from MSCs Overexpressing MicroRNA-223-3p Attenuate Cerebral Ischemia through Inhibiting Microglial M1 Polarization Mediated Inflammation.” *Life Sciences* 260(September):118403. doi: 10.1016/j.lfs.2020.118403.

Alternative Medicine and Herbal Drug Use in Turkey

Burcu SÜMER TÜZÜN

The use of natural resources in treatment began with the history of humanity. The Nineveh tablets (3000 BC), the first written document about the treatment in plants, show that the Mesopotamian civilizations Sumer, Assyria, and Akats had treatment with herbal medicines (Yıldırım, 2010; Onbasli et al., 2019:6). At present, between 50000-70000 plant species are used for therapeutic purposes worldwide. In general, the use of medicinal and aromatic plants in treatment is increasing worldwide. In developed countries, approximately 25% of prescription drugs are herbal-based active substances. While the rate of treatment with herbal products is around 40-50% in Germany, it is 42% in the USA, 48% in Australia, and 49% in France. In addition, 500 of 11000 plant species are used for medicinal purposes in Turkey. Herbal treatment methods are called 'alternative medicine or phytotherapy' today. French doctor Henri Leclerc (1870-1955) was the first to use the term phytotherapy. Today, herbal treatment is sometimes used as a supplement to chemical treatment and sometimes as a stand-alone treatment agent worldwide (Goktas and Gıdık, 2019:2). The World Health Organization defined herbal medicine in 2000 as it gives: "Parts of plants such as roots, leaves, flowers, bark, seeds or extracts prepared from these parts (aqueous or alcoholic) or materials obtained as a result of a process from plants (essential oil, fixed oil, resin, oleoresin, balsam)" (Yıldırım, 2010)

The herbal drug is known as a raw material of vegetable origin. The parts of a medicinal plant used for therapeutic purposes are known as extracts prepared from these parts or some products obtained from processing these parts. Herbal medicinal product, on the other hand, is a product whose active ingredients consist of one or more herbal drugs and are prepared in a particular dosage in a suitable pharmaceutical way (Djordevic 2017:).

In the last century, the use of plants has increased in Turkey and the rest of the world. Herbal remedies have become very popular because they are of natural origin and are considered safe for long-term use. However, pharmaceutical products, dietary/food supplements, and medicinal products are available to consumers without the knowledge and supervision of a physician. This situation has become dangerous to consumers due to the potential toxicity, contamination, and incorrect drug and food and drug interaction information in herbal medicine.

In our country, since 1985, the regulation published for the prohibition of the sale of potentially toxic herbal drugs and their control by the Ministry of Health has been implemented. In addition, herbal medicine licensing has been given to the Ministry of Health, but there are still problems as there are no clear lines on this issue(Suzgec-Selcuk and Eyisan, 2012:16).

In Turkey, the use of alternative medicine and herbal products for various diseases and support in daily life has been examined in many different studies.

According to studies conducted in various provinces of Turkey, it has been seen that one of the most frequently used methods is herbal treatment. In addition, demographic and socioeconomic data of the patients were collected, and it was evaluated whether there was a statistical difference in using or not using.

The most comprehensive study carried out is a survey of 19022 people over the age of 18 from 81 provinces in Turkey. According to the results, 13025 people completed the questionnaire, and 3132 had never applied herbal treatment before. The majority of the people are from Istanbul, Ankara, and Izmir. In general, they stated that they tried the alternative treatment for reasons such as hair loss, hair care, hemorrhoids, skin health, peptic ulcer, gastritis, and infertility. The most commonly used plants were nettle, St. John's Wort, rosemary, sage, and hawthorn. While 32.6% of them state that they use herbal treatment because they think it will help the treatment, 26.9% of them prefer to experiment because they are interested in herbal products even though they do not have health problems (Ogur et al., 2006: 4).

In a study conducted in the city of Konya, located in Central Anatolia, the use of alternative treatment was investigated with a questionnaire to 927 patients- at the age of 18. It has been observed that women tend to use herbal products more. In addition, as the working status, monthly income, and education level increased, it was determined that more herbal products were used. At the same time, no statistically significant relationship was found between age and use. It has been seen that tea is the most used plant. Then rosemary and ginger came. While most patients take herbal medicines to protect their health, other reasons are that they are of natural origin, they are believed to have few side effects, they are believed to be completely safe, they are more effective than medicines, they are easy to apply, and they are cheap. There is 11.3% of users think that they have experienced side effects in the last year. In order of frequency, these side effects are palpitations, abdominal pain, hot flashes, rash, diarrhea, vomiting-nausea, headache, and itching. While 44.7% used herbal medicine with the recommendation of a friend, only 10% started using herbal medicine after receiving advice from a pharmacist and medical doctor (Soner et al., 2013:5).

A cross-sectional study conducted in Aydın province investigated the effects of herbal medicine use. In the study, in which 873 people completed the survey, 58.1% indicated that they had used complementary therapy in the past year. 26.7% of the participants used alternative treatment methods in their children in the last year. 55.4% preferred the use of herbal medicine. The most frequently used plant was linden (88.1%). It is stated that the main reasons for using herbal treatment are both prevention and treatment. Health status, smoking, and the idea

that the use of CAM is superior to conventional medicine were determined as the factors affecting the use of CAM (Aydın et al., 2008:38).

In a cross-sectional study examining trends in traditional and complementary therapies conducted in Konya, the differences were evaluated by conducting the same survey between 2012 and 2018. In both studies, 405 people were surveyed. There was no significant difference between the two groups in terms of gender, age, and educational status. While there were 16.3% chronic patients in 2012, 25.7% had a chronic disease in 2018. These chronic diseases are similar in both periods, hypertension, diabetes, thyroid, chronic obstructive pulmonary disease, asthma, and rheumatic diseases. While the number of those who think that herbal medicine should not be used has decreased over the years, those who believe it should be used have increased. While the idea of using traditional and complementary therapy in treating the disease has increased, the idea of supporting it has decreased. The idea that it is effective in treating hypertension, digestive system diseases, skin diseases, and cancer has significantly reduced. While 70.1% of them used herbal medicine for themselves and their families in 2012, this rate decreased to 52.1% in 2018. There was a significant increase in traditional therapies such as blood-letting, cupping, leech application, and acupuncture and a decrease in therapies like Ayurveda osteopathy massage, music therapy, and breathing exercise. The influence of neighbors, relatives, and friends decreased, but the role of social and mainstream media increased significantly. At both points, participants declared that their recommendation source was not doctors. Also, the rate of people recommending decreased from 2012 to 2018 (Okka et al., 2022: 72).

The use of alternative treatments for certain diseases in Turkey has been tried to be clarified by various studies.

Studies have been conducted on weight loss, pregnant women, various types of cancer, chronic diseases, chronic viral hepatitis, allergies, pediatrics, covid-19 disease, cardiology, hemorrhoids, dermatology, chronic kidney diseases, Type 1 and type 2 diabetes, hypertensive patients, surgery. In addition, it is related to the use of alternative therapy in asthma and mental disorders.

1. WEIGHT LOSS

Obesity treatment is a very long-term process. For the treatment of obesity, the most essential thing the patient should do first is change his lifestyle and behaviors. Therefore, patients are looking for ways to lose weight easily and quickly. The research covers the period between June 2015 and September 2016 at Ege University Endocrinology Clinic. The mean body mass index of 358

female and 106 male participants was determined as 32.9. Only 24.1% of the participants stated that they used herbal products.

When the results were evaluated, it was seen that the mean age was low, but the BMI was high in the participants using herbal medicine. The tendency to use it in women and singles was found to be higher than in men and married people. No statistically significant correlation was found between education and use. It has been observed that herbal use is less in people with chronic diseases who use conventional drugs. The most preferred herbal medicines are indicated as mixtures. Often mate, green tea, thyme, rosemary, heather or mate, green tea, thyme, rosemary, heather, English plantain, puncture vire, puncturevine, alder buckthorn, Gymnema. Infusion is the most commonly used herbal preparation. Other methods include decoction, squeezing, mixing with water, and mixing with food. Although the use of the participants is usually familiar advice, only 2.7% of the participants use it with the advice of a doctor, 1.8% from a dietitian, and 0.9% from a pharmacist. While 56.3% of the patients obtain the plants they will use from herbalists, 24.1% buy them from the markets. While very few patients talked to their doctors about the alternative treatment method they used, most participants (72.5%) stated that they wanted the doctors to inform the patients about this issue (Bellikci-Koyu et al., 2020: 22).

2. PREGNANT WOMEN

In a study conducted in Mersin, there is a study on the use and results of "meizanc," a herbal mixture used in Chinese medicine, by three pregnant women. In the first case, a healthy 28-year-old woman used this drug during the first seven weeks of an unplanned pregnancy of which she was unaware. She used this herbal mixture, which is presented as a weight loss medicine, by taking one capsule a day for three months. No developmental problems or health malformations were observed in the baby, who was followed up throughout pregnancy and eight months after birth. In the second case, a 25-year-old obese patient started antenatal care in the 8th week. Stating that he had been using the drug for five months, the patient said that he continued to use the drug every day before learning about pregnancy in the first six weeks. Vaginal ultrasound showed consistent but non-viable intrauterine pregnancy with dates. In case 3, a 29-year-old woman who had two vaginal deliveries was examined. The patient, who has been using Meizanc for six months, realizes that she is seven weeks pregnant and has stopped taking the drug. However, since the heartbeat could not be seen in the following period and a normal miscarriage did not occur, abortion was required (Cayan et al. 2009:35).

In the research conducted in Samsun on alternative medicine and complementary therapy practice of pregnant women, it was determined that 41% of the patients applied these methods. 36.5% state that they apply the herbal treatment. 29.8% apply massage and therapy. Half of the users think that the use is relatively safe and do not inform their doctors. The reasons for use in pregnancy are nausea, abdominal pain, anemia, sore throat, and cold. The most frequently used plants were mint, linden, rosehip, and nettle (Koc et al., 2017: 24).

According to a study conducted in Tokat province, 47.3% of pregnant women used herbal products at least once during their pregnancy. It has been observed that there is a very significant statistical relationship between educational status, job, family structure, and plant use. However, there was no statistically significant relationship between age, social insurance, income, and the number of pregnancies planned or unplanned. While more than half of the participants started using herbal medicine without anyone's advice, only 13.9% started using it after getting advice from a professional. More than half of the respondents are concerned about its safety, but 36.1% argue it is safer than drugs. The most used plants were linden, ginger, chamomile, cranberry, and blueberry (Kıssal et al., 2017: 30).

3. CANCER

The tendency to use herbal medicine and complementary therapy increases, especially in cancer patients, when combined with the fear of death from the environment and the media. In the study, 472 patients were evaluated as a result of the questionnaire in Istanbul, one of the provinces that is thought to reflect Turkey most in the period between January 1, 2009, and May 31, 2011. It stated that 68.2% of the patients used herbal treatment. While 66% of them started using it by getting information from the media and the internet, only 24% informed their doctor. There were no significant statistical differences by age, gender, educational level, place of residence, income level, and disease stage (Tuna et al., 2013: 18).

The research was carried out in Çukurova University Gynecological Oncology Clinic with 67 people. Attention was paid to the fact that the patients participating in the survey were diagnosed at least one month ago. The mean age was determined as 58.2. The most common cancer was ovarian cancer, with a large proportion (80.6%). 62.7% of these people receive chemotherapy. No significant statistical relationship was found between education, marital status, work, and use. The type of cancer, the model of treatment, and the time of diagnosis could not be correlated. Forty-one of the participants apply complementary therapy, medicine37 of them apply herbal treatment,37 of them apply herbal therapy,

while 4 follow a diet regimen. Nettle, green tea, and licorice root were frequently used as herbal medicine. Although the primary reason for their use in cancer treatment is to treat cancer, they are also used to feel good physically and emotionally, reduce cancer treatment's side effects, and strengthen the immune system. It was determined that more than half of the patients informed their doctor. In cancer types, which is a serious diseases, it is striking that patients inform their doctors more (Nazik et al., 2012: 13).

In the province of Bursa, located in the Marmara region of our country, 271 cancer patients were surveyed, and their herbal medicine use was evaluated. 35.7% of the participants use herbal medicine and mostly prefer it as a mixture. Stinging nettle, ginger, bee oleni, green tea, garlic, and pomegranate juice are among the herbal drugs used. The age of the participants who used the most is between 40-50. It has been determined that as the education level increases, the product's use increases significantly. It is seen that the usage increases depending on the increase in income. 86.5% of the patients using it started herbal treatment after a cancer diagnosis. While 58.7% claim to have obtained positive results from this treatment, 11.3% state that they did not get any results. There was no significant relationship between the use of herbal products and localization and the grade of the tumor, administration of active medical therapy, or smoking. Only 12.3 uses advice from a Professional.

On the other hand, some people buy herbs from herbalists in general, and there is a minority who buy them from pharmacies. As in previous studies, more than half of the patients informed their doctor about its use. However, it was also noteworthy that most doctors did not ask their patients about this issue and did not bring up the case (Avcı et al., 2011: 6)..

In a study conducted in the Medical Oncology Department of Dicle University in eastern Turkey, 324 patients were examined. 173' of the patients were female, and 151 were male. Two hundred-one of the participants stated that they used alternative treatment methods at least once. 51.2% of all patients and 82.5% of those using alternative treatment said that they use herbal treatment methods. They specified the plants used frequently as nettle or seeds and green tea. Patients over the age of 60 use more alternative treatments than younger people. It is seen that patients receiving curative treatment use alternative treatment methods less, while those receiving palliative treatment prefer it more. In addition, while the rate of using alternative treatment is statistically significantly higher in patients who receive chemotherapy than those who do not, it is noteworthy that there is no significant relationship between using radiotherapy or undergoing cancer surgery. 47.3% of the patients use alternative treatment methods not to reduce their complaints but to eliminate the disease. There is a segment of 37.9% stating

that they benefit from the use of herbal medicine. 47.8% of them say that they have not seen any benefit. In addition, it was determined that most patients believed in the harmlessness of herbal treatment (Kucukoner et al., 2013: 10).

A cross-sectional study was conducted in Erzurum between December and January 2001. It is seen that 41.1% of 107 patients started herbal medicine after diagnosis. Generally, it was determined that women and young patients preferred alternative treatment more. No significant association was found between cancer characteristics and alternative therapy use. It was determined that more than half of the patients used herbal medicine without consulting a doctor. It was observed that they mostly received their advice from friends, relatives, and other patients in the clinic (Gozum et al., 2003: 26).

All studies on alternative therapy use in cancer treatment between 2001 and 2007 were compiled and summarized. The entire study consisted of 5069 adult and 183 pediatric cancer patients. The use of alternative treatment varies between 22.1% and 84.1%. The most used method was determined as herbal medicine in all studies. The factors affecting the use of the participants can be listed as follows. Gender, duration of illness, end-stage disease, socioeconomic status, education level, and benefit of use are believed. It has been determined that most patients get advice from their family, relatives, and other patients first. In general, it was observed that the patients started the treatment with herbal medicine after the cancer diagnosis (Kav et al., 2022: 32).

CHRONIC DISEASES

A cross-sectional study was conducted on using herbal medicines in 217 adult patients diagnosed with diabetes, hypertension, and hyperlipidemia in a hospital in Ankara between April and December 2014. In the study, it was seen that women are more prone to use herbal medicine than men. Most of the women in the study were housewives and were influenced by television. Inversely proportional to other studies, no relationship was found between education level and plant use. In other studies, it was observed that people with higher education levels were more likely to use it. Although the effect of herbal medicines on chronic diseases has not been proven, it has been observed that patients who use herbal and conventional medicine together are more likely to be non-compliance with medicines. This choice is very important as it has the potential to endanger human health. Within the scope of the study, it was observed that only seven patients knew that herbs could have side effects. A total of 10% have started using it more consciously by getting support from a healthcare Professional (Tulunay et al., 2015:4).

In a study conducted in the endocrinology clinic in Eastern Anatolia, the tendency and perspective of 316 diabetes patients to alternative treatment methods were evaluated. It was observed that 93% of the patients applied complementary treatment methods. Most of them, such as 97%, preferred plants as an alternative treatment. It has been observed that patients with diabetes blood values and complications have a higher tendency to use herbal medicine. It was stated that 56.3% of the participants were women, 41% were primary school graduates, and 69% were working (Cengiz and Budak, 2019: 36).

A study was conducted in Ankara, which included 390 patients, examining the effect of herbal medicine use on drug compliance of cardiology patients. 84.5% gave acceptable answers. The average age is over 55 years old. Also, 68.2% of the patients had hypertension, 46.2% had hyperlipidemia, 29.7% had diabetes, and 47.1% had coronary artery diseases. It has been stated that most of the patient population uses beta-blockers. 29.7% of the participants (116 patients) said they had used herbal medicine in the last 12 months. While 77 patients used only one herb, 29 used two plants, and eight declared that they used more than three plants. The most used herb was lemon juice. Afterward, it was stated that using fresh walnuts and green tea was quite common. The reasons for using these herbal remedies are to lower high cholesterol, maintain cardiac health, protect the heart vessels and treat hypertension. Only 3% of the patients obtained herbal medicines from the pharmacy, while the remaining part bought them from local herbal shops, television, and the internet. Most of them (81%) stated that they did not tell their doctor about the herbal medicines they used and thought they were harmless because they were natural. 13 people more consciously know that herbal remedies can cause interactions (Acikgoz et al., 2014:22).

In a study conducted in a hemodialysis center in Ankara on the use of herbal products in 114 end-stage renal disease patients who were on dialysis, it was determined that 28.1% of the patients started using herbal products after their kidney disease was diagnosed. Furthermore, it was determined that marital status was the only statistically significant variable with the use of herbal products. In addition, it was learned that 12.5% of the patients informed their doctor about the use of herbal products (Kara, 2009:18).

One hundred ninety-three patients participated in the survey conducted on hypertension patients in Izmir. 95.9% of the participants use conventional medicine for hypertension, while 51.3% also apply herbal therapy. They stated that they usually use herbal therapy when they feel sick, not to lower blood pressure. No significant statistical correlation was found between gender, education, marital status, health insurance, income, and herbal therapy (Bahar et al., 2013: 10).

In examining 100 chronic kidney patients in Samsun, the Black Sea region, it was determined that 37% of them used herbal products. Stinging nettle is the most used product. There was no significant difference between those who use herbal products and those who do not, regarding age, gender, education level, and marital status. Most patients stated that the monthly cost of the herbal products they used was less than 50 lira. About 80% of the patients did not inform their doctor about their used product (Bicen et al., 2012: 21).

In the use of herbal products in peritoneal dialysis patients in Istanbul, 13 out of 58 patients were found to be illiterate. While the average age is around 53, the duration of dialysis is around 41 months. While 19 people used herbal products before dialysis, 14 people started using herbal products after dialysis (Cebeci et al., 2016:25).

A survey was conducted on the alternative treatment practice in the province of Izmir for asthma patients. The survey study covers the period from December 2006 to June 2007. In the survey, there are 200 patients diagnosed in the last six months. It was stated that 63% of the participants applied alternative treatment. While 61.9% of these participants preferred herbal treatment, 53.2% stated that they applied an alternative treatment method by exercising, and 36.5% by praying. In addition, 58% of the participants who applied alternative treatment stated that their asthma complaints decreased. However, there was no significant difference between the use and the severity of disease, pulmonary functions test parameters, number of asthma attacks, and hospitalization (Tokem et al., 2011:21).

In a survey of 120 type 2 diabetes patients, it was stated that 60.8% were women, 87.6% were married, and 65% were primary school graduates. The average age was determined as 57 years. While 52.1% of them started using one or more herbs after the diagnosis of diabetes, 10% stated that they used them before the diagnosis. Only 6.5% of them started using herbal products with the advice of a healthcare professional. 82.3% did not even inform their physicians. No relationship was found between the use of herbal products, frequency, type, and the recommender, and between gender, marital status, and educational status (Ozturk et al. 2015: 53).

A total of 588 patients from cities in different regions of Turkey, such as Düzce, Rize, Erzurum, and Diyarbakır, participated in the study conducted on patients with chronic viral hepatitis. It was observed that 27% of the participants used alternative treatment methods. There was no significant relationship between residential area, educational level, or marital status. Herbal medicine use was reported as 63.6%. All participants stated that they could not get any information about the plant from the health worker. In this case, we understand that healthcare professionals should be more knowledgeable about this issue and especially inform patients. While 40.4% of

the participants took oral antiviral therapy, 27% said they applied herbal treatment (Ince et al., 2020: 48).

ALLERGY

The use of herbal products in atopic or non-atopic 395 patients with respiratory or skin diseases is 14.2%. It was observed that the education level of the participants was generally high. All characteristics were similar within herbal medicine users and non-users except for gender and age. It has been determined that women are more prone to use than men. It has also been found that people in their late 30s are more likely to use herbal medicine. The participants stated the reasons for using it as getting advice from someone, believing that the herbs are more beneficial, and believing that the herbs are completely reliable. Finally, about 70% of the participants stated that they did not know herbs can have side effects for allergic people (Caliskaner et al., 2010: 38).

A study conducted at the GATA allergy clinic revealed that 14% of people who applied to the polyclinic for various reasons used herbal products. It was also noted that this rate was quite low in allergy patients compared to other groups (Kartal and Caliskaner, 2007:27).

PEDIATRICS

In 2012, data were compiled by researching general pediatrics, pediatric oncology, asthma in pediatrics, and diabetes in pediatrics. It has been observed that alternative treatment is used between 26 and 87%. Although the first source was family and friends, it was seen that the majority did not inform their doctors. In a study involving 600 patients, it was seen that 57% of the families used their children at least once. It has been determined that the most used areas are respiratory and gastrointestinal system diseases. In another study, a face-to-face survey of 500 people determined that 87% of the families used alternative treatment and 52% of them used herbal medicine. Constipation was the most common reason for use. Without a doctor's advice, 31% of the participants used herbal tea due to constipation, while 28% used olive oil. In a study of 477 patients, 27% stated that they were using herbal medicine without a doctor's advice. The reasons for use were cough, colic, and constipation, respectively. Another study determined that 75.8% of 186 children aged 2-17 used alternative treatment methods, and herbal tea was the most preferred method, with 39%. In a survey conducted with 253 children with asthma, 67% of them used alternative treatment methods, and in another study, 49% of 304 patients with asthma used alternative methods. In a study conducted in Erzurum on 100 children who were treated for type 1 diabetes, it was determined that 52% of the participants tried alternative methods. It was also observed that 49% of the families

of 88 children with cancer in Erzurum tried alternative methods, and 52% of the families of 95 children with cancer who were treated in Ankara applied alternative methods. Lymphoma has been identified as the most common type of cancer in children. While most patients were satisfied with the conventional therapy, some stated that it did not work, and some stated that the side effects were very severe. It has been observed that nettle is one of the most used herbal products to support cancer treatment. All families using this herb stated that they were satisfied with the results. Very few of the patients' families asked healthcare professionals for their opinions (Ozturk et al., 2014: 10).

A survey was conducted with 95 pediatric oncology patients who were under treatment at Gazi University for at least three months between 1999 and 2000. According to the results, it was determined that a group of 51.6% used alternative treatment methods at least once. The most used method has been herbal treatment. The most commonly used herbal products are nettle, plant extract, and anzer honey. There was no correlation between alternative medicine use and parents' education status, income level, number of children, disease prognosis, and belief in the doctor's information about prognosis. It was observed that all patients used alternative therapy in addition to conventional therapy. It was observed that almost all participants considered alternative treatment completely harmless (Karadeniz et al., 2007:48).

In the study conducted with 88 pediatric cancer patients in the Pediatric Oncology Service of Yakutiye Hospital, it was stated that according to the answers received from the families, half of the patients applied complementary treatment and only 12 patients informed their doctors (Gozum et al., 2007:30).

DERMATOLOGY

A large study was conducted with 1610 dermatology patients in eastern Turkey between January 10 and April 30, 2011. Of the patients, 1008 were female, and 602 were male. The ages of the patients ranged from 1-92, and the families answered the questions of the patients under the age of 12. The five most common dermatological diseases are contact dermatitis, acne vulgaris, fungal infection, eczema, and warts. While the number of patients who used an alternative method at least once was 704, the number of those who used it twice or more was 335. Among the participants who tried alternative methods, the most common dermatological diseases were contact dermatitis, acne vulgaris, fungal infection, and viral infection. The most used alternative methods were henna, cologne, moisturizing cream, prayer, herbal therapy, and lemon juice. Quite unusual methods were also identified in the research. For anthrax, putting out cigarettes on the back skin, applying raw meat for boils, using fuel oil for contact dermatitis, eating hedgehogs for psoriasis, using pitch for itching, using leeches for contact dermatitis, and tinea pedis are given as examples. There was

no statistically significant difference between those who use alternative methods and those who do not regarding age, gender, educational status, and place of residence. It was observed that there was a significant relationship between the use of alternative methods and the frequency of admission to the clinic due to dermatological symptoms. In addition, it has been determined that mostly lemon juice is used for acne vulgaris, henna for fungal infection, cologne for eczema, grape molasses for aphthous stomatitis, and onion for bacterial infection. It was concluded that 43.7% of the participants tried alternative methods. At this point, it was also noteworthy that patients did not know that one of herbs' most important side effects is contact dermatitis (Bilgili et al., 2014:33).

COVID 19 (PANDEMIC)

In a study conducted at Istanbul University, a cross-sectional study was conducted with 871 patients, 670 of whom were women, on the use of herbal remedies. In the survey answered by people in a wide age range, it was determined that 97.7% of the participants lived in the urban living area, 58.2% were married, and 89.3% lived with family members. Only 80 people who answered the questionnaire were diagnosed with Covid19, and 274 said their first-degree relatives were diagnosed. While 28.9% of the participants said they did not take an additional vitamin, 32.4% stated that they used vitamins and supplements daily to protect themselves from Covid19. In the use of vitamin and mineral supplements, gender, age, marital status, occupation, monthly income, and the diagnosis of Covid19 in first-degree relatives were found to be statistically significant. It has been determined that married women between the ages of 46-55, healthcare workers, those with an income of 5000-10000, and those with a relative with a diagnosis of Covid 19 are more likely to use vitamins and minerals every day (Erarslan and Kultur, 2021: 25).

It has also been determined that being a health worker, being a woman, and being diagnosed with Covid19 are important in herbal therapy. It has been observed that two segments take herbal therapy as a diet and those that take the coarse extract. Eight of the 45 different herbs are ginger, linden, elderberry, sage, rosehip, thyme, echinacea, and turmeric. While 77.4% of the participants stated that they used more than one plant simultaneously, 49.3% stated that they used two or three plants simultaneously. Two hundred fifty-three participants, mostly in the 26-35 age range and with an income between 0-2500, said that they used it once a week, 30.6% said they used it every day, 10.5% said they used it when they felt bad, and 5.5% said they used it once a month. Mostly, patients receive information about their use from pharmacists or herbalists. In contrast, those between the ages of 46-55 receive 10000 lira per month and more, those with higher education and health workers get information from pharmacies, while those between the ages of 15-25 receive

information from the pharmacy with a middle school income of 0-2500 lira. It has been observed that graduate housewives prefer herbalists. Most participants informed their physicians about the herbs they used (81.9%) and consulted about the interaction. Only 11 participants mentioned that they had adverse effects(Erarslan and Kultur, 2021: 25)..

In the study covering dietitians in Turkey in May-June 2020, the data of 550 people were determined correctly. While the mean age was 30.6±9.1 years, 88.2% of the participants were women. While 82% had no chronic disease, 48.5% had endocrine system disease, and 39.6% had vitamin and mineral deficiencies. During the pandemic, 35% of dietitians said they regulated their eating habits. More than half of them argued that dietary supplements are necessary to prevent the disease. They stated that these supplements should include fish oil, vitamin D, multivitamin, probiotics, and vitamin C. During Covid, 62.9% declared that they received support once a day. 45.1% got an opinion by consulting a doctor. Herbal medicine use was found to be 44.5%. They stated that they particularly recommend consuming foods containing functional bioactive compounds. These foods are probiotics, prebiotic high-fiber foods, fruits, vegetables, whole grain foods, green tea, kefir, herbal teas, garlic, broccoli, and walnuts. The most used herbal products before the pandemic were stated as sumac and cinnamon. Approximately 75% declared that they used an herbal medicine during the pandemic. It has been observed that the preference for food consumption with function in women is higher than in men. It has been observed that the use increases with the increase in career years. However, as a result, no statistical difference was observed depending on gender, age and career (Kamarlı-Altın et al., 2020: 24).

In a study conducted in Adana on 11-30 April 2020 on the use and belief of traditional and complementary therapy during Covid 19, it was determined that 39.3% of 389 participants over the age of 18 tried these treatment methods. 30.8% used herbal products. 33.9% of users did not inform their doctors. Statistically, a significant difference was observed between use and gender, age, marital status, education level, income, and prior use. It has been determined that the tendency to use is higher in women, people over the age of 40, married people, those with better education, those with higher incomes, and participants who do not have chronic diseases. 33.7% of the participants said they thought traditional and complementary treatments were effective. 39% said that it should be used in COVID-19, and 54.8% stated there are side effects. Almost all participants declared that the products used should be tested for side effects (Karatas et al., 2021: 8).

MENTAL DISORDERS

A case report thought to be associated with herbal medicine use was presented. A 41-year-old married housewife with a child was urgently taken to psychiatry with complaints such as making the human face look like a devil, talking to himself, attacking people with a knife because they look like a devil, having insomnia, and not eating. Relatives of the woman stated that she has been using an herbal mixture given by a disciple for the last three months to relieve fatigue. They stated that they looked good at first, but then they got worse day by day. The fact that plants such as anise, turmeric, ginger, cardamom, fennel, clove, rosemary, cinnamon, and mint in this herbal mixture are nervous system stimulants suggested that they might interact with the drugs he used and accelerate the manic process. The interactions of herbal medicines with other preparations, their stimulating or sedative effects, or their power on the brain are not fully known. The idea that plants are harmless in our country is quite dangerous, and experience has supported this (Saatcioglu et al., 2007: 37).

As a result of the interviews with 1027 patients from 4 different cities of Turkey (Ankara, Rize, Afyon, Erzurum, Gaziantep), it was determined that 799 people used only conventional drugs, 81 people applied only complementary treatment, and 147 people applied both together. There was no significant difference between use and socioeconomic and demographic characteristics. Education level and income significantly differed between users and non-users. The most widely used complementary treatment method has been herbal products. People with higher incomes and higher education were more prone to use. The most common psychiatric disorders were anxiety disorders, depressive disorders, and somatoform disorders. 61 out of 325 depressed patients; 73 out of 390 patients with anxiety disorders; 19 out of 51 patients with psychotic disorders; 29 out of 67 patients with sleep disorders, and 20 out of 125 patients with somatoform disorders used traditional and complementary treatment methods (Bahceci et al., 2013:19).

Despite all the studies and their extensive use when diseases are evaluated, most herbs have an unproven efficacy and safety profile by today's standards. Data on active ingredients, pharmacokinetics, toxicology, side effects, use in certain patient groups, and contraindications are often missing. For this reason, people should definitely not use herbal products and stop conventional treatment in cases of illness or pregnancy without consulting their healthcare professionals and examining the interactions. At the same time, herbal products should not be purchased from places where unreliable products such as television, the internet, herbalists, and herbalists are sold. Only pharmacies should be preferred.

REFERENCES

1. Acikgoz, S.K., Acikgoz, E., Topal, S., Okuyan, H., Yaman, B., Er, O., Sensoy, B., Balci, M.M., Aydogdu, S. Effext of herbal medicine use on medication adherence of caridology patients, *Complematary Therapies in Medicine*, 22, 648-654.
2. Avcı, N., Canhoroz, M., Kanat, O., Gurun, M.S. (2011), The Prevalance of Herbal Product Use as a Alternative Medicine Among Cancer Patients in Turkey, *Journal of Clinical and Analytical Medicine*, 6(3), 327-330.
3. Aydın, S., Bozkaya, A.O., Mazıcıoglu, M., Gemalmaz, A., Ozcakir, A., Ozturk, A. (2008), What Influences Herbal Medicine Use? - Prevalence and Related Factors , *Turkish Journal of Medicinal Science*, 38(5), 455-463.
4. Bahar, Z., Kızılcı, S., Beser, A., Buyukkaya Besen, D., Gordes, N., Ersin, F., Kissal, A., Capik, C. (2013), Herbal therapies used by hypertensive patients in Türkay, *African Journal of Traditional and Complementary Alternative Medicine*, 10(2), 292-298.
5. Bahceci, B., Bagcioglu, E., Ozturk, A., Bulbul, F., Sahiner, I.V., Eryonucu Tuncer, B., Guzel, H.I., Hocaoglu, C. (2013), Complementary and alternative medicine use in patients with mental disorders in Turkey, *Complementary Therapies in Clinical Practice*, 19 (2013) 221-226.
6. Bellikci-Koyu, E., Sarer-Yurekli, B.P., Seckiner, S., Ozdemir, N., Buyuktuncer, Z. (2020), Use of herbal products for weight loss in Turkey, *Progress in Nutrition,* 22, 1-8.
7. Bicen, C., Erdem , E., Kaya, C., Karatas A., Elver, O., Akpolat, T. (2012), *Herbal Product Use in Patients with Chronic Kidney Disease, Türk nefroloji Diyaliz ve Transplantasyon Dergisi*, 21(2), 136-140.
8. Bilgili S.G., Ozkol, H.U., Karadag, A.S., Calka, O. (2014), The use of complementary and alternative medicine among dermatology outpatients in Turkey, *Human and Experimental Toxicology*, 33(2) 214–221.
9. Caliskaner, Z., Kartal, O., Gulec, M., Ozturk,S., Erel, F., Sener, O., Karaayvaz, M.(2010), Awareness of allergy patients about herbal remedies: a cross-sectional study of residents of Ankara, Turkey, *Allergologia Immunopathologia*, 38(2), 78–82
10. Cayan, F., Dilek, U., Akbay, E., Gen, R., Dilek, S. (2009), Use of Chinese herbal medicine 'meizitanc' in pregnancy: Report of three cases, *Journal of Obstetical. Gynaecology Resarch,* 35, 801-803.
11. Cebeci, E., Sevim, Y., Bektas, T., Gursu, M., Ozturk, S., Sumnu, A., Karadag, S., Uzun, S., Kazancioglu, S. (2016), Use of Herbal Products

in Peritoneal Dialysis Patients, *Turkish Nephrology Dialysis Transplantation*, 25 (2), 151-156
12. Cengiz, Z. And Budak, F. (2019)Use of complementary medicine among people with diabetes in eastern Turkey: A descriptive study, *Complementary Therapies in Clinical Practice*, 36, 120-124.
13. Djordjevic, SÇ. (2017), From Medicinal Plant Raw Material to Herbal Remedies, Intech: Aromatic and Medicinal Plants:16.
14. Erarslan, Z.B. and Kultur, S. (2021), A cross-sectional survey of herbal remedy taking to prevent Covid-19 in Turkey, *Journal of Research in Pharmacy*, 25(6), 926-936.
15. Ince, N., Kaya, S., Esen Yildiz, I., Parlak, E., Bayar, B.(2020), Use of complementary and alternative medicine in patients with chronic viral hepatitis in Turkey, Complementary Therapies in Medicine, 48, 102229.
16. Goktas Ö. And Gıdık, B. (2019), Tıbbi ve Aromatik Bitkilerin Kullanım Alanları, *Bayburt Üniversitesi Fen Bilimleri Dergisi,* 2, 135- 142.
17. Gozum, S., Arikan, D., Buyukavci, M.(2007), Complementary and Alternative
Medicine Use in Pediatric Oncology Patients in Eastern Turkey, Cancer Nursing, 30(1), 38-44.
18. Gozum, S., Tezel,A., Koc, M. (2003), Complementary Alternative Treatments Used by Patients With Cancer in Eastern Turkey, *Cancer Nursining*, 26(3), 230-236.
19. Karatas, Y, Khan, Z., Bilen, C., Boz, A., Goren Ozagil, E.S., Abussuutoglu, A.S., Rahman, H. (2021), Traditional and complementary medicine use and beliefs during COVID-19 outbreak: A cross-sectional survey among the generalpopulation in Turkey, *Advances in Integrative Medicine*, 8 (2021) 261–266.
20. Kamarli Altun, H., Karacil Ermumcu, M.S., Seremet Kurklu ,M. (2020), Evaluation of dietary supplement, functional food and herbalmedicine use by dietitians during the COVID-19 pandemic, *Public Health Nutrition,* 24(5), 861–869.
21. Kara, B. (2009), Herbal product use in a sample of Turkish patients undergoing
haemodialysis, *Journal of Clinical Nursing*, 18, 2197–2205.
22. Karadeniz, C., Pinarli, F.G., Oguz, A., Gursel, T., Canter, B. (2007), Complementary/alternative medicine use in a pediatric oncology unit in Turkey, *Pediatric Blood Cancer*,48(5), 540-543.
23. Kartal, O. and Calıskaner, A.Z.. (2007), Allerjik Hastalıklar ve Herbal Tıp, Turkiye Klinikleri Journal of Medicinal Science, 27, 876- 882.

24. Kav, S., Hanoglu, Z., Algier, L. (2022), Use of Complementary and Alternative Medicine by Cancer Patients in Turkey: A Literature Review, *International Journal of Hematology and Oncology*, 32(3), 32-38.
25. Kıssal, A., Cevik-Guner, U., Batkın Erturk, D. (2017), Use of herbal prıduct among pregnant women in Turkey, *Complemantary Therapies in Medicine,* 30, 54-60.
26. Koc, Z., Saglam, Z., Topatan, S. (2017), Determination of the usage of complemantary and alternative medicine among pregnant women in the northern region of Turkey, *Collegian,* 24, 533-539.
27. Kucukoner, M., Bilge, Z., Isikdogan, A., Kaplan, M.A., Inal, A., Urakci, Z. (2013), Complematray and alternative medicine usage in cancer patients in southeast of Turke, *African Journal of Traditional and Complementary Alternative Medicine*, 10(1), 21-25.
28. Nazik, E., Nazik, H., Api, M., Kale, A., Aksu, M., Complementary and Alternative Medicine Use by Gynecologic Oncology Patients in Turkey, *Asian Pacific Journal of Cancer Prevention*, 13, 21-25.
29. Ogur, R., Korkmaz, A., Bakır, B.(2006), Herbal Treatment Usage Frequency, Types and Preferences in Turkey, *World Family Medicine Journal/Middle East Journal of Family Medicine*, 4(3), 38-44.
30. Okka, B., Durduran, Y., Dumarn, I., Eryilmaz, A. (2022), Trends in traditional and complementary medicine practices in Turkey: Results of two consecutive surveys 2012-2018, *Journal of Pakistan Medicinal Association*, 72(4), 691-695.
31. Onbasli D., Yuvalı Celik, G., Ceylan, A., Dal, A. (2019), Türkiye'de eczanelerde bulunan bitkisel ilaçlar, *Erciyes Üniversitesi Sağlık Bilimleri Fakültesi Dergisi,* 6(1), 18-31.
32. Ozturk S. , Gundogdu Y. P. , Gursu M., Yamak M., Ozkan O., Sar F., Yenigun M., Kazancioglu, R. (2015), Use of Herbal Products in Type 2 Diabetic Patients, *Medical Bulletin of Haseki,* 53(3), 214- 219.
33. Ozturk, C., Karatas, H., Langler, A., Schutze, T., Bailey, R., Zuzak, T.J. (2014), Complementary and alternative medicine in pediatrics in Turkey, *World Journal of Pediatrics,*10(4),299-305.
34. Saatcioglu, O., Ugur, Z., Kamberyan, K., Yanik, M. (2007), A psychotic disorder related to use of herbal preparation: case report, *International Journal of Psychiatry Medicine*, 37(3):279-82.
35. Soner, B.C., Sahin, A.S., Sahin, T.K. (2013), A survey of Turkish hospital patients' use of herbal medicine, *European Journal of Integretive Medicine*, 5, 547-552.

36. Suzgec-Selcuk, S. and Eyisan, S. (2012), Türkiye'deki eczanelerde bulunan
bitkisel ilaçlar, *Marmara Pharmaceutical Journal*, 16, 164-180.
37. Tokem, Y., Aytemur, Z.A., Yildirim, Y., Fadiloglu, C. (2011), Investigation into the use of complementary and alternative medicine and affecting factors in Turkish asthmatic patients, *Journal of Clinical Nursing*, 21, 698-707.
38. Tulunay, M., Aypak, C., Yikilkan, H., Gorpelioglu, S. (2015), Herbal medicine use among patients with chronic diseases, *Journal of Intercultural Ethnopharmacology*, 4(3), 217-220.
39. Tuna, S., Dizdar, O., Calis, M. (2013), The prevalence of usage of herbal medicines among cancer patients, *JBUON*, 18(4): 1048-1051.
40. Yıldırım, T.Ş.(2010). *Terapötik etkili bazı gıdalar ve kullanım alanları.,* Bursa: Uludağ Üniversitesi Fen Bilimleri Enstitüsü.

Empathic Approach of Nurses on Pain Management

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Pain is an abstract concept that an individual experiences at certain time periods throughout his/her life, hinders daily living activities and reduces the quality of life (Erdine , 2007). In the reports of the World Health Organization (WHO, 1986), it is stated that three million patients suffer from treatable pain every day around the world, and the incidence and severity of postoperative pain is quite intense, especially in hospitals (WHO, 1986).

Pain is an unpleasant feeling, pain, or nuisance to the body and mind. Pain hurts and tires that person out. Pain; It is an abstract concept that can only be defined by the individual experiencing the pain and is affected by bio - physiological, psychological and sociocultural variables that vary according to its intensity and characteristics (Büyükyilmaz,2009). Stearnbeach defined pain as a unique sense of pain, a harmful stimulus that indicates possible tissue damage, and a response pattern that tries to protect the organism from harm. Today, the most valid definition has been made by the International Organization for the Study of Pain (IASP). According to this definition, pain is defined as an unpleasant sensory , sensory and emotional sensation and behavior related to past experiences of a person, originating from any part of the body, associated with actual or potential tissue damage. Pain includes the concepts of sensation and emotion together (Kılıç et al.,2012; Büyükyilmaz et al.,2009; Rajagopal ,2006; Aydınlı, 2005). It is seen that in both definitions made by Stearnbeach and the Taxonomy Committee of the International Organization for Pain Research, the subjective structure of pain, which can occur in different qualities and severity, is examined. However, McCaffery made the most clinically useful definition . "Pain; it is what the patient says, if he says it, it exists". Another definition is "Pain; it is the patient saying it hurts, not what others think it should be, but what the patient describes". These definitions; It covers the individual's verbal or nonverbal pain expression adequately and shows that the patient should be believed in order to develop the trust relationship, which is very important in pain management (Büyükyilmaz et al.,2009; Babacan et al., 1999; Güleç et al., 2006).

Pain is a health problem that is seen quite frequently both in the world and in our country, is increasing and is generally inadequately treated. Pain, which every person experiences at some point in his life, is now accepted as the 5th vital sign (Prince, 2001; Afşar et al., 2003; Özel et al., 2014). Pain is a problem that covers a large part of the society and significantly affects the daily life, psychosocial status and quality of life of the individual. It is also known that the pain experienced by individuals changes their sleep patterns, causes loss of role or role change in terms of family relations, causes decreases in work efficiency, and leads to despair with loss of productivity and self-confidence. In addition, it can sometimes be so severe that it prevents the most basic daily needs of the

individual and makes the individual dependent on other people for these basic activities (Kılıç et al.,2012; Özveren et al., 2009).

Pain management; requires a multidisciplinary approach and teamwork. However, there is a feature that distinguishes the nurse from other team members in pain management. This feature is that the nurse is with the patient with pain for a longer period of time and better observes and evaluates the patient (Aygin et al., 2012; Dallı, 1998).

The North American Nursing Diagnostic Association (NANDA) has included pain relief among its nursing goals. In order to achieve this goal, the nurse has an indispensable role in pain management. The nurse needs to learn the patient's previous pain experiences and methods of coping with pain, make use of them when necessary, teach the patient strategies for coping with pain, guide the patient, apply the planned treatment, monitor its effects and results, and approach it empathetically (Çöçelli et al., 2008; Eti Aslan, 2005; Aygin et al., 2012).

The Health Care Organization Committee (JCAHO), which develops standards for the assessment and management of pain, recommends evaluating pain as the fifth vital sign. The JCAHO standards state that “pain should be evaluated in all patients” and that “the patient himself is the most reliable source of pain assessment and management ”. These standards demonstrate the importance of pain management. The standards set by JCAHO are as follows;

The aim of care is not only to cure the disease, but also to treat the pain and treatment of other symptoms.

- Pain should be evaluated regularly.
- Health personnel should be trained in pain assessment and management.
- The importance of pain management in patient care should be emphasized.
- Active participation of the patient and family in pain management should be ensured.
- Pain assessment should be appropriate for the age of the patient.
- The severity and quality of the pain (characteristic, localization, frequency and duration) should be asked and

pain assessment should be recorded (JCAHO, 2018).

In pain management, the nurse should be able to identify, evaluate, monitor the pain, actively participate in the treatment, monitor the results of the treatment, use non-drug pain methods and keep the pain within livable limits and prevent problems that may develop. In order to do all these, the nurse must have the right knowledge and ability (Çöçelli et al., 2008; Aygin et al., 2012).

Today, pharmacological methods are widely used in pain management. Pharmacological methods involve controlling pain with different drugs. Analgesic treatment is the most commonly used treatment method for pain relief

in individuals because of its rapid effect and easy application (Özveren et al., 2009).

In pain control, many non-drug methods are used in addition to pharmacological treatment. Non-drug methods; These are applications that increase the effectiveness of drugs when used together with analgesics, and in cases where analgesics are not used, they help the pain heal by providing the body's natural morphine and endorphin release.Non-drug methods that can be applied to relieve or reduce pain; massage, aromatherapy , meditation, hot-cold treatments, bioenergy, imagination, biofeedback , herbal therapies, music therapy, prayer, hypnosis, reiki , acupuncture, chiropractic , yoga and spa treatments. After making a comprehensive pain assessment, the nurse can select patient-specific, non-drug methods that can be used in the management of the patient's pain, teach these methods to the patient, apply them together with the patient, and evaluate the results with this information (Özveren et al., 2009; Artan, 2012).

Today, the pain management program is accepted by nurses as a part of qualified patient care. The main goal here should be to provide exceptional care to all patients, regardless of their differences, and optimal results by using analgesics, as well as many non-drug applications that we can do independently (Dallı, 1998; Khorshid ,2005).

The concept of pain has become one of the important areas of interest of nursing in recent years. In effective pain management, nursing interventions include informing the patient about the causes and nature of pain, pain management, activities that can be done out of bed, expressing feelings and concerns, the importance of reducing anxiety , medicated and non-drug pain relief methods, pain assessment and pain control. (Eti Aslan, 2006; Erdine , 2007). However, today, effective pain management is still discussed by health professionals (Taylor and Stanbury , 2009; Ay and Alpar, 2010). In some studies, it has been determined that nurses prioritize drug administration, are reluctant to perform non- pharmacological applications, and only record the name and dose of the analgesic drug given for pain management in nurse observation forms (Eid and Bucknall , 2008; Bacaksız et al ., 2008).).

When the most important problems in pain management are examined; It is seen that the health care team does not have sufficient knowledge about pain, newly developed pain control methods and applications are not widely used, many patients accept pain as a natural process of the disease and do not report pain as a result, and a multidisciplinary team approach is not adopted in pain management. It was also found that nurses could not diagnose the patient's pain with correct/appropriate methods and could apply it independently to alleviate

pain, did not have knowledge and experience about non-drug methods, and did not provide any training to patients on these issues (Eti Aslan et al., 2005; Kılıç et al., 2012). It is known that it is important for nurses to have sufficient knowledge and practice skills about non-drug methods used in pain management and to demonstrate their independent functions.

Monitoring and evaluation of pain among healthcare team members, ensuring coordination with the physician and other healthcare team, evaluation of the patient with pain and quality of management; It is directly proportional to the knowledge, experience and abilities of the healthcare team, especially the nurses, who carry out the pain treatment. Nurses have a key role in pain management (Eti Aslan and Badır, 2005). The main points that distinguish the nurse's role in pain control from other team members are; Because the nurse is with the patient longer than the other team members, the patient learns about the previous pain experiences and coping methods and makes use of them when necessary, teaches the pain coping strategies to the patient, applies the planned analgesic treatment, monitors the results and provides an empathetic approach (Pasero & Mc Caffery, 2000; The Lion and the Badr, 2005). Nurses' correct evaluation of improved care interventions and pain control methods; It is necessary for the quality of pain management and ensuring patient comfort (Plaisance and Logan , 2006). Nurses are expected to actively participate in the treatment, monitor the treatment process, and spend effort to relieve the pain by using pharmacological and non - pharmacological methods in the care of a patient experiencing pain. Even if the pain cannot be completely relieved, keeping the pain within the limits that the person can tolerate is also a very important achievement for the nurse (Bacaksız et al, 2008; Çöçeli et al, 2008; Kılıç and Öztunç, 2012). Because pain is a subjective experience, the most reliable source for pain assessment is the patient himself. In addition to the data obtained from individuals experiencing pain, it is necessary to take a detailed history, keep it under constant observation, and use appropriate measurement and evaluation tools. Following such a path will also be useful for the evaluation of the onset and later stages of pain. The severity of the pain; Since it is a condition that is affected by psychological, physiological or social factors, the nurse should evaluate all these factors in detail and whether they interact with each other (Temiz & Özer, 2015; Kılıç & Öztunç, 2012; Çöçeli et al, 2008).

In addition to these, nurses' cultural backgrounds and their own experiences also affect pain control. In some studies (Özer and Bölükbaşı, 2001; Yücel, 2007; Demir et al., 2012) it has been observed that nurses are more affected by their own attitudes and misunderstandings about pain rather than describing the current state of the patients in pain management and approach to patients with pain. It is

thought that providing a care based on the multidimensional basis of pain by nurses will ensure success in pain management. These nursing approaches are as effective as drug applications (Babadağ & Alparslan, 2017). In order for the nurse, who has an important place in the care of the patient with pain, to provide an effective approach in the control and relief of pain, it is necessary to know the pain behaviors of the patients and how the nurses define the patient with pain (Baran, 2003). When we look at the literature, it is seen that one of the factors affecting pain control and approach to pain is pain beliefs, and some studies (Babadağ & Alparslan, 2017; Birge & Mollaoğlu, 2018; Koçoğlu & Özdemir, 2011; Walsh & Radcliffe, 2002). have been conducted on this subject in recent years. Pain coping skills, different emotional states, and the level of psychological functionality being affected by these beliefs have also formed the main subjects of studies in the field. As Sharp (2001) stated in his revised cognitive-behavioral model, pain beliefs are stated as one of the most important structures among the concepts related to pain (Erdine , 2007; Sharp, 2001; Sertel Berk & Bahadır, 2007). In various studies on pain beliefs (Demir et al., 2012; Babadağ & Alparslan, 2017; Koçoğlu & Özdemir, 2011; Walsh & Radcliffe, 2002) it has been stated that treatment processes and coping methods differ according to the beliefs of individuals. Nurses have various responsibilities in terms of questioning the patient's pain beliefs about approaching the patient with pain, evaluating how these beliefs affect the individual's ways of coping with pain, and determining an appropriate coping method for the individual (Erdine, 2007; Babadağ & Alparslan, 2017; Sertel Berk & Bahadır, 2007). Pain experiences and cultural backgrounds of the person affect pain control and approach to pain, as well as the degree of pain perceived by the person affects the approach to pain. Fear of pain occurs as a result of the degree of pain experienced and the excess of the damage that pain causes to the body, and leads to the belief that activities that cause pain should be avoided (Geisser et al., 2000). Pain and fear are inseparable. It can be said that pain causes fear, and fear increases pain by increasing sensitivity to pain (Potter, 2009; Eti Aslan, 2006). One of the terms emphasizing the importance of the relationship between pain and fear/ anxiety is " algophobia-algophobia " . This term refers to the combination of the concepts of pain and fear. Algophobia; fear of pain means an extreme fear of pain, an extreme fear of painful sensations and anything that may cause this sensation. Fear of pain is related to the individual's past experiences of pain, and it increases more as a result of painful situations that cannot be adequately managed (Ünver & Turan, 2018; McNeil & Rainwater, 1998). A fear of pain may develop in an individual who has experienced repetitive and unmanageable pain experiences (Ünver & Turan, 2018; McNeil & Rainwater, 1998). In studies (Demir et al.,

2012; Babadağ & Alparslan, 2017; Walsh & Radcliffe, 2002), it has been stated that nurses' own ways of coping with pain, based on their own experiences and showing an empathetic approach, are effective in the holistic approach to the patient and the approach to pain in the treatment process. In line with these results, the nurse's own pain beliefs, thus knowing whether the pain is caused by depression and anxiety or harm and injury, similarly, being aware of their own pain fears and knowing which of the mild, severe or medical events cause more pain can be seen in the patient with pain. It is thought that this may affect their approaches and practices.

Pain is considered a symptom , not a disease . While evaluating the patient, the location of the pain, its frequency, factors that reduce and increase it, accompanying diseases, drugs used, previous treatments, intensity and quality of pain, general physiological and psychological examination should be learned in detail and the patient should be followed up. While comprehensive evaluation of pain is performed, it is evaluated every day with vital signs in inpatients and every treatment course in outpatients (Levis et al., 2011; Potter & Perry, 2009; Eti Aslan, 2002). In the measurement of pain; Some scales are used by choosing the appropriate method for the type of pain and each patient. The commonly used scales today are listed below (Aşti & Karadağ, 2012; Eti Aslan, 2006).

One-dimensional scales,

*Verbal category scale

*Numerical scales

*Visual Comparison Scale (GLO)

* Burford Pain Thermometer (BAT)

Multidimensional scales,

* Mc gill Melzack Pain Questionnaire

* Dartmount Pain Questionnaire

*West Haven -Yale Multidimensional Pain Chart

*Reminder Pain Assessment Card

*Wisconsin Short Chart of Pain

*Pain Perception Profile is Behavior Models .

Turkish Nurses Nursing was defined as a health discipline consisting of science and art that aims to protect and improve the health of the individual, family and society in the definition of nursing made by the Turkish Association of Health (THD) in 1981 (Öz, 2010). Nursing Although it was a profession that initially met only the physical care needs of individuals , today it provides scientific and quality care services by addressing the social and emotional needs of people (Öz, 2010; Birol & Akdemir, 2011). The most important area of care is the nurse -patient interaction . Interaction is a must for the patient to be

understood correctly and to meet the needs. Interaction between nurse and patient therapeutic It is based on the relationship (Öz, 2010). A therapeutic relationship between the patient and the nurse . In order for it to occur, concepts such as interest, trust, empathy, sympathy, respect, love, dependency and independence must be taken into account. Therapeutic relationships are interpersonal to understand the patient's needs and perceptions. Communication It includes initiating, supporting, solving the patient's needs, reducing and solving problems. Therapeutic in relationships empathy, nurse - patient interaction It is important in terms of starting and supporting the nurse.3,4 Empathic in successful and easy communication approach is a key element (Birol & Akdemir, 2011; Tabak, 2006; Doğan, 2007).

Empathy is an important element in developing interpersonal relationships. According to Dökmen, empathy has two subcomponents, cognitive and emotional. The cognitive side of empathy is the individual's understanding of what the other person thinks by putting himself in the place of the other person, and the emotional side of the individual is to understand what the other person feels by putting himself in the place of the other person. In addition, empathy reduces the possibility of any conflict between them by enabling individuals to understand each other (Dökmen, 1997). Patients hospitalized in health institutions should have a quality communication with the health personnel who provide their care. Quality communication will make it easier to mingle with healthcare personnel and enable patients to express themselves more easily. The individual who knows that he/she is understood correctly by the nurse feels that he/she is important (Akgöz et al., 2005; Chan & Chau, 2005). Being able to communicate correctly and empathize ensures the acceptance of the patient and the development of a sense of trust, providing psychosocial integrity and the implementation of a quality nursing care (Ançel, 2006). Empathy in the concept of care is thought to be a key that enables nurses to respond appropriately to the needs of their patients. Although it is stated in the literature that the empathic disposition is an important facilitator in the nurse's perception of the patient's feelings and conveying them. There are publications on the subject stating that it is important to develop empathic disposition in order to communicate effectively (Davis et al., 2017; Strekalova et al., 2017; Vioulac et al., 2016). Clinical empathy, on the other hand, helps to understand the patient's situation, point of view, and feelings , to understand patients in a beneficial way and to make appropriate clinical decisions in determining the needs.

It may not always be possible to completely relieve pain. The important thing is to reduce the pain to the limits where the patient feels comfortable (Levis et al., 2011; Aygin et al., 2012). In a systematic study of 10 studies conducted by

Georgiou et al. (2015), it was determined that accurate pain assessment and pain management had a positive effect on length of stay in mechanical ventilation , morbidity and mortality . The pain management program is accepted by nurses as part of quality patient care. The main aim here is to provide better results by applying quality care to all patients and using pharmacological and non-pharmacological methods when necessary (Eti Aslan, 2006). Pain relief is often considered important in pain management. However, the approach to pain in nursing is more important than the term pain relief (Aşti & Karadağ, 2012; Çöçelli et al., 2008; Aygin et al., 2012). Successful management of pain depends on establishing a relationship of trust between the nurse, patient, and family. After making pain assessment, the nurse can determine the appropriate non-drug methods in pain management, teach these methods to the patient, apply them together with the patient, and evaluate the results (Özveren et al., 2009). Accurate pain assessment is essential for effective pain management. Pain has now become the fifth vital sign. Comprehensive assessment of the patient's pain (physiological, behavioral, psychological, social, and emotional) provides the basis for optimal pain control (Berman et al., 2016). The individual characteristics of the patient and the nurse are an important determinant in the interaction of the two, and the meaning of pain is important for both. The nurse's attitude towards pain is very important in the success of the treatment (Kocaman, 1994). Culture, values, and beliefs differentiate people's responses to pain. Therefore, all these factors; It can be effective on the nurse, who is a member of the society in which she lives, in coping with pain, her attitude and approach towards pain. By determining how the patient perceives himself and his pain, what the pain means for the patient, the nurse trains and provides support to the patient in coping with the pain. Therefore, a trust relationship is established between the nurse and the patient in the care of the patient with pain, and this is important for the successful maintenance of pain management (Çöçelli et al., 2008; Erdine , 2007; Potter, 2009).

In addition, the nurse should evaluate her attitude towards the patient with pain by reviewing her own feelings and beliefs about pain. With the evaluation made in this way, the nurse's own beliefs and experiences about pain prevent the negative effects of care (Khorshid &Yapucu, 2005). At the same time, it is known that the pain experience and cultural background of the person affect pain control and approach to pain (Potter, 2009). Based on this information, it is extremely important that the nurse, who cares for the patient and plays an important role in pain, as well as the patient's pain beliefs, knows her own beliefs and gives more effective care to the patient in this direction.

REFERENCES

1. Afşar, F., & Pinar, R. (2003). Kanser hastalarında ağrı ve ağrı ile başetme yöntemlerinin değerlendirilmesi. *Atatürk Üniversitesi Hemşirelik Yüksekokulu Dergisi*, *6*(3), 19-28.
2. Ançel, G. (2006). Developing empathy in nurses: An inservice training program. *Archives of psychiatric nursing*, *20*(6), 249-257.
3. Akgöz, S., Özçakır, A., Atıcı, E. (2005). The emphatic of phisicians employing in health, application an research center of Uludağ Universty. *Türk Psikiyatri Dergisi,*13(2), 97-104.
4. Artan, A. Kocaeli İlinde Çocuk Hastalıkları Kliniklerinde Çalışan Hemşirelerin Çocuklarda Ağrı Kontrolüne İlişkin Bilgi Durumları. Sağlık Bilimleri Enstitüsü, Hemşirelik Anabilim Dalı. Yüksek Lisans tezi, İstanbul: Haliç Üniversitesi, 2012.
5. Aşti, A.T., Karadağ, A.(2012). Hemşirelik Esasları. 1. Baskı. İstanbul, Akademi Basın ve Yayıncılık, 625-661.
6. Ay, F. ve Alpar, .Ş.E. (2010). Postoperatif Ağrı ve Hemşirelik Uygulamaları. Ağrı, 22, 21-29
7. Aydınlı, I. (2005). Ağrının fizyopatolojisi. *Türk Fiz Tıp Rehab Derg*, *5*, 8-13.
8. Aygin, D., & Var, G. (2012). Travmalı hastanın ağrı yönetimi ve hemşirelik yaklaşımları. *Sakarya Tıp Dergisi*, *2*(2), 61-70.
9. Babacan, A., Akçalı, T.D. (1999).Ağrı ve Tedavisi 2. Baskı . Ankara, Gazi Üniversitesi *Tıp Fakültesi 20. Yıl Yayınları*, 5-24.
10. Babadağ, B., Alparslan, G.B.(2017). Hemşirelik öğrencilerinin ağrı inançları. *Sürekli Tıp Eğitimi Dergisi*, 26(6), 245.
11. Bacaksiz, B.B., Çöçelli, P.L., Ovayolu, N. ve Özgür, S. (2008). Hastaya bakım veren sağlık çalışanlarının ağrı kontrolünde uyguladıkları girişimlerin değerlendirilmesi. *Ağrı,* 20(3), 26-37.
12. Baran, G. (2003). Hemşirelerin ameliyat sonrası ağrıya ilişkin değerlendirmeleri. *Hemşirelik Forumu Dergisi*, *6*(5), 24-9.
13. Berman, A., Snyder, J.S., Frandsen, G.(2016). Kozier & Erb's Fundamentals of Nursing. 10. Baskı. England, Global Edition, 1112-1151.
14. Birge, A. Ö., & Mollaoğlu, M. (2018). Hastaların ağrı inançları ve ağrıyı yönetmede kullandıkları ilaç dışı yöntemler. *Ağrı*, *30*(2), 84-92.
15. Birol, L, Akdemir, N. (2011). İç hastalıkları ve hemşirelik bakımı. Ankara: Sistem.
16. Büyükyilmaz, F., & Aşti, T. (2009). Ameliyat sonrası ağrıda hemşirelik bakımı. *Atatürk Üniversitesi Hemşirelik Yüksekokulu Dergisi*, *12*(2), 84-93.
17. Chan, J. N. H., & Chau, J. (2005). Patient satisfaction with triage nursing care in Hong Kong. *Journal of advanced nursing*, *50*(5), 498-507.

18. Çöçelli, L. P., Bacaksız, B. D., & Ovayolu, N. (2008). Ağrı tedavisinde hemşirenin rolü. *Gaziantep Tıp Dergisi*, *14*(2), 53-58.
19. Dallı, D. (1998).Postoperatif Ağrı Yönetiminde İntravenöz Hasta Kontrollü Analjezi ve Aralıklı İntramüsküler Analjezi Yöntemlerinin Karşılaştırılması. Sağlık Bilimleri Enstitüsü, Hemşirelik Anabilim Dalı. Doktora tezi, İzmir: Ege Üniversitesi.
20. Davis, C., Jiang, L., Williams, P., Drolet, A., & Gibbs, B. J. (2017). Predisposing customers to be more satisfied by inducing empathy in them. *Cornell Hospitality Quarterly*, *58*(3), 229-239.
21. Demir, Y., Usta, Y.Y., İnce, Y., Türken, K., Kaya, M. (2012). Hemşirelerin ağrı yönetimi ile ilgili bilgi, davranış ve klinik karar verme durumlarının belirlenmesi. *Çağdaş Tıp Dergisi*, *2*(3), 162-172.
22. Doğan, O. (2007). Sağlık hizmetlerinde iletiŞim. Ankara: Songür Yayıncılık.
23. Dökmen, Ü. (1997). Sanatta ve günlük yaşamda iletişim çatışmaları ve empati. Sistem.
24. Eid, T. ve Bucknall, T. (2008). Documenting and implementing evidence based postoperative pain management in older patients. *Journal of Orthopeadic Nursing*, 12, (90-98).
25. Erdine, S. (2007). Ağrı Mekanizmaları ve Ağrıya Yaklaşım. Erdine S. *Ağrı,* 37-48. 3.Baskı. Nobel Tıp Kitabevi. İstanbul.
26. Eti Aslan, F. (2002). Ağrı değerlendirme yöntemleri, *C.Ü. Hemşirelik Yüksekokulu Dergisi*, 2, 6(1).
27. Eti Aslan, E. (2005). Akut ağrı. *Hemşirelikte Eğitim ve Araştırma Dergisi*, 2: 24-31.
28. Eti Aslan, F. (2006). Postoperatif Ağrı. Ağrı Doğası ve Kontrolü, 159-90. 1. Baskı, Mart Matbaacılık Sanatları Ltd. Şti., İstanbul.
29. Eti Aslan, F., & Badır, A. (2005). Ağrı kontrol gerçeği: Hemşirelerin ağrının doğası, değerlendirilmesi ve geçirilmesine ilişkin bilgi ve inançları. *Ağrı,* 17: 44-51.
30. Geisser, M. E., Haig, A. J., & Theisen, M. E. (2000). Activity avoidance and function in persons with chronic back pain. *Journal of occupational rehabilitation*, *10*(3), 215-227.
31. Georgiou, E., Hadjibalassi, M., Lambrinou, E., Andreou, P., & Papathanassoglou, E. D. (2015). The impact of pain assessment on critically ill patients' outcomes: a systematic review. *BioMed research international*, *2015*.
32. Güleç, G., & Güleç, S. (2006). Ağrı ve ağrı davranışı. *Ağrı dergisi*, *18*(4), 5-9.
33. Joint Commission On Accreditation of Healthcare Organizations. Pain Assessment and Management Standards for Hospitals. Accessed at:

https://www.jointcommission.org/standards/r3-report/r3-report-issue-11-pain-assessment-and-management-standards-for-hospitals/#.Y5r6Zn1ByUk.
34. Kılıç, M., & Öztunç, G. (2012). Ağrı kontrolünde kullanılan yöntemler ve hemşirenin rolü. *Fırat Sağlık Hizmetleri Dergisi*, *7*(21), 35-51.
35. Khorshid, L., & Yapucu, Ü. (2005). Tamamlayici tedavilerde hemşirenin rolü. *Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi*, *8*(2), 124-130.
36. Kocaman G. (1994). Ağrı Hemşirelik Yaklaşımları. 1. Baskı. İzmir, Saray Tıp Kitabevleri.
37. Koçoğlu, D., & Özdemir, L. (2011). Yetişkin nüfusta ağrı ve ağrı inançlarının sosyo-demografik ekonomik özelliklerle ilişkisi. *Ağrı*, *23*(2), 64-70.
38. Levis, L.S., Dirksen, R.S., Heitkemper, M.M., Bucher, L., Camera, M.I. (2011). Medical Surgical Nursing:assesment and management of clinical problems, 8th ed. USA, Elsevier Mosby, 127-250.
39. McNeil, D. W., & Rainwater, A. J. (1998). Development of the fear of pain questionnaire-III. *Journal of behavioral medicine*, *21*(4), 389-410.
40. Öz, F. (2010) Sağlık alanında temel kavramlar. Ankara: Mattek.
41. Özel, F., Yıldırım, Y., & Fadıloğlu, Ç. (2014). Huzurevinde yaşayan yaşlılarda ağrı yönetimi. *Ağrı*, *26*(2), 57-64.
42. Özer, N., & Bölükbaş, N. (2001). Postoperatif dönemdeki hastalarin ağriyi tanimlamalari ve hemşirelerin ağrili hastalara yönelik girişimlerinin incelenmesi. *Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi*, *4*(1), 7-17.
43. Özveren, AGH., & Uçar, H. (2009). Öğrenci hemşirelerin ağrı kontrolünde kullanılan farmakolojik olmayan bazı yöntemlere ilişkin bilgileri. *Hacettepe Üniversitesi Hemşirelik Fakültesi Dergisi*, *16*(3), 59-72.
44. Pasero, C., Mc Caffery, M. (2000). When patient can't report pain. *A J Nurs*, 100, 22-3.
45. Plaisance, L., Logan, C.(2006). Nursing students' knowledge and attitudes regarding pain. *Pain Manag Nurs,* 7, 167-75.
46. Potter, P. A., Perry, A. G. E., Hall, A. E., & Stockert, P. A. (2009). *Fundamentals of nursing*. Elsevier mosby.
47. Prince, V. (2001). Pain management in patients with substance-use disorders. *Chronic illnesses*, 171-85.
48. Sharp, T. J. (2001). Chronic pain: a reformulation of the cognitive-behavioural model. *Behaviour research and therapy*, *39*(7), 787-800.
49. Sertel Berk, H. Ö., & Bahadır, G. (2007). Kronik ağrı yaşantısı ve ağrı inançları. *Ağrı Dergisi*, *19*(4), 5-15.
50. Strekalova, Y. A., Krieger, J. L., Neil, J., Caughlin, J. P., Kleinheksel, A. J., & Kotranza, A. (2017). I understand how you feel: The language of

empathy in virtual clinical training. *Journal of Language and Social Psychology*, *36*(1), 61-79.
51. Rajagopal, M. R. (2006). Pain–basic considerations. *Indian J Anaesth*, *50*(5), 331-4.
52. Tabak, RS. (2006). Sağlık iletiĢimi. Ġstanbul: Literatür Yayıncılık.
53. Taylor, A. ve Stanbury, L. (2009). A Review Of Postoperative Pain Management And The Challenges. Current Anaestehesia & Critical Care, 20, 188-194.
54. Temiz, Z., & Özer, N. (2015). Ameliyat sonrası ağrı şiddetinin dört farklı ağrı ölçeği ile karşılaştırılması.*Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi*, 18:4.
55. Ünver, S., & Turan, F. N. (2018). Ağrı Korkusu Ölçeği-III'ün Türkçe geçerlilik ve güvenilirlik çalışması. *Ağrı Dergisi*, *30*(1), 18-27.
56. Vioulac, C., Aubree, C., Massy, Z. A., & Untas, A. (2016). Empathy and stress in nurses working in haemodialysis: a qualitative study. *Journal of Advanced Nursing*, *72*(5), 1075-1085.
57. Yücel A. Ağrı kontrolünde hemşirenin rolü, S Erdine (Ed), Ağrı, 3. Baskı. Ankara, Nobel Tıp Kitabevleri Ltd. Şti., 2007: 37-40.
58. Walsh, D. A., & Radcliffe, J. C. (2002). Pain beliefs and perceived physical disability of patients with chronic low back pain. *Pain*, *97*(1-2), 23-31.
59. World Health Organization. Cancer Pain Relief. Worl Health Organization, 1986.

Evaluation of Plant Extracted Exosomes and their Effect on Neuroblastoma Cells

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INTRODUCTION

Cell homeostasis is achieved through the coordinated trafficking of biological material across membranes via several active and passive means including exosomes. Exosomes are extracellular vesicles released by the cell, and in contrast to intracellular vesicles, exosomes contain small molecules ranging from nucleic acids, proteins, and lipids which play key physiological and pathological roles. They have numerous advantages over liposomes or other carriers like nano-particles owing to their heterogeneity and immunomodulatory roles. indicating that they are invaluable in aiding disease diagnosis and treatment (1). Additionally, exosomes may participate in the removal of drugs and other intracellular therapeutics impeding the action of these therapies. Exosomes cross-talk has been shown to play a vital role in influencing many tumour-related pathways of various cell types contributing to poor outcomes by enhancing cancer pathogenesis (2).

Exosomes being evolutionarily conserved can open new routes for drug delivery especially useful for penetrating the blood-brain barrier. Overall, the current application of exosomes in treatment is limited by a lack of information on their stability while in storage, in addition to consistent issues with the quantities extracted as well as their purity. The work done on exosome-like vesicles found in plants shows promise as a drug delivery method showing that they can be loaded with drugs for uptake by recipient cells. It is important to investigate how these findings can be used to deliver drugs to hard-to-reach cells like brain cells(3).

Compared with other cancers of the brain neuroblastomas are more often than not fatal, part of the reason is that they arise as a result of improper developmental processes. These immature nerve cells found in several areas of the body give rise to neuroblastomas commonly seen to arise in and around the adrenal glands, sitting at a top of the kidneys because they share embryonic origins with the kidneys(4).

Most neuroblastomas are sporadic, although the presence of germline, or heritable, alterations can modify the risk of developing neuroblastoma. The tumours are thought to start in tissues of the sympathetic nervous system causing either an asymptomatic mass in the neck, chest and abdominal regions or progressing into a fatality-inducing tumour(2).

.Latest data indicates that the odds of a neuroblastoma diagnosis is conservatively 1 case per every one hundred thousand children under the age of 15, and it is the most common cancer diagnosed during the first 12 months after birth.(4)

Hence the need to evaluate the effectiveness of such techniques on Neuroblastoma cell lines. Currently, it is not easy to delineate exosomes, considering that their contents usually vary depending on their cells of origin. Thus opening avenues for weak targeting eventually leading to a slower pace of clinical application. In this study, we delivered safflower exosomes to neuroblastoma cells(5).

MATERIALS AND METHODS

The study was done at Gazi University biophysics Lab. First, we obtained the exosomes from a plant source using the Low PH-based Exosome Isolation method, which involved brewing the plant extracts filtering the extract and then isolating the exosomes via a series of centrifugation steps. The final step of the extraction and purification step process involved the passing of the obtained solution through a 0.45um filter to obtain a pure exosome solution which is then confirmed using Bicinchoninic Acid Analysis (BCA)

Using the BCA technique we established the protein content of purified exosomes and then we measured the exosome sizes using a Transmission electron Microscope and images taken. The number of exosomes was determined by Nanoparticle Tracking Analysis using the Nanosight NS300 device that helped determine the number of extracellular vesicles by reading using a 488 nm filter.

A neuroblastoma Cell Line was used and the. cells were treated by adding 10% fetal bovine serum in Dulbecco's Modified Eagle's Medium (DMEM) which we maintained as a nutrient medium. Additionally L-glutamine, and benzyl. Penicillin and gentamicin sulphate. (Safflower exosomes,) were added to the mixture. DMEM was diluted in a growth medium and taken into Petri dishes for cytotoxic testing and control the cell suspensions will be handled in the same way and cytotoxic tests will be performed. Cell viability was determined by the Annexin V Method and the results were obtained via flow cytometric analysis.

Statistical Analysis

Data analysis was done using Stata version 13 special edition. GraphPad Prism was used to plot the bar charts in figure 2 and Figure 3. The One way ANOVA was used to test for any statistically significant differences between the control and SDELNs treated samples. Afterwards, Tukey’s post-hoc test was conducted to compare all possible pairings emanating from the control and SDELNs test sample pairings. The significance level was set to 0.05.

RESULTSSafflower-derived exosome-like Nanoparticles Exosome, Characterization

Scanning Electron Microscope (SEM) images showed the size of the exosomes and the SEM images showed that the exosomes were spherical, and circular structures. (Fig 1). Further analysis revealed that on average SDELNs were 70.22 nm in diameter(see figure 2).

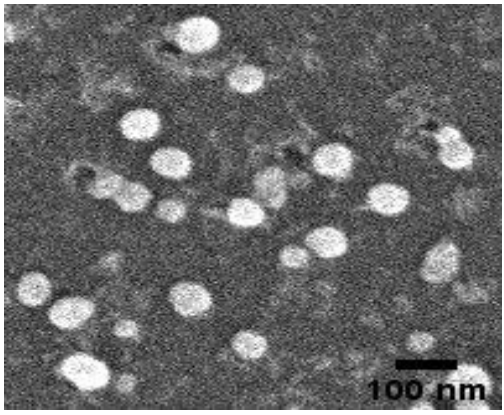

Figure 1: SEM image of SDELNs
SDELN's: safflower-derived exosome-like nanoparticles

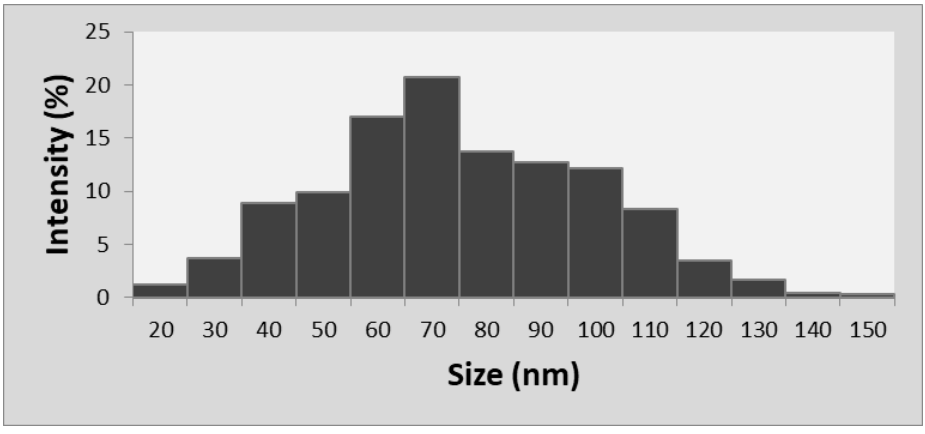

Figure 2: Size distribution of SDELNs average SDELNs diameter: **70.22 nm**

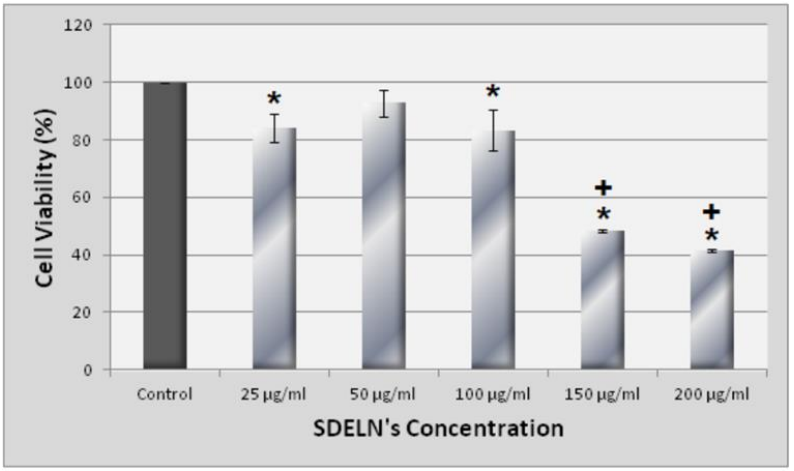

Figure 3: The viability of SH-SY5Y cells treated with different concentrations of SDELNs (MTT test). *: Statistically significant compared to the control group. +: Statistically significant compared to the other treatment groups.

Cytotoxicity analysis MTT Cytotoxicity analysis of SH-SY5Y cells

Figure 3; shows the viability of SH-SY5Y cells treated with different concentrations of SDELNs using the MTT assay for viability testing. Cell viability was highest in the Exosome 50 ng/ml group compared to all the other groups except the control group. We observed that SH-SY5Y cell proliferation was significantly reduced with higher concentrations of the sidelines. Our results show a correlation with the literature(6).

However, this association is not seen when comparisons are made to 50ng/Ml treatment groups. Seemingly at higher SDELN concentrations, there is a statistically significant difference demonstrating the effectiveness of SDELNs against SH-SY5Y cells.

Based on our results SDELN demonstrated the ability to decrease cell proliferation in SH-SY5Y cells significantly with increasing concentration when compared to the control group and across the other treatment groups with lesser SDELN concentrations thus showing their cytotoxic effect on the Neuroblastoma cancer cells. A finding supported by recent evidence collected in the new field of plant-derived exosome-like nanoparticles (7,8)

DISCUSSION

Over millennia plant materials have been used both in the human diet and as medicines. Plant extracts or various bioactive constituents in plants possess medicinal properties. In fact estimates by the Food and Agriculture Organization

put the number of therapeutic plants at about 60000 (9). In this regard, the number of bioactive compounds that can be extracted or found naturally occurring within medicinal plants has played a massive role in the clinical treatment of infectious diseases and/or providing relief for many other ailments (8) Therefore our study was driven by evidence demonstrating that exosome-like nanoparticles derived from plants are associated with anti-cancer activity(8,10).

In this study, we proposed a strategy for isolating exosome-like nanoparticles from Safflower which could be useful in creating protocols for mass production of SDELNs which involves the ultracentrifugation exosome purification method and has long been regarded as the gold standard for isolating purpose tested means for extracting exosomes of relatively homogeneous sizes as was demonstrated by the average size of the exosomes we extracted of 70.22 nm in diameter(fig: 2) with a spherical shape (fig 1) (1)

Despite ultracentrifugation being the gold standard for exosome isolation, it has been associated with the possibility of sample loss depending as such it is heavily reliant upon operator skill since repeated ultracentrifugation steps may cause damage to vesicles and reduce yields, thereby potentially impacting the quality of protein and RNA content of the isolated exosomes. This could be the reason for the discordant result obtained within the 50ug/ml treatment group where the cell viability increased despite the increasing concentration of the SDELNs(11).

Thus, in our study, we aimed to investigate the anticancer activity of Safflower-derived exosome-like nanoparticles (SDELNs) on Neuroblastoma cell line SY5Y cells, after characterizing the extracted SDELNs obtained from the rigorous extraction phase, we designed in vitro experiments to evaluate their anticancer properties. We then targeted the SY5Y cells under various test conditions such as under increasing concentrations of SDELNs and examined their cytotoxicity-inducing abilities against a control group

Safflower extract was used to provide the exosome-like nano-particles because like other useful medicinal plants it has been used as a therapeutic agent and/or for augmenting treatment over a long period. Therefore despite the few studies investigating exosome-like nanoparticles for specific biomolecules against various treatments. The current evidence has demonstrated that. they cannot only localize at target tissues, their component proteins and lipids, can bind to receptors and act as recognition elements for distant sites which may trigger the body's immune system when used in vivo.

The demonstrated decrease in Neuroblastoma cell proliferation with increasing SDELN concentration in the treatment groups demonstrates that the SDELNs can be actively transported into the cells to access the cancer

microenvironment and that also they penetrate the cells with ease. When we look at the MTT assay results from our experiment, at lower SDELN concentrations the Cytotoxic effect is weaker than at higher SDELN concentrations as seen by the effect on cell viability. The reason for this is that SDELNs just like other exosomes possess the ability to be effectively transported into the cell. Ha et al (12) showed in their study in 2016 explore the usefulness of exosomes in pharmacology as drug delivery vectors.

Indeed it has been demonstrated that high doses of exosome-like nanoparticles as in the case of SDELNs led to an increase in the cytotoxicity of cancer cells (13). However, from our result, we inferred that resistance to SDELNs caused a decrease in cell death rate, despite the increasing SDELN concentration seen in the 50ug/ml treatment group. It was determined that the discordant results in the 50ug/ml group can be ascribed to operator error, SDELN quality, and cellular stress. Nonetheless, the highest Cytotoxicity activity in the SDELN group was observed between the 150ug/ml and 200ug/ml groups. At the highest dose, Cytoxicty activity increased by an impressive approximately 50-fold when compared to that of the control treatment group.

Neuroblastoma requires multimodal therapy ranging from chemotherapy, surgery and radiotherapy to biological and immunological treatments for optimal outcomes which so far are not associated with a cure or even improved outcomes for those with advanced disease(14). Hence the need for innovative treatment approaches, currently some of the best treatment options are associated with life-limiting toxicity as highlighted in several studies(4,5). Nemati et al(15) in their review indicated that plant-derived exosomes like nano-particles, can promote effective activation of the immune system since they are derived from natural sources which also means that they show less toxicity synthetic nanoparticles and liposomes The data we obtained from our study indeed supports the evidence so far that plant-derived exosome like nano-particles can be a useful addition in anti-cancer therapies hence they can be seen as a new hope in the treatment of many diseases, including Neuroblastoma(7,8,13,16).

Conclusions

Data from the study demonstrated that SDELNs show significant levels of cytotoxicity against the Neuroblastoma cell line SY5Y cells in nearly all of the treatment groups. Thus indicating the usefulness of SDELNs as a therapeutic tool against the SY5Y cell line and by extension neuroblastoma. The prevailing consensus in the field of plant-derived exosome-like nano-particles is that not all of their aspects have been fully identified and described as it relates to exosomes. Available evidence so far shows that various plant-derived exosomes have been

extensively applied in the development of novel drugs to treat specific diseases or maintain healthy body functions of which SDELNs are a part.

Recommendations

The potential future application of exploiting the findings of our study should be conducted to explore the application of SDELNs in the treatment of Neuroblastoma in robust studies that can cater to eliminating any confounding factors like operator error and incorporate efficient exosome extraction methods.

References.

1. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications . Int J Nanomedicine [Internet]. 2020 Sep 22 [cited 2022 Oct 4];15:6917–34. Available from: https://www.dovepress.com/exosome-a-review-of-its-classification-isolation-techniques-storage-di-peer-reviewed-fulltext-article-IJN
2. Pegtel DM, Gould SJ. Exosomes. Annu Rev Biochem. 2019 Jun 20;88:487–514.
3. Pinnell JR, Cui M, Tieu K. Exosomes in Parkinson Disease. J Neurochem [Internet]. 2021 May 1 [cited 2022 Oct 5];157(3):413. Available from: /pmc/articles/PMC8863192/
4. Richard H, Pokhrel A, Chava S, Pathania A, Katta SS, Challagundla KB. Exosomes: Novel Players of Therapy Resistance inNeuroblastoma. Adv Exp Med Biol [Internet]. 2020 [cited 2022 Oct 11];1277:75. Available from: /pmc/articles/PMC7775408/
5. Calderoni D, Barone G, Elliott M, Tweddle DA, Ramanujachar R, Gray JC. Neuroblastoma: a master of disguise and a challenge to cure. Paediatr Child Health (Oxford). 2022 May 1;32(5):171–7.
6. Kim J, Lee YH, Wang J, Kim YK, Kwon IK. Isolation and characterization of ginseng-derived exosome-like nanoparticles with sucrose cushioning followed by ultracentrifugation. SN Appl Sci [Internet]. 2022 Feb 1 [cited 2022 Oct 23];4(2):1–11. Available from: https://link.springer.com/article/10.1007/s42452-022-04943-y
7. Cai Y, Zhang L, Zhang Y, Lu R. Plant-Derived Exosomes as a Drug-Delivery Approach for the Treatment of Inflammatory Bowel Disease and Colitis-Associated Cancer. Pharmaceutics [Internet]. 2022 Apr 1 [cited 2022 Oct 11];14(4). Available from: https://pubmed.ncbi.nlm.nih.gov/35456656/
8. Kim J, Li S, Zhang S, Wang J. Plant-derived exosome-like nanoparticles and their therapeutic activities. Asian J Pharm Sci. 2022 Jan 1;17(1):53–69.
9. Schindler, C., Heral, E., Drinkwater, E., Timoshyna, A., Muir, G., Walter, S., Leaman, D.J. and Schippmann U. Wild check – Assessing risks and opportunities of trade in wild plant ingredients. 2022 [cited 2022 Oct 23]; Available from: http://www.wipo.int/amc/en/mediation/rules
10. Tordesillas L, Berin MC. Mechanisms of Oral Tolerance. Vol. 55, Clinical Reviews in Allergy and Immunology. Humana Press Inc.; 2018. p. 107–17.

11. Patel GK, Khan MA, Zubair H, Srivastava SK, Khushman M, Singh S, et al. Comparative analysis of exosome isolation methods using culture supernatant for optimum yield, purity and downstream applications. Sci Reports 2019 91 [Internet]. 2019 Mar 29 [cited 2022 Oct 23];9(1):1–10. Available from: https://www.nature.com/articles/s41598-019-41800-2
12. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharm Sin B [Internet]. 2016 Jul 1 [cited 2022 Oct 26];6(4):287. Available from: /pmc/articles/PMC4951582/
13. Zhang Z, Yu Y, Zhu G, Zeng L, Xu S, Cheng H, et al. The Emerging Role of Plant-Derived Exosomes-Like Nanoparticles in Immune Regulation and Periodontitis Treatment. Front Immunol. 2022 Jun 10;13:2885.
14. Qiu B, Matthay KK. Advancing therapy for neuroblastoma. Nat Rev Clin Oncol 2022 198 [Internet]. 2022 May 25 [cited 2022 Oct 11];19(8):515–33. Available from: https://www.nature.com/articles/s41571-022-00643-z
15. Nemati M, Singh B, Mir RA, Nemati M, Babaei A, Ahmadi M, et al. Plant-derived extracellular vesicles: a novel nanomedicine approach with advantages and challenges. Cell Commun Signal 2022 201 [Internet]. 2022 May 23 [cited 2022 Oct 23];20(1):1–16. Available from: https://biosignaling.biomedcentral.com/articles/10.1186/s12964-022-00889-1
16. Shinge SAU, Xiao Y, Xia J, Liang Y, Duan L. New insights of engineering plant exosome-like nanovesicles as a nanoplatform for therapeutics and drug delivery. Extracell Vesicles Circ Nucleic Acids [Internet]. 2022 Jun 29 [cited 2022 Oct 23];3(2):150–62. Available from: https://evcna.com/article/view/4995

Vascular Access Education In Hemodialysis Patients

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VASCULAR ACCESS

Vascular access is the golden key to hemodialysis treatment for end-stage renal failure patients. Ensuring safe and continuous vascular access in hemodialysis can be achieved through the coordination of healthcare professionals, patients diagnosed with chronic kidney disease (CKD), patients receiving dialysis treatment and their relatives. A multidisciplinary team trained and working in coordination is required to successfully establish, use and maintain optimal vascular access for each patient. Patient education should be tailored to the patient's treatment goals by the individual's characteristics. It is the responsibility of the multidisciplinary healthcare team to ensure that patients are evaluated in the early period and receive vascular access training and provide training updates in the future. Training interdisciplinary team members are one of the indispensable steps to achieving the best results in establishing and maintaining vascular access (Kumwenda et al, 2015; Moist, et al, 2013).

Patient education programs about vascular access increase the use of arteriovenous fistula at the beginning of the first dialysis treatment. Vascular access education in hemodialysis is essential in terms of growing dialysis efficiency, reducing mortality and morbidity, increasing patient compliance with treatment, reducing complications of administration and use, supporting multidisciplinary teamwork, and reducing costs (Moist, et al, 2013; Kidney School).

EDUCATION

It is a dynamic process that envisages the acquisition of knowledge, skills, attitudes and behaviours that will meet the needs of the individual and bring solutions to their problems, as well as the acquisition of certain behaviours, values and perspectives by the individual and reaching a more rational imaginary view in their approach to them (Taşocak, 2012).

Patient and Family Education Process

The stages of the patient education process are similar to the steps of the nursing process. This process is limited to hospitalisation and covers the period after discharge. The patient education process refers to the actions that serve to learn how to improve the health status and continue for this purpose. The patient education process consists of data collection, diagnosis, development of the education plan, implementation and evaluation and recording stages that interact with each other (Gedük, 2018; Taşocak, 2012).

Data Collection

The data collection stage is the first step of the nursing process in which the patient's needs are identified, and the education content is determined. The patient's learning needs, characteristics, learning style preference, and whether the patient can read and write should be selected at this stage. The nurse should consider how the patient wants to be educated before planning education for the patient. There are four basic learning styles. These are

- Visual learning; by reading the blackboard and notes
- Visual and auditory learning; looking at pictures, videos, etc.
- Learning in the presence of an authority figure; conversation, lecture, group discussion, etc.
- Tactile, gestural learning is physical, gestural education with a preference for the instructor (Gedük, 2018; Taşocak, 2012).

Diagnostics

In this step, information about the patient's learning preparations for information needs is collected systematically. At this stage, the nurse analyses, interpret and evaluates the data collected for learning and determines the patient's learning needs. Nursing diagnoses are determined by analysing the collected data (Gedük, 2018; Taşocak, 2012).

Training Plan Development

The planning stage is crucial for the successful outcome of patient education. The education plan prepared by considering all elements of the patient education process is the stage of deciding what to do, in what order, how and when to do it, and how to evaluate it. It is necessary to plan the duration, objectives, methods, and tools for the patient and their family. Learning objectives should be determined while preparing the education plan. Since the goals are related to each step of the education process, it is essential to decide on them correctly and reflect them in practice. It is aimed at the patient and their family to acquire behaviours in the desired direction after the education. Therefore, the objectives should be observable, measurable, consistent, and cover learning areas. Learning domains are the cognitive domain, affective domain and kinesthetic domain. These domains are intertwined, and learned behaviour can include them simultaneously. Still, it is named cognitive behaviour, affective behaviour and kinesthetic behaviour according to the dominant quality of the behaviour. Since these three learning domains are present in every step of the education process, it will be effective for the nurse to know the characteristics of these domains in planning and implementing an adequate education (Gedük, 2018; Taşocak, 2012).

Application

The implementation phase is where the planned teaching activities are implemented to support the patient and their family. In the implementation phase of the patient education process, the nurse demonstrates an individual approach and uses appropriate teaching methods. She ensures that the education is transferred to the patient/family with the tools she will use for the target she has determined. In the implementation phase, the nurse ensures that the education is exciting and permanent by using her creativity and experience. In this process, the nurse establishes eye contact with the patient (Gedük, 2018; Taşocak, 2012). While transferring the education prepared in advance, the nurse;

- Presents the training with a clear expression using simple words
- Avoids confusion by using plain language
- Pay attention to volume during training, and speak as loudly as necessary
- Arranges the topics in a logical order
- They pay attention to the ordering of topics from general to specific.
- Places visual aids so that they can be easily seen by the patient and their family
- Removes distractions from the environment and makes education their sole focus
- Ensures that directives are clear and precise
- He is open to giving feedback throughout this process and is always patient (Taşocak, 2012)

Evaluation

The evaluation phase is the last component of the patient and family education process, although it takes place at every stage. It gives the educator an idea about the extent to which the educational objectives have been achieved. The results are compared with the goals to determine the learning effectiveness and whether the expected results have been achieved. The evaluation process should be completed at the end of each teaching-learning process. This allows the nurse to quickly provide positive and constructive feedback to the patient and family and revise the education plan to meet ongoing learning needs. At the same time, the nurse sees their success in education and provides feedback. There are observation, interview, verbal and written evaluation methods. The nurse uses some techniques to assess that learning has taken place. She asks questions to the patient

and family. The act of asking questions is an interactive process that helps evaluate whether the patient and family have received the information. For example, they may ask the patient if they can list the symptoms of a heart attack. Educate the patient and family about the effects and side effects of the medication and ask about the effects and side effects of the drug a day later to assess its persistence. Attitudes, beliefs and lifestyle changes are often difficult to determine because patients may say they have changed their lifestyle when they have not. The nurse knows that the best way to determine this is through observation (Gedük, 2018; Taşocak, 2012).

Recording

Recording the training is very important and mandatory to communicate the training effort to the patient and family, the health care team and the institution. All teaching-learning activities during exercise should be documented in line with the institutional policy. These documents are communication tools that show progress from shift to shift, area to area and day to day. Documentation should cover the training from admission to discharge. The nurse must also recognise that the informal teaching that takes place at the bedside is, in fact, education (Gedük, 2018; Taşocak, 2012).

Education Methods

Various methods are used to ensure learning. The forms show different dynamics depending on the trainer, learner or group. A single process can be used, or training with several methods can be planned together. Training methods include lectures, groups, demonstrations, question and answer, coaching and simulation learning methods (Taşocak, 2012).

Lecture Method: It is a method in which an educator teaches a subject by explaining and explaining it to a large number of people in a sequence and includes discussion. Most of the subject matter is theoretical and is presented verbally by the lecturer. During the training, learners can ask questions and have the opportunity to share their thoughts or problems. It is one of the most used methods in teaching. It is essential in terms of integrating theoretical knowledge into practice (Hacıalioğlu, 2013; Hinkle & Cheever 2015).

Group Learning: This training method provides teamwork with the active participation of individuals in learning, providing not only the information they need but also making them feel comfortable in the group (Hinkle & Cheever 2015). It is a training method with positive aspects, such as allowing group members to benefit from each other's knowledge and the emergence of different per-

spectives. Group work is used to solve a problem raised by the trainer or participants, to conduct case studies, and to prepare roles to be played in role-playing (Hacıalioğlu, 2013).

Demonstration: Visuality and practice have an important place in the learning process. The best practice method is to repeat the skill after it is demonstrated. It is the effective teaching of a new skill or tool using slides, videos, models or in a natural environment. It is essential in terms of creating a visual image of the individual. The application is shown as a whole, divided into small parts, shown again in single pieces, and finally shown again as a whole (Hinkle & Cheever 2015).

It is a method frequently used by both the patient and team members in vascular access training. Team members learn by both seeing and hearing. Training should be done in small groups, and the activity should be carefully explained and practised. No mistakes should be made during the demonstration, and the process steps should be followed. For example, new practices in catch care can be used effectively in training team members with the demonstration method (Hacıalioğlu, 2013).

Question and Answer Method: It is a training method based on the trainer asking questions to the participants and directing the subject according to the answer given (Hacıalioğlu, 2013).

Coaching: This training method is used to learn a skill related to manual dexterity or the psycho-motor field. It corresponds to the "master-apprentice" relationship traditionally used in our country. The trainer must be qualified in their teaching skill (Hacıalioğlu, 2013). An example is the training of other nurses on this vascular access after the first cannulation of new grafts and fistulas by experienced nurses (Lok et al., 2020).

Simulation: Simulation applications have been widely used in health education today. Although its use is limited for arteriovenous fistul (AVF) and arteriovenous graft (AVG) structures, which may differ from patient to patient, it is considered easy to apply for catheter use. Computer-assisted simulations or models improve psychomotor skills and hand manipulation, determine entry angles, and accurately perform procedure steps (Philips et al, 2011).

Training Materials: Books, brochures, pictures, models, videos, phone applications, etc... can be used as training materials (Hinkle & Cheever 2015).

VASCULAR ACCESS EDUCATION

Nurses are responsible for continuous education and continuity of care quality. Considering the rapidly developing technology and the conditions of our profession, it has become mandatory for healthcare professionals who have difficulty

in meeting patient care in hemodialysis, which is a particular branch of hemodialysis, with the education they receive at school, to be trained within the institution within the current information (Virani at al., 2005).

In-service training or continuing education programs organised in line with the knowledge and needs of the healthcare team provide benefits in terms of preventing occupational accidents, increasing individual and institutional productivity, developing the sense of professional confidence of employees, and strengthening internal relations (Gedük et al., 2018; McCann et al., 2008; Lok et al., 2020).

It is essential to systematically teach the knowledge, skills and behaviours required for effective vascular care from the moment individuals start working in the institution and to ensure the continuity of education. Orientation training is organised for new employees, and their duties, authorities and responsibilities are introduced. The aim is to gain knowledge, attitudes and behaviours for creating, protecting and maintaining vascular access to provide hemodialysis treatment at the highest level. In addition, the acclimatisation of newly recruited employees to their new job responsibilities supports the old team members to renew themselves by improving their professional skills and increasing the quality of care (Hacıalioğlu, 2013).

Benefits of training for team members;

- It ensures that the employee does their job knowingly and correctly,
- Supports the employee in developing a positive attitude,
- It creates the employee's ability to problem solve and think creatively,
- It facilitates teamwork by improving communication.
- Employees can better protect themselves against work accidents,
- It motivates the employee to learn new knowledge,
- It makes it easier for new hires to adapt (Hacıalioğlu, 2013).

TRAINING OF HEALTH PERSONNEL

Healthcare team members should be aware of all the steps to be taken before the creation of vascular access, in the maintenance of vascular access and in the event of complications in patients who need or have vascular access. For this purpose, following current publications, organising in-service training, and case discussions are extremely important (Moist et al., 2013).

The patient should be referred to a nephrologist at the right time to establish early vascular access. The patient who is evaluated by the nephrologist should be

provided with pre-dialysis training and referred to surgery early to verify optimal vascular access for the patient. Healthcare team members should be familiar with vascular access monitoring, approaches to prevent complications that may develop, and treatment management when complications develop. It should be kept in mind that evaluating vascular access at appropriate intervals can be used for a more extended period without complications (Moist et al., 2013).

Vascular access cannulation, maintenance, monitoring and evaluation are skills that can be learned and improved with practice. Inadequate cannulation skills can lead to damage to AVFs and AVGs, infection, hematoma formation and permanent damage to vascular access. Two experienced nurses should evaluate a newly opened vascular access as easy, intermediate and complex. They should help themselves until the vessels can be cannulated well and then help other nurses perform this cannulation best (Moist et al., 2013; McCann et al., 2008).

Establishing vascular access written protocols in dialysis centres is recommended in the guidelines. This practice will be effective in ensuring standardisation, preventing errors as well as preventing vascular access complications. The knowledge of the employees should be regularly monitored in line with the protocols (Daugirdas et al., 2015; Tordoir et al., 2007). Research and use of evidence-based practices should be ensured. By recording the procedures performed in the institution and providing a basis for the studies to be planned, research on the development and protection of vascular access should be encouraged (Chronic Kidney Disease: Vein Preservation, 2020; Lok et al., 2020).

Patient Education

Patient education is an integral part of nursing practices. Especially in chronic diseases, education significantly affects the patient's disease process. Family and patient education is essential to IV use and should be repeated regularly. While planning the education, the nurse should provide the patient and the patient's relatives with appropriate, sufficient and not more confusing information than necessary. Health knowledge and education status should be questioned while evaluating the individual. Inadequate health knowledge will cause them to need help understanding the subject well enough. Disruptions will also be observed in the communication of individuals without adequate education or health knowledge with health team members (Virani et al., 2005).

Patient education and the appropriate education method for the patient and their family should be determined, and implementation should be started. Studies have shown that training with the participation of family members is more effective. A suitable environment should be prepared for education, and the patient

should be made to feel comfortable. Attempts should be made to reduce stress and anxiety. Nurses apply adult education methods during education and encourage patients and their relatives to communicate and act independently (Moist et al., 2013; Virani et al., 2005).

- The best time for adults to learn is when they need to,
- Adults learn best when they are active in education,
- Allow adults to try out a new skill,
- Have adults reinforce the new skill,
- In adult education, move from what the individual already knows to what they do not know,
- Progress from simple to more complex sections in training,
- Take feedback promptly and correct any inaccuracies immediately.

Topics that can be addressed in patient education (Kumwenda et al., 2015; McCann et al., 2008; Lok et al., 2020);

- Training on vascular access selection before dialysis treatment,
- Teaching to preserve vessels for the arm where an AVF can be created,
- Training before the vascular access operation,
- Education in the postoperative period,
- Training on vascular access monitoring,
- Training the look, listen, and feel method for AVF,
- Training on protecting the watercourse,
- Hand hygiene training,
- Training on bleeding control,
- Education on vascular complications,
- Training on the care of catheter dressings,
- Training on what to do in case of catheter dislodgement,
- Training on what to do in case of fistula or graft arrest or reduction in a thrill,
- Training on signs and symptoms that may occur in the presence of infection (Kumwenda et al., 2015; McCann et al., 2008; Lok et al., 2020).

RESOURCES

1. Chronic Kidney Disease: Vein Preservation, (2020) Vascular Access Guideline, BC Renal Agency. http://www.bcrenal.ca/resource-gallery/Documents/VA-Chronic_Kidney_Disease-Vein_Preservation.pdf Access Date: 15.11.2022.
2. Daugirdas, J.T., Blake, P.G., Ing, T.S. Handbook of Dialysis. 5th ed. USA. Wolters Kluwer. 2015. p. 121-154.
3. Gedük, E. (2018). Hemşirelik Mesleğinin Gelişen Rolleri. Sağlık Bilimleri ve Meslek Dergisi, 5 (2), 253-258.
4. Hacıalioğlu N. Hemşirelikte Öğretim Öğrenme ve Eğitim.2. Baskı. Nobel Tıp Kitabevleri. 2013.
5. Hinkle JL, Cheever KH. (2015). Brunner & Suddarth's Textbook of Medical-Surgical Nursing. 13th Edition. Lippincott, Williams &Wilkins. 2015.
6. Kidney School, https://kidneyschool.org/pdfs/KSModule8.pdf Access Date:20.11.2022.
7. Kumwenda, M., Mitra, S. ve Reid, C. Clinical Practice Guideline, Vascular Access for Haemodialysis, 6th. Edition, UK Renal Association. (2015). https://renal.org/sites/renal.org/files/vascular-access. Access Date: 24.11.2022.
8. Lok, C.E., Huber, T.S., Lee, T., Shenoy, S., Yevzlin, A.S., et al. National Kidney Foundation KDOQI Clinical Practice Guideline for Vascular Access: 2019 Update. American journal of kidney diseases: the official journal of the National Kidney Foundation. 2020;75(2):1–164.
9. McCann, M., Einarsdóttir, H., Van, Waeleghem, J.P., Murphy, F., Sedgewick. J. Vascular access management 1: an overview. Journal of Renal Care. 2008;34(2):77-84.
10. Moist, L.M., Lee, T.C., Lok, C.E., Al-Jaishi, A., Xi, W., Campbell, V., Graham, J., Wilson, B., Vachharajani, T.J. Education in Vascular Access. Seminars in Dialysis. 2013; 26(2);148-153.
11. Philips, S., Collins, M., Dougherty, L. Venepuncture and Cannulation. Wiley-Blackwell. 2011.
12. Taşocak, G. Hasta Eğitimi. İstanbul. İstanbul Üniversitesi Basın ve Yayınevi Müdürlüğü. 2012.
13. Tordoir J, Canaud B, Haage P, Konner K, Basci A, Fouque D, et al The European Renal Best Practices (EBPG) on Vascular Access. Nephrol Dial Transplant. 2007;22(2): 88-117.
14. Virani T, Schouten JM, McConnell H, McConnell S, Santos J, Russel B, Scott C, Burris J, Powell K. Care and Maintenance to Reduce Vascular

Access Complications. https://sigma.nursingrepository.org/bitstream/handle/10755/334906/Care_and_Maintenance_to_Reduce_Vascular_Access_Complications.pdf?sequence=6&isAllowed=y Access Date: 24.11.2022.

Health Education

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1. INTRODUCTION

Health education is an indispensable element of quality health care to give individuals the habit of protecting and improving health, teach the rules of healthy living, restore health in case of illness, and improve the quality of life. In recent years, health education has become an essential part of the health system in solving health-related problems worldwide, especially in developed countries (Smith *et al.,* 2006). In 1978, with the Alma-Ata Declaration, within the scope of Primary Health Care Services, "educating the public on issues related to common health problems in a society, their prevention and control" was determined as an essential health service accepted by the member countries of the World Health Organization (WHO) (WHO,1978).

A plan should be made for health education, and the results should be evaluated. In the selection of methods and techniques to be used in individual/patient education, the learning needs of the individual/patient, the characteristics and preferences of the individual/patient and nurses, the content of the education, the environment in which the education will take place, the duration of the education and the availability of available resources are essential. In education, simple terminology and plain language that the individual/patient and their family can understand should be used, and good communication should be established (Grad, 2002).

2. HEALTH EDUCATION AND GENERAL OBJECTIVES

According to the World Health Organization (WHO), health education is to make people adopt the measures to be taken for a healthy life and to make them believe in the necessity of their practices, to get them used to the correct use of health services, and to take decisions as individuals and as a community in order to improve their health status and environment (Hacıalioğlu, 2020).

With adequate health education, the knowledge, beliefs and behaviours that have been adopted with what needs to be known to improve health can be changed. The most important feature of health education is the willing participation of individuals in planning their health practices (Hacıalioğlu, 2019).

The primary purpose of health education is to make health a social value and to provide each individual with the correct information they can use to solve health problems. The general objectives of health education are as follows (Hacıalioğlu, 2019; Bijayalaskhmi, 2017):

- To explain and convince individuals of the importance of health
- To enable individuals to make the right decisions about health
- Supporting individuals' behaviours that will create a healthy lifestyle
- Accustoming individuals to solve their health problems on their own

- Supporting the patient to regain health and independence
- To maintain the necessary health care of the sick or healthy individual at home
- Accustoming individuals to healthy living and improving the quality of care
- Reducing the cost of health services

Individuals' socio-demographic, socioeconomic and psychosocial characteristics effectively create behavioural changes at the end of health education. Health training planned and implemented without considering these characteristics causes failure. Individuals' behavioural change on a health-related issue depends on their level of education, knowledge of that subject, sociocultural factors, personal beliefs and health perceptions (Hacıalioğlu, 2019; Bijayalaskhmi, 2017).

3. STAGES OF HEALTH EDUCATION PRACTICE

The stages of health education practices are as follows; (Bijayalaskhmi, 2017);

Awareness: Awareness means knowing the facts about health. It is the first step of the health education process. At this stage, where the health educator will provide health education, the client will first listen, be motivated and show more interest in listening.

Areas of Interest: This is the second step in the health education process. At this stage, the mentees will be well motivated and only be interested in knowing the details of health-related behaviours.

Evaluation: In this stage, the individual will analyse the advantages and disadvantages of the health education provided. They will also analyse the barriers to the implementation of health behaviour.

Trial: After examining the advantages and disadvantages of health education, they will be motivated and try to implement it when they think health behaviour is more advantageous.

Change: A healthy habit will be created by engaging in behaviours that promote health.

4. PRINCIPLES OF HEALTH EDUCATION

The characteristics of the population to whom health education will be provided are different. Some principles of health education consider this and ensure the right to a healthy life for all individuals in society. These principles are as follows; (Özvarış 2011; Öztemel 2018; Tekbaş *et al.* 2005; Kıssal 2021);

Principle of Target Population Formation: The characteristics of individuals with different characteristics and needs need to be known. Various questions can

be asked when determining this. It should be determined to whom and why education will be given and why education is needed. In this way, a suitable target group is determined, in which the differences between individuals are reduced, and the commonalities are increased (Özvarış, 2011; Öztemel, 2018; Tekbaş *et al.,* 2005; Kıssal, 2021).

Principle of Relevance: In training given to adults, even if the subject is for their benefit, they may not turn what they listen to into behaviour. For this reason, one of the essential principles of adult education is to make it relevant. In order to realise this, the characteristics and needs of individuals should be known, and needs should always be taken into account for interest (Özvarış, 2011; Öztemel, 2018; Kıssal, 2021).

Principle of Applicability: After the individual's interest in the subject is ensured, translating what they listen to into behaviour is realised by providing opportunities and removing obstacles. For this reason, in health education, it is necessary to remove the factors that hinder the aim and to create opportunities (Özvarış, 2011; Öztemel, 2018; Tekbaş *et al.,* 2005; Kıssal, 2021).

Continuity Principle: One of the objectives of health education is to create behavioural change. Behaviour change in adults is a time-consuming issue. Therefore, planned training should be continued until behavioural change occurs (Özvarış, 2011).

The Principle of Covering Everyone in Society: Despite all individual differences, health education has the principle of covering everyone. The need for health education is social as well as individual. Since individuals in society affect each other, collective change is needed to improve health as a society (Özvarış, 2011; Öztemel, 2018; Tekbaş *et al.,* 2005; Kıssal, 2021).

The Principle of Collaboration Between Leaders and Sectors: Health issues are multifactorial and diverse to be solved by health professionals. It concerns the cooperation of all sectors. The priority in health education is to educate community leaders and to influence individuals in this way. Political leaders, administrators, religious leaders, teachers, artists, and journalists should be trained, and cooperation with voluntary, private and public organisations should be ensured (Özvarış, 2011; Öztemel, 2018; Tekbaş *et al.,* 2005; Kıssal, 2021).

The Principle of Delivering Education with a Service: It is beneficial to carry out health education. Health education is provided to the individual, and the health service is transformed into behaviour quickly. Nurses and physicians need to provide health education in addition to medical treatment and care (Özvarış, 2011; Öztemel, 2018; Tekbaş *et al.,* 2005; Kıssal, 2021).

Principle of Avoiding Punishment: Punishment has never been effective in developing positive behaviour. Instead of punishment in adult education, it is

more important to emphasise the importance of the subject through education and to provide treatments by persuasion (Özvarış, 2011; Öztemel, 2018; Tekbaş *et al.,* 2005; Kıssal, 2021).

Principle of Quality of Educators: In health education, the characteristics of the person providing the education are also fundamental. Health education is a planned education, and training should be planned and implemented using educational methodology and technology principles. Therefore, there are some characteristics that the trainer should have. These are (Özvarış, 2011; Öztemel, 2018):

- Must be someone trusted by society
- Know the culture, values, and problems of the society
- He/she must first believe in the need to teach the subject
- Speak in a language that society understands
- Behave by the rules of communication and body language
- Must be restrained in their clothing and, at the same time model
- Respect people's values and beliefs
- Should not show an authoritarian attitude in the educational environment
- Create a safe learning atmosphere
- Receive feedback during and after the training (Özvarış, 2011; Öztemel, 2018).

1.

2. FACTORS AFFECTING HEALTH EDUCATION

When we look at the masses to be given health education, it is seen that many factors affect education. If the trainer considers these factors, more success can be achieved with the education given. These factors are as follows; (Özvarış, 2011; Tekbaş *et al*., 2005; Kıssal, 2021).

Intrinsic Factors: It develops through physiological and psychological changes that continue from birth to death. In parallel with physiological changes and body development, complex psychological events such as thinking and feeling also develop. Towards the end of life, this development results in decline in function. In order to develop any function and gain knowledge and skills, the individual must have matured to a certain level and gained some experiences, knowledge and skills in this field. This is called physiological and psychological readiness. A person cannot fully acquire a skill for which he/she is not physiologically and psychologically ready, no matter how hard he/she tries. In addition to intelligence, creativity, attention, perception, forgetting and other mental characteristics of individuals, psychological states, and behaviours such as interest, need, interest, commitment, excitement, fear, anxiety, and inhibition are factors

affecting the education process (Özvarış, 2011; Öztemel, 2018; Tekbaş *et al*., 2005; Kıssal, 2021).

External Factors: Human being is an entity that needs to be handled by its environment. The physical, social and cultural environment that affects human behaviour must be considered, which constitutes external factors. The most important external factors are cultural factors. The personality of a human being in a particular environment takes shape according to the characteristics, traditions and customs of the society in which he/she lives. While maturing in terms of biological aspects, humans also adopt the value judgments and behaviours of the society in which they live. People adopt the culture of the society in which they interact socially. This factor also affects health education as it affects the health behaviours of individuals (Özvarış, 2011; Öztemel, 2018; Tekbaş *et al*., 2005; Kıssal, 2021).

6. HEALTH EDUCATION METHODS

There are various methods that trainers can use when providing health education. It is incorrect to say that there is a method that is always successful for everyone or in every situation. As in every planning stage, the trainees' characteristics and educational status should always be considered. The most commonly used methods in health education are individual education, group education, community education, and regional and local health education (Özvarış, 2011; Öztemel, 2018; Tekbaş et al., 2005; Kıssal, 2021).

Individual Education Methods: It is an effective but time-consuming and costly method of health education. It is used when the individual comes together with health professionals when the individual applies to social institutions and in the education of community leaders and people who have shown resistance to education (Özvarış, 2011; Öztemel, 2018; Tekbaş et al., 2005).

Creating an appropriate environment to protect the individual's privacy while providing health education is essential. Home visits are the most suitable environment for this method. Home visits ensure positive communication between the trainer and the individuals, problems can be seen on-site and developments in practices can be easily monitored. Confidence should be given in the first meeting, and opportunities should be prepared for subsequent visits. Methods such as lecturing, answering questions, demonstrating, and sharing responsibility can be used. The methods applied should be repeated several times for what is taught to be permanent. Counselling is also one of the most frequently used approaches to inform and help individuals and families. People who need help, who need sup-

port and encouragement, and who need to gain confidence in solving their problems on their own are interviewed (Özvarış, 2011; Öztemel, 2018; Tekbaş et al., 2005; Kıssal, 2021).

Group Education Methods: Group training is preferred when individual training cannot be given to everyone. Although group training results are lower than personal training, this method is more practical and comprehensive. Groups are small communities that come together for a purpose and are tried to be formed homogeneously in terms of their characteristics. Groups are divided into two ready and unready groups (Öztemel, 2018; Tekbaş et al., 2005; Kıssal, 2021).

- **Ready groups:** Classrooms in schools, workers in factories
- **Groups that are not ready:** Groups of 6-25 people brought together for the training to be conducted

Lecture, discussion, role-play, brainstorming, and question-and-answer methods can be used in group training. In addition, group training provides support and encouragement, helps to share experiences and skills, and working in a group makes the workforce efficient by pooling the resources of all members (Özvarış, 2011; Öztemel, 2018; Tekbaş et al., 2005; Kıssal, 2021).

Methods of Community Education: Community education aims to bring about social change. The change desired to be brought about by health education in society is necessarily based on a health need. What is essential is the level of the need and how the organisation will meet the requirements. These needs can be at national, regional, local and mass levels. At whatever level the need is, social change should be at that level. In determining needs, knowing society and revealing social behaviours is essential. To assess health needs in community education, it is necessary to know the characteristics of the society and to select social behaviours (Özvarış, 2011; Öztemel, 2018; Tekbaş et al., 2005; Kıssal, 2021).

Community education to meet nationally planned health needs is considered part of the service. However, independent studies are necessary for regional, local or mass community education studies. The level of community education in health education depends on the magnitude of the health problem and its impact on the community. Social data (such as maternal mortality rate and infant mortality rate) can be used to determine this (Özvarış, 2011; Öztemel, 2018; Tekbaş et al., 2005; Kıssal, 2021).

Health Education Methods at Regional and Local Level; Health institutions and employees should plan and implement health education for the community at the regional, local or mass level, taking into account the characteristics and needs of the community in which they work within the framework of general

principles. There are some questions to be asked about this. These are (Özvarış, 2011; Öztemel, 2018; Tekbaş et al., 2005; Kıssal, 2021):

- What is the health status of society?
- What are the reasons for this level of health?
- What are the Ministry of Health and the community doing to improve health?
- What more can be done? What are the recommendations and expected results?
- Which criteria are necessary to evaluate the interventions and health status?

The evaluation of society in terms of health education is a dynamic process that needs to be carried out every day. How these changes affect society, which is constantly changing, should be monitored. In community education, a preliminary assessment should be made, and information on social characteristics, diseases, deaths, beliefs, existing health services, and their utilisation should be obtained (Özvarış, 2011; Öztemel, 2018).

What individuals in the community think about health problems and solutions can be learned. Home visits are an essential setting for collecting information. During home visits, the health professional can talk to the family and observe the family's life (Tekbaş et al., 2005; Kıssal, 2021).

Characteristics that need to be known and acted upon for the education of a community at the local level (Kıssal, 2021):

- Population characteristics of society
- General information about the community, such as physical and social conditions
- Persons and organisations that can participate in community education
- Service opportunities available for the community in the immediate and distant environment
- The utilisation of health services
- Community participation in health services
- Health and disease status
- Risk status and health-related behaviours

After obtaining this information, social support for community education should be provided. Community support is the use of community resources (such as teachers, television, radio, newspapers, brochures, magazines, municipalities, and public and private institutions) to bring about the behavioural change we aim to achieve in solving the problem (Özvarış, 2011; Öztemel, 2018; Tekbaş et al., 2005; Kıssal, 2021).

7. HEALTH EDUCATION ENVIRONMENT

It is known that the physical environment, interpersonal relationships, and organisational environment are effective in people's learning process. For this reason, physical, individual, and corporate environments should be considered when creating a health education environment (Kıssal, 2021).

Physical Environment: This includes temperature, room temperature, light, sound system, chairs, and display devices. The physical space's size and appearance affect the learning quality, with bright colours tending to create a cheerful and optimistic mood and dark and dull colours the opposite. It should be oval, round, or U-shaped in group work to encourage interaction between learners (Öztemel, 2018).

Interpersonal Relationships: Reinforcement of positive behaviours is significant, especially in motivation and the realisation or maintenance of learning. In an environment where self-improvement is acknowledged and even rewarded, adults will be more motivated to participate in learning activities and more likely to practice the new behaviour. In an educational environment, a clear and precise definition of goals, a careful explanation of expectations and possibilities, an environment open to questioning and exploration, and objective feedback are essential to creating psychological regularity (Öztemel, 2018).

Organisational Environment: In the development of human resources, the management philosophy, policy, organisational structure, financing policies, and reward system of the organisation organising the training affect the self-development, motivation and thus learning. Especially in adult education, the learning environment is the most crucial element. Suppose the learning environment is not conducive to learning. In that case, the organisation does not value people and does not reflect the understanding that development is the most productive motivation; all process elements are jeopardised. Therefore, training with an organisational structure that supports training, positive interpersonal relationships, and a suitable physical environment will achieve tremendous success (Öztemel, 2018; Kıssal, 2021).

8. MATERIALS USED IN HEALTH EDUCATION

Educational tools and materials provide essential benefits by facilitating the learning process. In the education of an abstract concept, instruments that will replace it and make it meaningful should be used. This way, the abstract concept becomes concrete, and the education is correctly understood and interpreted. The materials used for such purposes are called educational tools and materials. They are divided into three according to the sense organs they address (Bijayalaskhmi, 2017; Özvarış, 2011).

- *Visual tools and materials:* Posters, brochures, exhibitions, silent movies, pictures, etc.
- *Audio tools and equipment:* CDs, radios, records
- *Audiovisual tools and equipment:* Television, video, audiotape slides, projector, etc.

Audiovisual tools used in health education help to develop relationships between the trainer and the trainee. The more sensory organs it appeals to, the more successful the education is. It increases the effectiveness of education, makes the subject permanent and facilitates the transformation of what is learned into a habit (Özvarış, 2011).

9. PLANNING HEALTH EDUCATION

The health education planning process is as follows;

Identification of Educational Needs; First, data about the individual, family or community are collected. In data collection, the knowledge, attitudes and behaviours to be gained by the target group are taken into consideration. The collected data are evaluated, and the problem or problems are identified. The difference between the current knowledge, attitudes and behaviours of the individual and the desired health behaviours is called the need for health education (Hacıalioğlu, 2020; Bijayalaskhmi, 2017; Özvarış, 2011; Kıssal,2021).

Determination of the Target Group and Objectives: The group whose educational needs are determined is called the target group. Objectives are the knowledge, skills, and behaviours to be gained by the target group (Hacıalioğlu, 2020; Bijayalaskhmi, 2017; Özvarış, 2011; Kıssal, 2021).

Preparation of Health Education Plan: The education level and socioeconomic and cultural status of the society should be considered. Therefore, attention should be paid to the methods used in education and equipment selection. The language to be spoken by the educator should be in a way that individuals can understand. In addition, the place of implementation and the duration of the training should also be specified in the plan (Hacıalioğlu, 2020; Bijayalaskhmi, 2017; Özvarış, 2011; Kıssal, 2021).

Programming the Health Education Plan: The trainer, target group, subject, learning objective, how the topics will be covered, and when and where the training should be included in the program (Bijayalaskhmi, 2017).

Implementation of Health Education Programs: In educational practices, care should be taken to ensure that the programs are carried out to achieve the purpose. At this stage, the program should be supervised and evaluated. Social

and cultural characteristics of the individual and value judgments of the individual should also be taken into consideration in health education practices (Bijayalaskhmi, 2017; Özvarış, 2011; Kıssal, 2021).

Evaluation of Health Education: It is possible to evaluate the success of health education by measuring the extent to which the intended goals have been achieved. What was the individual's knowledge, attitude and skills on any subject before the training? What has been achieved after the training? To what extent has the knowledge gap been eliminated? Has attitude change been achieved? Have skills been acquired? Evaluation can be made with questions such as (Hacıalioğlu, 2020; Bijayalaskhmi, 2017).

10. CONCLUSION

Education is a dynamic process. In summary, data collection, planning, teaching, and evaluation stages follow each other in a cycle. The current health status of the individual/patient, risk factors, willingness to solve problems, and change behaviour should be evaluated together with the patient/individual or family (Özvarış, 2011; Grad, 2002).

The patient's level of understanding should be assessed when preparing the education plan. Sometimes family members should also participate in the education. The necessary information for patient/health education planning can be obtained quickly with the appropriate history-taking method or previously prepared forms (Hacıalioğlu, 2020).

The critical point is to determine to what extent the individual/patient can comply with the recommendations. It is also necessary to have information about possible obstacles or support points such as family, social environment, profession, income, working hours, etc., in the individual's decision whether to perform the behaviour (Kıssal, 2021).

11. REFERENCES

3. Bijayalaskhmi, D. (2017). A Comprehensive Textbook of Community Health Nursing (1st ed.). New Delhi: Jaypee Brothers Medical Publishers.
4. Grad, F. P. (2002). The Preamble of the Constitution of the World Health Organization. Bulletin of the World Health Organization, 80 (12), 981–984.
5. Hacıalioğlu, N. (2020). Sağlık Eğitimi. İçinde B. Erci (Ed.) Halk Sağlığı Hemşireliği, (3. Baskı, s. 180-200). İstanbul: Nobel Tıp Kitabevleri.
6. Hacıalioğlu, N. (2019). Hemşirelikte Öğretim Öğrenme ve Eğitim (4. Baskı). İstanbul: Nobel Tıp Kitabevleri.
7. Kıssal, A. (2021). Sağlık Eğitimi. İçinde İ. Erefe (Ed.) Halk Sağlığı Hemşireliği, (s. 1071-1090). Antalya: Çukurova Nobel Tıp Kitabevi.
8. Öztemel, E. (2018). Eğitimde Yeni Yönelimlerin Değerlendirilmesi ve Eğitim 4.0. Üniversite Araştırmaları Dergisi, 1(1), 25-30.
9. Özvarış, Ş.B. (2011). Sağlığı Geliştirme ve Sağlık Eğitimi (1. Baskı). Ankara: Hacettepe Üniversitesi Yayınları.
10. Smith, B.J., Tang, K.C., Nutbeam, D. (2006). WHO Health Promotion Glossary: new terms. Health Promotion International, 21(4), 340–345.
11. Tekbaş, Ö.F., Ceylan, S., Oğur, R., Göçgeldi, E. (2005). Sağlık Eğitiminde Kullanılan Materyaller ve Etkin Kullanımı. Ankara: Genel Kurmay Lojman Başkanlığı Sağlık Şube Müdürlüğü.
12. World Health Organization (WHO) (1978). Primary health care: report of the International Conference on Primary Health Care, Alma-Ata, USSR, 6-12 September 1978 / jointly sponsored by the World Health Organization and the United Nations Children's Fund. In. Geneva: World Health Organization.

Anti-Cancer Effect Mechanisms of Chalcone Derivatives

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1. Introduction

Cancer is the general name given to a group of diseases caused by the uncontrolled division and proliferation of cells in an organ or tissue. Cancer is named according to the tissue in which it occurs or the type of cell from which it originates. It is known that there are more than 100 types of cancer (1). According to the GLOBOCAN 2020 database, part of the International Center for Cancer Research (IARC), there were 19.3 million new cancer cases and 10.0 million cancer deaths in 2020. In 2020, the most frequently diagnosed cancer was lung in men (25.8%), followed by prostate (14.6%) and colorectal cancer (9%). In women, breast cancer (23.9%) is the leading cause of death, followed by thyroid (10.9%) and colorectal cancer (9.1%) (2,3). According to studies conducted by IARC, 21.8 million people are expected to be diagnosed with cancer in 2030, and it is estimated that 13.2 million people will die from cancer in the world in 2030 (1).

Cancer cells attract attention with very different characteristics compared to healthy cells. These features are; The receptors on the cell surface are more sensitive to signals and receive signals more frequently, they have their own signal systems that cause them to multiply uncontrollably, they do not stop dividing after contact with neighboring cells, and they continue to grow and multiply. In addition, while normal cells continue their lives using all types of nutrients, cancer cells can only use glucose from glycolysis. They take glucose from the blood about 100 times more than in healthy cells, and by producing lactate, they maintain the continuity of their energy metabolism. Cancer cells can form new vessels by affecting the stroma around them to get the nutrients and oxygen they need, and they can multiply indefinitely by fixing telomere regions at the ends of chromosomes or by providing continuous telomerase activity. At the same time, cancer cells can enter the circulatory system and settle in distant tissues and organs and spread the cancer to different organs and tissues of the body. Another feature of cancer cells is their ability to escape from controlled cell death, that is, apoptosis (4). It is known that cancer cells are not genetically and epigenetically stable, and their cell profiles vary phenotypically (5). Hanahan (2022) added four new features to the ten basic features of cancer cells in his latest review. These are non-mutational epigenetic reprogramming, polymorphic microbiomes, senescent cells, and decoding of phenotypic plasticity (Fig. 1), (6).

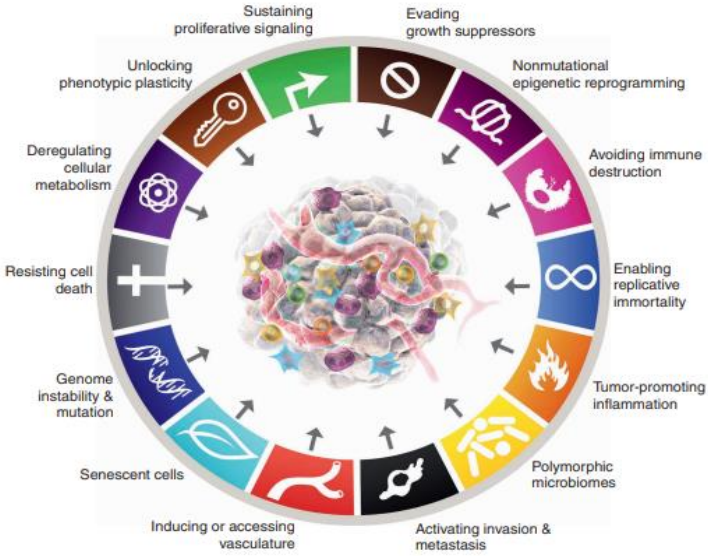

Figure 1. Hallmarks of Cancer-New Additions (Hanahan 2022), (6).

There are many chemotherapeutic drugs developed for molecular targets in cancer treatment. However, multi-drug resistance (MDR) and the side effects of drugs constitute the most important obstacles in the cancer treatment process. For this reason, in addition to scientific treatments, the use of herbal or other natural products has become increasingly important in cancer treatment in recent years (7,8). Vegetables and fruits, which are among our daily nutritional needs, are extremely important in the prevention of balanced cell proliferation and carcinogenesis with the flavonoid components they contain (9). Belonging to the flavonoid family, chalcones are intermediates in the biosynthesis of flavonoids and exhibit structural heterogeneity, and have the potential to act on various drug targets and be potent anti-cancer agents. However, due to its biological activities such as anti-inflammatory (10), anti-diabetic (11), anti-oxidant (12), anti-microbial (13), anti-leishmanial (14) and anti-malarial (15). are important phytochemicals (16). Some chalcone compounds can affect important molecular reactions and inhibit carcinogenesis (17). Chalcones have a 1,3-diaryl-2-propen-1-one chemical skeleton that can be modified accordingly so that the molecules can target different biological activities (Fig. 2), (18).

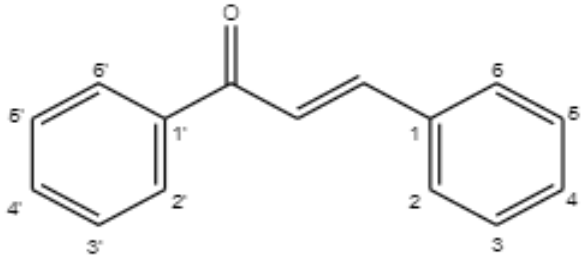

Figure 2. Chalcone Structure (Taskin 2016), (18)

In addition, they exhibit a broad spectrum of drug design that is more biologically effective with the addition of various functional groups (aryls, halogens, hydroxyls, carboxyl, phenyl, etc.) that enable chalcone skeletons to bind with different molecular targets and interact with other molecules as compounds (19). Therefore, chalcones are important plant-derived compounds for the development of new anti-cancer agents. In addition, the combination of chalcone moiety with other anti-cancer agents produces hybrids with the potential to overcome drug resistance and improve therapeutic selectivity, making chalcones a promising strategy for developing new anti-cancer agents. In this context, researchers have intensified their studies on different molecular mechanisms to synthesize new and more effective therapeutic anti-tumor drugs by utilizing the knowledge about the continued use of traditional medicinal plants and natural products (20-22).

2. Effect Mechanisms of Chalcones

Many scientific studies have been conducted to reveal the mechanism of action of chalcone compounds. The extensive biological activity spectrum of chalcones demonstrates effective therapeutic potentials in targeted therapy, leading to positive advances in clinical development. Therefore, understanding the molecular mechanisms of chalcones and their direct targets will be crucial for future development of clinically useful chalcone compounds.

2.1. In-Vitro and In-Vivo Effects of Chalcones on Cell Viability

Cell viability tests are methods used to measure the cytotoxic effect of anti-cancer agents with drug potential applied in cell culture for cancer research (23). By using these methods, anti-cancer activities of natural or synthetic chalcone derivatives have been determined so far. For example; Anti-tumor activities of newly synthesized ligustrazin-chalcone complexes were evaluated in-vitro and in-

vivo. Most of these chalcone complexes have been shown in-vitro to kill approximately 50% of cells at very low concentrations and to exhibit significant cytotoxicity against breast (MCF-7, MDA-MB-231), lung (A549) and liver (HepG2) cancer cell lines. In addition, another in-vivo study reported that chalcones caused a remarkable reduction in tumor growth in a triple-negative breast cancer model (24). In another study, the anti-proliferative activity of Imidazole-chalcone was evaluated in certain human cancer cell lines, including human lung (A549), breast (MCF-7) and liver (HepG2). It was determined that the imidazole-chalcone derivative showed higher cytotoxicity on A549 cancer cells compared to the other two cell lines (25). Hussaini et al. (2016), in their study on 1, 2, 3-triazole-linked pyrazoline chalcone derivatives, stated that pyrazoline and chalcone derivatives have high cytotoxic effects on human HepG2 and prostate (DU145) cancer cell lines (26). Another chalcone derivative, 3, 4, 4, 5-tetramethoxy chalcone, was found to be an effective anti-proliferative agent on various cancer cells in a study. This compound was chosen as the main chalcone and a series of five chalcone-polyamine conjugates were obtained using 4-bromopropyloxy-3',4',5'-trimethoxy chalcone as the key intermediate. The core and polyamine tails of this obtained chalcone are fused with an amine bond. These conjugates were found to have a pronounced anti-proliferative effect against in-vitro colon cancer (HT-29, HCT-116) and prostate cancer (PC-3, DU-145) cell lines (27). Finally, new series of benzofuran chalcone derivatives, 1-(7-ethoxy-1-benzofuran-2-yl) (3a-j) were synthesized by Coşkun et al. (2017). The anti-growth effect of these compounds was tested by SRB and ATP cell viability assays in MCF-7, A549 and PC-3 cell lines. The results showed that chalcone derivatives have cytotoxic effects on cancer cells, and especially chalcone derivative 3a has strong anti-cancer activity (28).

2.2. Effects of Chalcones on the Cell Cycle

Stages of the cell cycle; It is regulated together with proteins such as growth factors, cytokines, oncogenes, cyclins, CDK (Sklin Dependent Kinase) and when DNA damage occurs in any of these stages, the cycle is stopped at the G-2 stage without entering the M stage. In the G-1 stage, if the detected DNA damage is moderate, p21 protein is synthesized by the tumor suppressor gene (p53). By inhibiting the cyclin CDK complex, the cycle is stopped or suspended in the G-1 or G-2 phase (29, 30). In a study, it was seen that Ligustrazin-chalcone complexes affect cell viability in MDA-MB-231 cells in a concentration-dependent manner, inhibit the growth cycle regulation of cells, and block the cell growth cycle in the G0/G1 phase (31). Oskuee et al. (2021) performed cell cycle analysis after testing the anti-proliferative activity of imidazole-chalcone on A549 cells. Flow cytometry analysis of cancer cells showed that these compounds induce cell cycle arrest in the

G2/M phase at low concentrations and increase the number of apoptotic cells at higher concentrations (32). In another study, it was reported that trans-chalcone administration caused mitochondrial membrane damage on hepatocellular carcinoma (HCC) cells, halted the cell cycle G0/G1 phase, and induced cell death (33). The effects of newly synthesized ciprofloxacin chalcone [7-(4-(N-substituted carbamoyl methyl) piperazine-1 yl)] on proliferation, migration and metastasis in MCF-7 and MDA-MB-231 cells were investigated. The new ciprofloxacin chalcone initiated apoptosis in MCF-7 and MDA-MB-231 cells in the G2/M and S phase, respectively, and caused cell cycle arrest (34). In studies focused on the effects of 3, 4, 4, 5-tetramethoxy chalcone and 4-bromopropyloxy-3',4',5'-trimethoxy chalcone, a different chalcone derivative, on cell cycle distribution, these compounds have been shown to induce cell cycle cycles in the G1 and G2 phases, respectively. It has been shown that it can inhibit the proliferation of human colon and prostate cancer cells by blocking it. The information in the literature emphasizes that chalcone derivatives activate the induction of apoptosis by blocking the cell cycle in G1 and G2 phases (27).

2.3. Effects of chalcones on p53 Induction

In addition to mutations in the p53 gene found in approximately 50% of human cancers, overexpression of MDM2 and MDMX is one of the mechanisms leading to suppression of p53 activation (35). MDM2 is known to be the main negative physiological regulator of p53 expression by stimulating ubiquitin-proteasome degradation of p53 and keeping its expression at a low level (36,37). In cells exposed to genotoxic stress, MDM2 catalyzes the mono-ubiquitinization of p53, causing its transfer from the nucleus to the cytosol. Another protein required for inhibition of p53 function is MDMX, a homologue of the MDM2 gene, which is also overexpressed in many tumor types. Therefore, disruption of the three protein complexes (p53/MDMX/MDM2) offers another option for the pharmacological treatment of p53-induced tumors (38). In one study, Butein, a natural chalcone derivative, was shown to have an anti-proliferative effect by reducing the levels of phospho MDM2 and other key proteins involved in cell proliferation. p53-dependent apoptosis has been detected in chronic myeloid leukemia cells treated with butein. This effect has been associated with disruption of MDM2. Therefore, butein blocks the interaction between MDM2 and p53, resulting in suppression of MDM2-mediated p53 ubiquitination. A similar effect on the p53 pathway, namely increased p53 expression and decreased MDM2 expression, has also been demonstrated in other native chalcones (39, 40). Eisa et al. (2021), found that Ciprofloxacin 3, 4, 5 tri-methoxy chalcone caused a concentration- and time-dependent decrease in the viability of HepG2 and MCF7 cells. They also proved to

result in significantly higher p53 and protein expression levels. However, it was reported that significantly lower COX2 mRNA and protein expression levels were also observed. Based on these data, a clear anti-proliferative and p53-induced apoptosis-inducing effect of ciprofloxacin 3, 4, 5 tri-methoxy chalcone is noticed in both cell lines (41).

2.4. Effects of Chalcones on Tubulin Inhibition

Microtubules play an important role in the cell mitosis process and form a spindle in the mitotic prophase of the cell. As a result of this situation, it pulls the chromosomes to the extreme poles of the cell, allowing it to divide into two equal cells to complete the mitosis process (42,43). Tubulin inhibitors suppress cell proliferation by inhibiting microtubule dynamics and disrupting microtubule homeostasis. Thus, a cell cycle arrest is induced in the G2/M phase and the mitotic process is interfered with (44). In a study, it was found that various chalcone derivatives can bind to microtubule proteins and disrupt the dynamic balance of microtubules, inhibit the proliferation of tumor cells, and show anti-tumor effects (45). In other studies cited as examples, the A or B ring of chalcone was found to be naphthalene, benzopyran, benzofuran, indole, benzothiazole, benzoxazole, quinoline, etc. The heterocycle substitution has been reported to have very high cytotoxicity and inhibitory effects against tubulin polymerization (46). Chalcone has an α,β-unsaturated ketone structure with a single rotatable bond, but the α-position at the carbonyl group facilitates the compound to maintain its trans configuration and better bind tubulin to the colchicine binding site. Therefore, incorporation of thiophene, thiazole, imidazole, and benzothiophene into the alkenone system similarly forces the double bonds in alkenones to maintain a trans conformation, which increases the compounds' affinity for tubulin (47,48). In general, it is thought that the inhibitory effects of chalcone derivatives against tubulin polymerization and tumor cells can be increased by structurally modifying them (49). Liu et al. (2021) found that six types of chalcones exhibit anti-proliferative activity against different acute myeloid leukemia (AML) cell lines. Based on the results of immunofluorescence staining, tubulin polymerization test, it was discovered that 4′-O-Methylbroussochalcone B is a novel colchicine domain tubulin polymerization inhibitor. As a tubulin polymerization inhibitor, 4′-O-Methylbroussochalcone B has been shown to inhibit the proliferation and migration of AML cells via the MAPK and Wnt/β-Catenin pathways. The fact that 4′-O-Methylbroussochalcone B becomes a new drug to treat AML is considered promising by Liu et al. (49).

2.5. Effects of Chalcones on Endoplasmic Reticulum Stress

Hydrogen Peroxide (H2O2), derived from the Endoplasmic Reticulum (ER), originates from NOX4 and causes ER stress (50). Therefore, it has been reported that IRE1α sulfenylation is activated by NOX-ER and the mitochondrial ROS axis associated with cell death (51). Considering cancer features that require high protein synthesis for cell proliferation, protein dysfunction based on protein redox imbalance and eventual cell death may be potential anti-cancer therapeutic mechanisms. Therefore, an effort was made to elucidate the molecular mechanism behind the anti-tumor activity of chalcone and its derivatives through NOX4-IRE1a sulfonation. Here, chalcone has been shown to trigger ER stress-induced apoptosis through sulfonation of IRE1α by ER-localized NOX4. In this study, chalcone, which is an important structure of isoflavonoids, was applied to a cancer model, where ER stress and ROS accumulation are highly increased via NOX4 and lead to cell death. Chalcone-induced ROS production enhanced the classical ER stress response, particularly from H2O2 to NOX4. In particular, IRE1α sulfonation is greatly increased with the RIDD phenomenon. Furthermore, miR-23b has been identified as a substrate for IRE1α RNase activity, which appears to cause degradation of miR-23b in the chalcone-treated condition. Reducing miR-23b expression induced NOX4 expression and thus resulted in ROS accumulation. It is suggested that IRE1α sulfonation and NOX4-related redox defect is a novel mechanism to explain the anti-cancer effect caused by chalcone and its structural analogue. With these findings, chalcone and its derivatives are suggested as a precursor anti-cancer compound acting along the NOX4-IRE1α-RIDD-miR-23b axis, which plays a promising role in the anti-cancer mechanism (52). In addition, Nedungadi et al. (2021) showed for the first time that 2′-hydroxy-retrochalcone, one of the six synthetic chalcone derivatives they tested, was effective in inducing extensive cytoplasmic vacuolation-mediated death, called paraptosis, in malignant breast and cervical cancer cells. Immunofluorescence with the ER marker protein calreticulin indicates that the formed cytoplasmic vacuoles originate from the ER. This ER expansion is due to ER stress as evidenced by the increase in polyubiquitinated proteins, BIP and CHOP. Cell death by 2′-hydroxy-retrochalcone was also triggered by the collapse of mitochondrial membrane potential and depletion of ATP. Therefore, the data suggest that 2′-hydroxy-retrochalcone can effectively kill cancer cells through an alternative death pathway, paraptosis, and may be a potential precursor molecule for anti-cancer therapy (53).

2.6. Effects of chalcones on the NF-kB Signaling Pathway

The Nuclear Factor Kappa B (NF-Kb) pathway plays an important role in regulating the expression of different proteins involved in immune and

inflammatory responses. In addition, many genes related to apoptosis and cell survival are also under the control of the NF-κB pathway. Constitutive activation of this pathway has been found to promote cell proliferation and survival of cancer cells, protection against tumor invasion or apoptosis (54). Bordoloi et al. (2019) demonstrated Butein isolated from the plant Oxicodendron vernicifluum as an inhibitor of the phosphorylated NF-kB form (p-p65) in human oral squamous cancer cells. They also showed that the buteinanti-apoptotic factor survivin, a different chalcone derivative, downregulates several NF-kB-regulated proteins, such as COX-2 and the adhesion molecule MMP-9 (55). In another study, isoliquiritigenin, a different natural chalcone, was mentioned several times as a potent apoptosis inducer or angiogenesis inhibitor. One of the possible mechanisms of its action is modulation of the NF-κB pathway. Recently, the anti-proliferative effect of isoliquiritigenin in human hepatocellular carcinoma cells has been studied. Isoliquiritigen appears to regulate NF-κB signaling through potentiation of the inhibitory effect of IκB (a natural inhibitor of NF-κB) as well as reduction of NF-κB expression. These effects are associated with mitochondrial apoptosis, cell cycle arrest and ROS production (56). In support of the previous study, two new chalcone derivatives, dihydrotriazine-chalcone compounds, have been evaluated for their anti-invasive and anti-inflammatory abilities, and it has been reported that these compounds, at non-toxic concentrations, suppress the in-vitro migration of MDA-MB-231 cells. Mechanistically, inhibition of cancer cell invasion and inflammation by the compounds is known to be mediated by suppression of the NF-κB signaling pathway, which is confirmed by the reported mechanism of action of chalcones. With their ability to target multiple biological mediators involved in multistage carcinogenesis and with bioactivities stronger than those of the main chalcone scaffold, further analysis of dihydrotriazine-chalcone compounds for use in the pharmacological intervention of aggressive cancers may be promising (57).

2.7. Effects of Chalcones on Angiogenesis

The formation of new blood vessels is dependent on many anti-angiogenic factors and their associated receptors. The main proangiogenic factors include vascular endothelial growth factor (VEGF), whose expression is regulated by hypoxia. In addition, these factors stimulate endothelial cell proliferation and migration, vascular permeability and expression of MMPs. Other important factors are fibroblast growth factor 2 (FGF-2), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), angiopoietins, ephrins, apelin and chemokines (58). In one study, xanthohumol, a chalcone investigated as a potential anti-angiogenic compound, was shown to inhibit the secretion of VEGF and Interleukin-8 (IL-8) in pancreatic cancer cells due to suppression of NF-κB activity.

In addition, co-cultivation of pancreatic cancer cells with human umbilical cord endothelial cells (HUVEC) increased tube formation in HUVECs. Xanthohumol appeared to significantly inhibit the formation of capillary-like tubes by HUVECs, even at nanomolar concentrations (59). In a study conducted in 2019, it was reported that the chalcone derivative Izoliquiritigenin inhibited angiogenesis stimulators including VEGF, FGF-2 and TGF-β (60). In addition, Rizeq et al. (2021) reported that the new nitrogen chalcone-based compounds they produced in the laboratory had specific effects on triple negative breast cancer cells. Therefore, the effects of these compounds (DK-13 and DK-14) on two HER2-positive breast cancer cells, SKBR3 and ZR75, were investigated. The data show that these two chalcone compounds cause a significant reduction in cell invasion ability of SKBR3 and ZR75 cancer cells. In parallel, it was noticed that DK-13 and DK-14 inhibited colony formation of both cell lines compared to their matched controls, while these two compounds inhibited angiogenesis in the chorioallantoic membrane model. Molecular pathway analysis of cells exposed to chalcone compounds reveals that these compounds inhibit the expression of both JNK1/2/3 and ERK1/2 major molecular pathways. These findings suggest that chalcone-based DK-13 and DK-14 have effective chemotherapeutic results against HER2-positive breast cancer via the ERK1/2 and JNK1/2/3 signaling pathways (61).

3. Conclusion

Cancer is a complex disease and it is thought that simultaneous modulation of different targets in this disease may be associated with better therapeutic response. In this context, chalcones appear as perspective compounds for the development of a multi-targeted anti-cancer drug. It has been determined that chalcones, which belong to the flavonoid family, have anti-cancer activity as well as targeting different molecular pathways in cancer treatment. Especially in recent years, chalcones have become the focus of studies on potential anti-cancer compounds. Chalcones can play an anti-cancer role through tumor cell anti-proliferation, apoptosis induction, microtubule polymerization, anti-angiogenesis and cell cycle arrest. This property makes chalcones very attractive as basic building blocks for the synthesis of cancer molecule targeting agents. However, despite the significant results obtained in this research area, it seems that further experimental and clinical studies are needed to reveal the mechanism of action of chalcone compounds in cancer in more detail.

4. References

1. Steck SE, Murphy EA. Dietary patterns and cancer risk. Nature Reviews Cancer. 20(2): 125-138, 2020.
2. Parton M, Mitchell D, Ian S. Studies of apoptosis in breast cancer. Clinical Review. 322(7301): 1528-1532, 2001.
3. Adhami M, Haghdoost AA, Sadeghi B, et al. Candidate miRNAs in human breast cancer biomarkers: a systematic review. Breast Cancer. 25(2): 198-205, 2018.
4. Park MT, Lee SJ. Cell cycle and cancer. BMB Reports. 36(1): 60-65, 2003.
5. Baykara O. Current approaches in cancer treatment. Balikesir Journal of Health Sciences. 5(3): 154-165, 2016.
6. Hanahan D. Hallmarks of cancer: New dimensions. Cancer Discovery. 12(1): 31-46, 2022.
7. Sezgin C. Example applications in the treatment of cancer with herbs. Herbal Therapy Symposium. 172, 2010.
8. Hussain S, Singh A, Nazir SU, et al. Cancer drug resistance: A fleetto conquer. Journal of Cellular Biochemistry. 120(9): 14213-14225, 2019.
9. Yao H, Xu W, Shi X, Zhang Z. Dietary flavonoids as cancer prevention agents. Journal of Environmental Scienceand Health. 29(1): 1-31, 2011.
10. Mahapatra DK, Bharti SK, Asati V. Chalcone derivatives: Anti-inflammatory potential and molecular targets perspectives. Current Topics in Medicinal Chemistry. 17(28): 3146-3169, 2017.
11. Rocha S, Ribeiro D, Fernandes E, FreitasM. A Systematic review on anti-diabetic properties of chalcones. Current Medicinal Chemistry. 27(14): 2257-2321, 2020.
12. Lin Y, Zhang M, Lu Q, et al. A novel chalcone derivative exerts anti-inflammatory and anti-oxidant effects after a cute lungin jury. Aging (Albany NY). 11(18): 7805-7816, 2019.
13. Henry EJ, Bird SJ, Gowland P, et al. Ferrocenyl chalcone derivatives as possible anti-microbial agents. The Journal Of Antibiotics. 73(5): 299-308, 2020.
14. de Mello MVP, Abrahim-Vieira BA, Domingos TFS, et al. A comprehensive review of chalcone derivatives as anti-leishmanial agents. European Journal of Medicinal Chemistry. 150: 920-929, 2018.
15. Cheng P, Yang L, Huang X, et al. Chalcone hybrids and their anti-malarial activity. Archiv der Pharmazie. 353(4): 1900350, 2020.

16. Gao F, Huang G, Xiao J. Chalcone hybrids as potential anti-cancer agents: Current development, mechanism of action, and structure-activity relationship. Medicinal Research Reviews. 40(5): 2049-2084, 2020.
17. Jandial DD, Blair CA, Zhang S, et al. Molecular targeted approaches to cancer therapy and prevention using chalcones. Current Cancer Drug Targets. 14(2): 181-200, 2014.
18. Taskin M. Synthesis of benzofuran substituted chalcones. Master's thesis, Adıyaman University, Institute of Science and Technology, Adiyaman, 2016.
19. Gomes MN, Muratov EN, Pereira M, et al. Chalcone derivatives: Promising starting points for drug design. Molecules. 22(8): 1210, 2017.
20. Aiello P, Sharghi M, Mansourkhani SM, et al. Medicinal plants in the prevention and treatment of colon cancer. Oxidative Medicine and Cellular Longevity. 2019: 2075614, 2019.
21. Imperatore C, Della Sala G, Casertano M, et al. In vitro anti-proliferative evaluation of synthetic meroterpenes ınspired by marine natural products. Marine Drugs. 17(12): 684, 2019.
22. Manzo E. Synthesis of marine natural products and molecules inspired by marine substances. Marine Drugs. 19(4): 208, 2021.
23. Riss TL, Moravec RA, Niles AL, et al. Cell viability assays. Assay Guidance Manual. 2016.
24. Luo Y, Wu W, Zha D, et al. Synthesis and biological evaluation of novel ligustrazine-chalcone derivatives as potential anti-triple negative breast cancer agents. Bioorganic & Medicinal Chemistry Letters. 47: 128230, 2021.
25. Oskuee SR, Mirzaei S, Jafari-Nik MR, et al. Design, synthesis and biological evaluation of novel imidazole-chalcone derivatives as potential anti-cancer agents and tubulin polymerization inhibitors. Bioorganic Chemistry. 112: 1-30, 2021.
26. Hussaini SM, Yedla P, Babu KS, et al. Synthesis and biological evaluation of 1, 2, 3-triazole tethered pyrazoline and chalcone derivatives. Chemical Biology & Drug Design. 88(1): 97-109, 2016.
27. Rioux B, Pinon A, Gamond A, et al. Synthesis and biological evaluation of chalcone-polyamine conjugates as novel vectorized agents in colorectal and prostate cancer chemotherapy. European Journal of Medicinal Chemistry. 222: 113586, 2021.
28. Coskun D, Erkisa M, Ulukaya E, et al. Novel 1-(7-ethoxy-1-benzofuran-2-yl) substituted chalcone derivatives: synthesis, characterization and

anti-cancer activity. European Journal of Medicinal Chemistry. 136: 212-222, 2017.
29. Pediconi N, Ianari A, Costanzo A, et al. Differential regulation of E2F1 apoptotic target genes in response to DNA damage. Nature Cell Biology. 5(6): 552-558, 2003.
30. Kopnin BP. Targets of oncogenes and tumor suppressors: key for understanding basic mechanisms of carcinogenesis. Biochemistry. 65(1): 2-27, 2000.
31. Luo Y, Wu W, Zha D, et al. Synthesis and biological evaluation of novel ligustrazine-chalcone derivatives as potential anti-triple negative breast cancer agents. Bioorganic & Medicinal Chemistry Letters. 47: 128230, 2021.
32. Oskuee SR, Mirzaei S, Jafari-Nik MR, et al. Design, synthesis and biological evaluation of novel imidazole-chalcone derivatives as potential anti-cancer agents and tubulin polymerization inhibitors. Bioorganic Chemistry. 112: 1-30, 2021.
33. Siqueira ES, Concato VM, Tomiotto-Pellissier F, et al. Trans-chalcone in duces death by autophagy mediated by p53 up-regulation and β-catenin down-regulation on human hepatocellular carcinoma HuH7.5 cell line. Phytomedicine. 80: 1-9, 2021.
34. Alaaeldin R, Abuo-Rahma GEDA, Zhao QL, Fathy M. Modulation of apoptosis and epithelial-Mesenchymal transition E-cadherin/TGF-β/Snail/TWIST pathways by a new ciprofloxacin chalcone in breast cancer cells. Anti-cancer Research. 41(5): 2383-2395, 2021.
35. Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. Nature Reviews Cancer. 9(10): 749-758, 2009.
36. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature. 387(6630): 296-299, 1997.
37. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. Nature. 387(6630): 299-303, 1997.
38. Chao CCK. Mechanisms of p53 degradation. Clinica Chimica Acta. 438: 139-147, 2015.
39. Woo SM, Choi YK, Kim AJ, et al. p53 causes butein-mediated apoptosis of chronic myeloid leukemia cells. Molecular Medicine Reports. 13(2): 1091-1096, 2016.
40. Zhou Y, Wang K, Zhou N, et al. Buteinactivates p53 in hepatocellular carcinoma cells via blocking MDM2-mediated ubiquitination. Onco Targets and Therapy. 11: 2007-2015, 2018.

41. Eisa MA, Fathy M, Abuo-Rahma GEDA, et al. Anti-proliferative and pro-apoptotic activities of synthesized 3, 4, 5 tri-methoxy ciprofloxacin chalcone hybrid, through p53 up-regulation in hepg2 and mcf7 cell lines. Asian Pacific Journal of Cancer Prevention. 22(10): 3393-3404, 2021.
42. Sorger PK, Dobles M, Tournebize R, Hyman AA. Coupling cell division and cell death to microtubule dynamics. Current Opinion in Cell Biology. 9(6): 807-814, 1997.
43. Jordan MA, Wilson L. Microtubules as a target for anti-cancer drugs. Nature Reviews Cancer. 4(4): 253-265, 2004.
44. Risinger AL, Giles FJ, Mooberry SL. Microtubule dynamics as a target in oncology. Cancer Treatment Reviews. 35(3): 255-261, 2009.
45. Canela MD, Noppen S, Bueno O, et al. Antivascular and anti-tumor properties of the tubulin-binding chalcone TUB091. Oncotarget. 8(9): 14325, 2017.
46. Liu W, He M, Li Y, et al. A review on synthetic chalcone derivatives as tubulin polymerisation inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry. 2022; 37(1): 9-38.
47. Peyrot V, Leynadie, D, Sarrazin M, et al. Interaction of tubulin and cellular microtubules with the new anti-tumor drug MDL 27048: a powerful and reversible microtubule inhibitor. Journal of Biological Chemistry. 264(35): 21296-21301, 1984.
48. Peyrot V, Leynadier D, Sarrazin M, et al. Mechanism of binding of the new anti-mitotic drug MDL 27048 to the colchicine site of tubulin: equilibrium studies. Biochemistry. 31(45): 11125-11132, 1992.
49. Liu Z, Wang C, Wang Y, et al. 4'-O-Methylbroussochalcone B as a novel tubulin polymerization inhibitor suppressed the proliferation and migration of acute myeloid leukaemia cells. BMC Cancer. 21(1): 1-13, 2021.
50. Wu RF, Ma Z, Liu Z, Terada LS. Nox4-derived H2O2 mediates endoplasmic reticulum signaling through local Ras activation. Molecular and Cellular Biology. 30(14): 3553-3568, 2010.
51. Hourihan JM, Mazzeo LEM, Fernández-Cárdenas LP, Blackwell TK. Cysteine sulfenylation directs IRE-1 to activate the SKN-1/Nrf2 anti-oxidant response. Molecular Cell. 63(4): 553-566, 2016.
52. Kim HK, Lee HY, Riaz TA, et al. Chalcone suppresses tumor growth through NOX4-IRE1α sulfonation-RIDD-miR-23b axis. Redoxbiology. 40: 101853, 2021.

53. Nedungadi D, Binoy A, Pandurangan N, et al. Proteasomal dysfunction and ER stress triggers 2′-hydroxy-retrochalcone induced paraptosis in cancer cells. Cell Biology International. 45(1): 164-176, 2021.
54. Xia L, Tan S, Zhou Y, et al. Role of the NFκB-signaling pathway in cancer. Onco Targets and Therapy. 11: 2063, 2018.
55. Bordoloi D, Monisha J, Roy NK, et al. An ınvestigation on the therapeutic potential of butein, a tretra hydroxy chalcone against human oral squamous cell carcinoma. Asian Pacific Journal of Cancer Prevention: APJCP. 20(11): 3437-3446, 2019.
56. Wang JR, Luo YH, Piao XJ, et al. Mechanisms underlying isoliquiritigenin-induced apoptosis and cell cycle arrest via ROS-mediated MAPK/STAT3/NF-κB pathways in human hepatocellular carcinoma cells. Drug Development Research. 80(4): 461-470, 2019.
57. Gan FF, Zhang R, Ng HL, et al. Novel dual-targeting anti-proliferative dihydrotriazine-chalcone derivatives display suppression of cancer cell invasion and inflammation by inhibiting the NF-κB signaling pathway. Food and Chemical Toxicology. 116: 238-248, 2018.
58. Mirossay L, Varinska L, Mojzis J. Anti-angiogenic effect of flavonoids and chalcones: an up date. International Journal of Molecular Sciences. 19(1): 27, 2017.
59. Saito K, Matsuo Y, Imafuji H, et al. Xanthohumol inhibits angiogenesis by suppressing nuclear factor-κB activation in pancreatic cancer. Cancer Science. 109(1): 132-140, 2018.
60. Wang C, Chen Y, Wang Y, et al. Inhibition of COX-2, mPGES-1 and CYP4A by isoliquiritigenin blocks the angiogenic Akt signaling in glioma through ceRNA effect of miR-194-5p and lncRNA NEAT1. Journal of Experimental & Clinical Cancer Research. 38(1): 371, 2019.
61. Rizeq B, Gupta I, Kheraldine H, et al. Novel nitrogen-based chalcone analogs provoke substantial apoptosis in HER2-positive human breast cancer cells via JNK and ERK1/ERK2 signaling pathways. International Journal of Molecular Sciences. 22(17): 9621, 2021.

Use of Virtual Reality in Midwifery Education

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Virtual reality; It is derived from the words virtual and reality, which are opposite to each other. According to the Turkish Language Association (TLA), the word virtual, which has no place in reality but is designed in the mind, has meanings such as conjecture, hypothetical, predictive, comes from the Latin virtualis root. Reality, on the other hand, means real, all things that exist, truth, senility, reality, according to the Turkish Language Association.

Virtual Reality (VR) is a state-of-the-art simulation designed to create a lifelike virtual world for users using computer graphics. VR is a technology that creates a visual experience environment with 3D videos that a person in the virtual environment thinks is in the real world. Although computers and tablets are the basis of virtual reality, virtual reality glasses are also used for this purpose. VR glasses show the same image on the phone screen by dividing the screen into two, right and left. However, thanks to the lenses in the glasses, the person wearing the glasses sees it as a single screen as if the two screens came together, and thus she feels herself in the image.

History of Virtual Reality

It is possible to understand these technologies by discovering the milestones that led to the emergence of virtual reality technology and many current computing ideas. Euclid, by making three-dimensional studies in the 19th century, found that the left and right eyes perceive the same image from different perspectives and used the concept of stereo for the first time. In 1838, Charles Wheatstone invented the stereoscope and made history as the first stereo viewer. He invented the machine called Kinematoscope and received a US Patent. With this camera, Coleman created the impression of a movie by using rotating discs to set in motion a series of stereoscopic photographs. The first head-mounted periscope (optical distance observing instrument) was developed by Albert B. Pratt in 1916 and granted a US patent. In 1929, Edward Link developed a simple mechanical flight simulator to train pilots in an enclosed space. The product called View-Master, which is a vision simulator, was developed in 1939 and made it possible to see the films placed inside with the help of light. Ray Bradbury, an American writer, is described as the creator of the concept of virtual reality with his extraordinary story published in 1950. he first electronic digital computer ENIAC was developed at the University of Pennsylvania in 1946 and delivered to the US military. Inspired by the Sinerama (very widescreen cinema format), Morton Heilig developed the Sensorama in 1956. The sensormachine, patented in 1962 and consisting of multiple sensors such as sound, odor, wind, vibration, was the first way to discover the VR System. In 1968, Ivan Sutherland developed the first example of a helmet, called the Head-Mounted Three-

Dimensional Display‖, which inspired the virtual reality glasses used today. This helmet was a heavy, primitive system suspended from the ceiling and worn on the head by the wearers. This system, also called the Sword of Damocles, has been accepted as the first hardware of Virtual Reality. The term cyberspace became popular in William Gibson's 1984 novel Neuromancer. This term is defined as an artificial world and is an abstraction that describes the virtual reality environment where there is no time-space boundary, with the communication between the computer and the user in an internet network. Jaron Lanier first used the term Virtual Reality in 1989. A company called VPL Research was founded by Lanier to create a visual programming language. The company used this work to create DataGlove in 1985 and EyePhones in 1989 under grants from the NASA VIEW laboratory shortly thereafter. In addition, the VPL company equipped these products, produced and offered for sale as the first commercial product of virtual reality. LCD screen In 1991, the Sega VR product with a head-movement sensor and stereo speakers appeared. Philip Rosedale, the founder of Linden Lab, made the first study of 360-degree images in 1999.

In 2007, Google introduced the Street View‖ service, which allows users to navigate the streets with 360-degree panoramic views. Oculus VR, a virtual reality technology company, was founded in 2012 by Palmer Luckey and Brendan Iribe. The company was acquired by Facebook in 2014. Virtual reality glasses such as Card Board, developed by Google and used with smartphones, are important in terms of showing us the point this technology has reached from past to present. The Gear VR, a controllable device with a Samsung License, was presented to users in 2015.

Usage Areas of Virtual Reality

Although it is an expensive technology, it can be used in many areas. Among the areas of use of virtual reality, the world of entertainment and games are in the first place. Following these, it is possible to use it for simulation purposes in education, rehabilitation, treatment, tourism and travel sectors, virtual exercises in the military, and pilot trainings. The use of virtual reality in formal education, especially in medicine, military-aviation, mathematics and science education, can provide many benefits in terms of the quality of education offered and is extremely important. For example, the easy creation of laboratory environments using virtual reality allows costly, potentially dangerous, and complex experiments to be safely and seamlessly performed. In addition, thanks to virtual reality glasses and sensors, it is expected that people will use this application widely in electronic virtual market applications (to have fun surfing) over time. This rapidly developing technology has also started to be used in patient

education. Children with communication and mobility difficulties can realize potential problems that they cannot do in real life and that they may encounter in their daily lives, by using virtual reality environments, by being educated through these environments. Use in the field of health: One of the areas that are rapidly affected by new technology products such as VR is undoubtedly health services. Technologies used while providing health services to individuals provide convenience to health workers such as midwives, nurses and physicians, while also increasing the quality and efficiency of the service. VR technologies, which are widely used in the world, have been approved for clinical use. For example, procedural interventions such as vaccination or blood draw commonly cause pain and anxiety in children. Recent reports have identified VR use as a distraction during these procedures. Göksu (2017), in her study, had children watch videos with virtual reality glasses during the blood collection process and determined that virtual reality glasses were an effective method in reducing pain. In another study, a 4-minute virtual reality video showing the operating room and explaining the perioperative process was shown to reduce preoperative anxiety in children and it was found to be effective in alleviating preoperative anxiety. Similar studies can be found in different fields. For example, it was found that the video watched with virtual reality glasses during breast biopsy, which is a painful procedure, is effective on pain and anxiety. Similarly, in the treatment of burns, in acute procedural pain management, in the treatment of conditions that distort the body image such as obesity and eating disorders, in lower and upper extremity rehabilitation, in pediatric oncology patients, in the treatment of psychological disorders (post-traumatic stress disorder, anxiety disorder, obsession, etc.) and phantom. Used for extremity pain. One of the recent uses of virtual reality is labor pain. Studies in this area have found that virtual reality is an effective non-pharmacological method to reduce pain and anxiety during labor. It is stated that during labor, daydreaming or music has an effect on distraction and relaxation. However, because sometimes dreaming and dreaming can be difficult for women, watching videos with virtual reality glasses is a good alternative. There is also a study showing that it reduces pain during episiotomy repair in the postpartum period. Virtual reality is an ideal method for pain control. Studies have scanned the brain activities of subjects with and without virtual reality glasses using functional magnetic resonance imaging (fMRI), and it has been shown that there is a great increase in pain-related activity in brain regions known to be involved in pain perception in subjects who do not use virtual reality glasses. In addition to pain and other applications, the use of VR for educational purposes is increasing in applications such as medicine and surgical interventions. It is a good method for inexperienced physicians to gain experience by performing surgery

on virtual patients in a virtual reality environment and to reduce vital errors for the patient. VR technologies, which can also be used for virtual surgical interventions, is a technology called teleoperation that will allow the surgeon to perform remote surgical interventions with the help of a robot for minimally invasive applications. It is also possible to talk about virtual cadavers in medical education. Students can dissect virtual cadavers over the internet and do the work they want.

The Role of the Midwife in Virtual Reality Applications

Virtual reality is a technology that continues to grow in popularity in the obstetric field. Increasing adoption of new technologies such as virtual reality in midwifery practice and midwifery education is helping to bring positive changes and potential benefits in midwife-client relationships. Virtual reality technology is being used more and more, in line with the widespread adoption of simulation learning. It has led to the potential use of virtual reality in midwifery education to create innovative and memorable learning opportunities. Simulation appears to be particularly important when practicing skills that are rarely encountered in practice, such as in clinical emergencies. The virtual reality application can allow students to practice technical skills such as sterile technical and emergency response skills one by one, at their own pace and time. Virtual reality application has a lot to offer to midwives in both education and practice environments. It is very valuable to use virtual reality application in the trainings that midwives will give to their clients.

Referans

1. Aktamış, H., Arıcı, V. A. (2013). Sanal Gerçeklik Programlarının Astronomi Konularının Öğretiminde Kullanılmasının Akademik Başarı ve Kalıcılığına Etkisi. Mersin Üniversitesi Eğitim Fakültesi Dergisi, Cilt 9, Sayı 2, ss.58-70. Erişim 05.10.2022 http://dergipark.gov.tr/download/article-file/160868
2. Alqahtani, A. S., Daghestani, L. F., Ibrahim, L. F. (2017). Environments and System Types of Virtual Reality Technology in STEM: A Survey. (IJACSA) International Journal of Advanced Computer Science and Applications, Vol. 8, No. 6, Erişim 26.10.2022 https://thesai.org/Publications/ViewPaper? Volume=8&Issue=6&Code=I JA CSA&SerialNo=10
3. Arane, K., Behboudi, A., Goldman, R. D. (2017). Virtual reality for pain and anxiety management in children. Child Health Update. Canadian Family Physician VOL 63(12): p.932-934 Erişim 08.10.2022 https://www.ncbi.nlm.nih.gov/pmc/articles/ PMC5729140/pdf/0630932.pdf
4. Aslan, R. ve Erdoğan, S. (2017). 21. Yüzyılda Hekimlik Eğitimi: Sanal Gerçeklik, Artırılmış Gerçeklik, Hologram. Kocatepe Veterinary Journal 10(3): 204-212. DOI:10.5578/kvj.57308. Erişim 04.10.2022 http://dergipark.gov.tr/download/article-file/387229
5. Bayraktar, E., Kaleli, F. (2007). Sanal gerçeklik ve uygulama alanları. Akademik Bilişim, Dumlupınar Üniversitesi, Kütahya. Erişim 08.10.2022 https://www.researchgate.net/publication/237599951_SANAL_GERCEKLIK _VE_ UYGULAMA_ALANLARI
6. Bukola, I. M., Paula, D. (2017) The Effectiveness of Distraction as Procedural Pain Management Technique in Pediatric Oncology Patients: A Metaanalysis and Systematic Review. Journal of Pain and Symptom Management, Vol. 54 No. 4 Erişim 09.10.2022 http://dx.doi.org/10.1016/j.jpainsymman.2017.07.006
7. Chan, E., Foster, S., Sambell, R., Leong, P. (2018). Clinical efficacy of virtual reality for acute procedural pain management: A systematic review and metaanalysis. PLOS ONE, p.1-13 Erişim 09.10.2022 https://doi.org/10.1371/journal.pone.0200987
8. Chirico,A., Lucidi, F., Laurentiis, M., Milanese, C., Napoli, A., Giordano, A. (2015). Virtual Reality in Health System: Beyond Entertainment. A MiniReview on the Efficacy of VR During Cancer Treatment. Journal of Cellular Physiology. 231: 275–287, DOI: 10.1002/jcp.25117Erişim 04.10.2022 https://www.ncbi.nlm.nih.gov /pubmed/26238976
9. Dean S, Halpern J, McAllister M, Lazenby M. (2020). Nursing education, virtual reality and empathy?. Nursing open, 7(6): 2056–9. Erişim 10.11.2022 https://www.researchgate.net/publication/342609925_Nursing_education_virtual_reality_and_empathy

10. Demirci, Ş. (2018). Sağlık hizmetlerinde sanal gerçeklik teknolojileri.İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu Dergisi, ISSN: 2147-7892, Cilt 6, Sayı 1(2018) 35-46. Erişim 08.10.2022 http://dergipark.gov.tr/download/articlefile/473654
11. Durmaz, A., & Kakaşçı, Ç. G. (2022). The Effects of Simulation-Based Training on Midwifery Student Malpractice Trends and Student Perceptions of Care Behaviors: A Randomized Controlled Trial. Adnan Menderes Üniversitesi Sağlık Bilimleri Fakültesi Dergisi, 6(3), 405-418. https://doi.org/10.46237/amusbfd.940905
12. Durmaz, A., Elem, E., Unutkan, A., Keskin, N. (2017). Simülasyonun Normal Doğum Becerilerine ve Öz-Yeterlilik Düzeylerine Etkisi. Journal of Current Researches on Health Sector , vol.7, 41-52.
13. Ferhat, S. (2016). Dijital Dünyanın Gerçekliği, Gerçek Dünyanın Sanallığı Bir Dijital Medya Ürünü Olarak Sanal Gerçeklik. TRT akademi. ISSN 2149-9446 Cilt 01 Sayı 02, Dijital Medya Sayısı. Erişim 07.10.2022 http://dergipark.gov.tr/download/article-file/218567
14. Frey, D. P., Bauer, M.E., Bell, C. L., Low, L.K., Hassett, A. L., Cassidy, R.B., Boyer, K. D. and Sharar, S. R. (2018). Virtual Reality Analgesia in Labor: The VRAIL Pilot Study—A Preliminary Randomized Controlled Trial Suggesting Benefit of Immersive Virtual Reality Analgesia in Unmedicated Laboring Women. Brief Report. International Anesthesia Research Society. DOI: 10.1213/ANE.0000000000003649, Erişim 13.10. 2022. https://www.ncbi.nlm.nih.gov/pubmed/30004931
15. Göksu, F. (2017). Çocuklarda Venöz Kan Alımı Sırasında Kullanılan Sanal Gerçeklik Gözlüğünün Hissedilen Ağrı Üzerine Etkisi. Yüksek lisans Tezi. (Danışman: Ayyıldız, T. K.) Bülent Ecevit Üniversitesi, Sağlık Bilimleri Enstitüsü, Çocuk Sağlığı ve Hemşireliği Hemşireliği Anabilim Dalı, Zonguldak
16. Hoffman, H. G., Richards, T. L., Bills, A. R., Oostrom, T. V., Magula, J., Seibel, E. J. ve ark. (2006). Using fMRI to Study the Neural Correlates of Virtual Reality Analgesia. CNS Spectr.11(1):45-51 Erişim 09.10.2022 https://pdfs.semanticscholar.org/aa90/ed5ded6891d2847c42c00694fb6628344 f50.p df
17. Hoffman, H. G., Richards, T. L., Coda, B., Bills, A. R., Blough, D., Richards, A. L. ve ark. (2004). Modulation of thermal pain-related brain activity with virtual reality: evidence from fMRI. NEUROREPORT, Vol 15, No 8, DOI: 10.1097/01. wnr.0000127826.73576.91 Erişim 23.10.2022 https://www.ncbi.nlm.nih.gov/pubmed/ 15167542
18. Karahan, A. Y. (2016). İnme Rehabilitasyonunda Sanal Gerçeklik. Türkiye Klinikleri J PM&R-Special Topics 9(1):100-6 Erişim 06.10.2022

http://www.turkiyeklinikleri.com/ article/tr-inme-rehabilitasyonunda-sanal gerceklik-73563.html
19. Karaman, D. (2016). Meme Biyopsisi Sırasında Sanal Gerçeklik Uygulamasının Ağrı ve Anksiyete Üzerine Etkisi. Yüksek lisans Tezi. (Danışman: Taşdemir, N.) Bülent Ecevit Üniversitesi, Sağlık Bilimleri Enstitüsü, Hemşirelik Anabilim Dalı, Cerrahi Hastalıkları Hemşireliği Programı, Zonguldak
20. Kayabaşı, Y. (2005). Sanal Gerçeklik ve Eğitim Amaçlı Kullanılması. The Turkish Online Journal of Educational Technology – TOJET, ISSN: 1303-6521 volume 4 Issue 3, s. 151-158 Erişim 26.10.202 http://www.tojet.net/articles/v4i3/4320.pdf
21. Kurbanoğlu, S. S. (1996). Sanal Gerçeklik: Gerçek mi, Değil mi?. Türk Kütüphaneciliği 10, 1(1996): 21-31. Erişim 02.10.2022 http://www.tk.org.tr/index.php/TK/article/view/1012
22. Li, A., Montaño, Z., Chen, V. J., Gold, J. I. (2011). Virtual reality and pain management: current trends and future directions. Pain Manage. 1(2):147–157. ISSN 1758-1869. Erişim 05.10.2022 https://www.ncbi.nlm.nih.gov/pubmed/21779307
23. Malloy, K. M., Milling, L. S. (2010). The Effectiveness of Virtual Reality Distraction for Pain Reduction: A Systematic Review, Clinical Psychology Review 30, p.1011- 1018. Erişim: 09.10.2022. https://www.ncbi.nlm.nih.gov/pubmed/20691523
24. Öztürk E, Sondaş A. (2020). Sanal sağlık: sağlıkta sanal gerçekliğe genel bakış. Kocaeli Üniversitesi Fen Bilimleri Dergisi, 3(2):164-9. Erişim 04.10.2022 https://dergipark.org.tr/tr/download/article-file/1217212
25. Pazar, B., İyigün, E. (2016). Hasta Eğitiminde Bilişim Teknolojilerinin Kullanımı. Turkiye Klinikleri J Surg Nurs-Special Topics, 2(1):22-6. Erişim 08.10.2022. http://www.turkiyeklinikleri.com/article/en-hasta-egitiminde-bilisimteknolojilerinin-kullanimi-75127.html
26. Pratiw, I. G., Husin, F., Ganiem, A. R., Susiarno, H., Arifin, A., Wirahkusum, F. (2017). The Effect of Virtual Reality on Pain in Primiparity Women. International Journal of Nursing and Health Science. 4(4): 46-50. Erişim 15.10.2022 http://www.openscienceonline.com/journal/ijnhs ISSN: 2381-4861 (Print); ISSN: 2381-4888 (Online)
27. Riva, G. (2011). The Key to Unlocking the Virtual Body: Virtual Reality in the Treatment of Obesity and Eating Disorders. Journal of Diabetes Science and Technology, 5(2): 283-292. Erişim 09.10.2022 https://www.ncbi.nlm.nih.gov/ pubmed/21527095

28. Ryu, J.H., Oh, A. Y., Yoo, H. J., Kim, J. H., Park, J. W., Han, S. H. (2018). The effect of an immersive virtual reality tour of the operating theater on emergence delirium in children undergoing general anesthesia: A randomized controlled trial. Pediatric Anesthesia/ Volume 29, Issue 1, p.98-105 Erişim 09.10.2022 https://doi.org/10.1111/pan.13535
29. Shahoi, R., Shahghebi, S., Rezaei, M., Naqshbandi, S. (2017). The effect of transcutaneous electrical nerve stimulation on the severity of labor pain among nulliparous women: A clinical trial. Complementary Therapies in Clinical Practice 28, p.176-180 Erişim 26.10.2022 https://www.sciencedirect.com/science/article/pii/ S1744388117301342
30. Sherman, W. R., Craig, A. B. (2003). Understanding Virtual Reality: Interface, Application and Design (Second Edition). U.S.A: Morgan Kaufmann Publishers, Elsevier Science (USA) p.5-536.
31. Şahan, T. Y., Erbahçeci, F. (2018). Transtibial amputelerde sanal gerçeklik uygulamasının kinezyofobi, depresyon ve ağırlık aktarma üzerine etkileri. Journal of Exercise Therapy and Rehabilitation. 5(2):82-88 Erişim 09.10.2022 http://dergipark.gov.tr/download/article-file/507070
32. Şahin, Ö. Y., Saltan, H., Alp, A. T., Özel, B., Sanatçı, S. (2016). Eğitimde Sanal Gerçeklik, Artırılmış Gerçeklik. Sakarya Üniversitesi. Eğitim Fakültesi, Bilgisayar ve Öğretim Teknolojileri Öğretmenliği. Erişim 26.10.2022. http://kodyazar.net/wpcontent/uploads/2016/05/ Makale-1.pdf
33. Şekerci, C. (2017). Sanal Gerçeklik Kavramının Tarihçesi. Uluslararası Sosyal Araştırmalar Dergisi 10(54): 1126-33, Issn: 1307-9581 Erişim 06.10.2022 http://dx.doi.org/ 10.17719/jisr. 2017 5434681
34. Üzümcü, E., Akın, B., Nergiz, H., İnözü, M., Çelikcan, U. (2018). Anksiyete Bozukluklarında Sanal Gerçeklik. Psikiyatride Güncel YaklaĢımlar-Current Approaches in Psychiatry, 10(1):99-117, eISSN:1309-0674. doi: 10.18863/pgy.336593 Erişim 02.10.2022 http://www.cappsy.org/archives/vol10/no1/cap_10_01_07.pdf
35. Williams J, Jones D, Walker R. (2018). Consideration of using virtual reality for teaching neonatal resuscitation to midwifery students. Nurse Educ Pract, 31: 126-9. doi: 10.1016/j.nepr.2018.05.016. Epub 2018 May 28
36. Yıldırım, G., Şahin, N. H. (2003). Doğum ağrısının kontrolünde hemşirelik yaklaşımı. Cumhuriyet Üniversitesi HemĢirelik Yüksek Okulu Dergisi, 7 (1); 14-20. Erişim 05.10.2022. http://eskidergi.cumhuriyet.edu.tr/makale/618.pdf

Diabetes Mellitus in Cats: An Update On Physiology, Etiopathogenesis, Clinical Diagnosis, Treatment

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1. INTRODUCTION

Diabetes mellitus is defined as persistent hyperglycemia caused by relative or absolute insulin deficiency. Insulin is produced only by β cells in the islets of Langerhans (islet) of the pancreas. Insulin deficiency occurs when beta (β) cells are destroyed or their functions are impaired (Rand, 2013). This disease is one of the most common endocrinopathies of cats. Diabetes is characterized by disturbance of carbohydrate, lipid and protein metabolism. Diabetes mellitus of cats is similar in many features to type 2 diabetes of humans. Although the prevalence of the disease varies, it has increased significantly in recent years (O'Neill et al. 2016; Osto et al., 2013). Classification of diabetes is based on the mechanisms that cause loss of function in β-cells (Rand, 2013). In feline diabetes mellitus, insulin therapy is currently the most effective treatment for achieving glycemic control (Osto et al., 2013). Cats are true carnivores and should be handled differently from omnivorous dogs. For this reason, diets is very important in cat's food. Feeding with low-carbohydrate and high-protein diets is more appropriate for cats. In cats with diabetes mellitus, the average survival time is 13-29 months. However, cats that are well stabilized may have a longer survival time (Ford & Lynch, 2013)

1.1. THE PREVALENCE OF THE DISEASE

Diabetes mellitus (DM) is one of the two most common endocrinopathy among feline diseases. While the prevalence of the disease in the United States was 0.25% in the 1990s, it increased to 1.24% in 1999. In the studies in the 2000s, the prevalence of the disease was found to be 0.5% and 0.74% in Australia. Among cats, the highest prevalence was found in Burmese cats with 2.24%. The disease is commonly seen in Burmese, Maine Coon, Russian Blue, Norwegian Forest and Siamese cats (Reusch, 2012; Bloom &Rand, 2014; Ohlund et al., 2015; Perez-Lopez et al, 2019). Common risk factors for the development of type 2 diabetes mellitus in humans and cats are age, obesity and physical activity. Age is defined as the single most important risk factor. Diabetes mellitus can occur in cats over a wide age range, and they are usually over 6 years of age when diagnosed. The mean age in cats diagnosed with the disease is 10 years, with a peak incidence of 9-13 years. Diabetes occurs more frequently in older cats (Herrtage, 2009). The increase in prevalence of the disease in recent years is probably related to the prevalence of obesity in cats. In addition to obesity, many risk factors such as age, sterilization and gender play a role in this increase (Bloom & Rand, 2014). Obese cats are 3-5 times more likely to develop diabetes (Herrtage, 2009). Male cats have a higher incidence of diabetes than female cats, since spayed cats are prone to obesity (Davidson, 2015; Ohlund et al., 2015)

1.2. PHYSIOLOGY OF THE PANCREAS IN CATS

The pancreas is both an exocrine and an endocrine gland. The exocrine part (acinar), which makes up a large portion (82%) of this gland, is responsible for the production and release of digestive enzymes. In the other part of the pancreas, groups of endocrine cells called islets of Langerhans form. While enzymes such as trypsinogen, chymotrypsinogen, proelastase, carboxypeptidase, phospholipase, lipase are released from acinar cells, hormones such as insulin, glucagon and somatostatin are secreted from the islets of Langerhans (Turgut & Ok, 2001; Başoğlu & Sevinç, 2004).

Produced insulin from beta cells of the pancreas, promotes cellular glucose uptake for the energy needs of most cells in the body, especially muscle, adipose tissue and liver. In the absence of insulin, cells cannot uptake and use glucose. Therefore, it results in hyperglycemia. Brain cells are unique in that they are permeable to glucose and can use glucose without insulin. Most cells in the body can use free fatty acids (FFA) as an energy source in the absence of glucose. Cells with absolute glucose requirements are the germinal epithelium of the brain, retina, and gonads. Insulin also slows down lipolysis. In the absence of insulin, lipolysis begins in adipocytes and FFAs are released into the circulation. Circulating FFAs are taken up by the liver for the production of triglycerides, as well as for the production of ketone bodies, which can be an additional energy source for many cells in the body. In uncomplicated diabetes mellitus, triglyceride production is predominant and ketone production is slow that tissues can use it as an energy source. Therefore, hyperketonemia is not observed (Turgut & Ok, 2001)

1.2.1. Classification of Diabetes in Cats

Type 1 diabetes; It occurs as a result of the destruction of immune-derived β-cells and usually causes absolute insulin deficiency. Unlike dogs, type 1 diabetes mellitus is rare in cats. Lymphocytic infiltration of islet cells as a marker of immune-related diseases has been reported in only a few cats, but recently, in studies in diabetic cats and control cats, it was determined that lymphocytic infiltration into islet cells was higher in diabetic cats compared to control cats. However, it has been reported that this infiltration is mild and may reflect an inflammatory condition as in type 2 diabetes (Elliot, 2005; Herrtage, 2009; Nelson & Reusch 2014)

Type 2 diabetes; It is characterized by insulin resistance with β-cell dysfunction. A significant proportion of diabetes in cats is type 2. When diagnosed, most cats have absolute insulin deficiency due to β-cell dysfunction.

Some of these may be reversible if rapid glycemic control is achieved (Elliot, 2005; Herrtage, 2009).

Other specific types of Diabetes (Secondary Diabetes); Secondary forms of diabetes mellitus may develop as a result of pancreatic destruction from pancreatitis or may occur as a result of other diseases such as other conditions (drug-induced diabetes mellitus) (Elliot, 2005; Herrtage, 2009).

Gestational diabetes; It is not a major cause of diabetes in cats because most cat owners have their pets spayed.

Diabetes mellitus in cats is clinically and pathologically quite similar to type 2 diabetes mellitus in humans. No single mechanism is responsible for the development of the disease (Herndon et al., 2014). At least two primary pathogenic mechanisms are required for type 2 diabetes mellitus, a) progressive deterioration of pancreatic islet cell function resulting in decreased insulin secretion, and b) insulin resistance, known as decreased metabolic response to insulin (Carrera-Boada & Martinez-Moreno, 2013; Kleinert et al., 2018). Insulin resistance alone is not a good indicator for the development of diabetes in pets as in humans. Impairment of fasting and postprandial plasma glucose metabolism is required as a result of loss of function in beta cells (Hoenig, 2014). The main pathological processes may continue for weeks, months or even years until the clinical signs of feline diabetes mellitus appear. Cellular processes such as diet, genetic factors, physical activity, obesity, oxidative damage, inflammation, and defective protein formation eventually cause functional β-cell loss, resulting in significant hyperglycemia (Slingerland et al., 2009; Herndon et al., 2014; Nelson & Reusch 2014; Behrend et al, 2018; Kleinert et al., 2018). Insulin requirement is determined by peripheral insulin sensitivity, dietary glucose uptake, and mechanisms of gluconeogenesis. In the initial stage of type 2 diabetes mellitus, relatively compensatory hyperinsulinemia occurs in response to peripheral insulin resistance. With prolonged duration of this phase, the insulin production capacity of β-cells is depleted and a continuous relative or absolute hypoinsulinemic phase develops. Apart from insulin, the other major secreted product of pancreatic β-cells is islet amyloid polypeptide (IAPP), also known as amylin. In healthy individuals, the storage ratio of IAPP and insulin in secretory granules is 1:50 (Herndon et al., 2014)

1.3. POTENTIAL FACTORS INVOLVED IN ETIOPATHOGENESIS

Potential factors involved in the etiopathogenesis of feline diabetes mellitus are islet amyloidosis, insulin resistance, obesity, pancreatitis, other concomitant hormonal diseases (hyperadrenocorticism, acromegaly, hyperthyroidism), drugs (progestogens, glucocorticoids), concomitant infections (kidney diseases, heart

diseases), hyperlipidemia, genetic factors (Burmese cats and their relatives) and immune related disease (Nelson & Reusch 2014).

1.3.1.İslet Amyloidosis

Apart from insulin, the other major product secreted from pancreatic β-cells is islet amyloid polypeptide (IAPP), also known as amylin. Amylin is the major amyloid structure stored in the pancreas of diabetic cats. Amylin is secreted by beta cells together with insulin. Therefore, any stimulant that stimulates insulin secretion (obesity, insulin resistance, acromegaly) also stimulates amylin secretion. The role of this neuroendocrine polypeptide is that it is a glucoregulatory hormone that inhibits the effects of insulin during satiety (in the postprandial state). The ratio of IAPP/insulin stored in the secretory granules of the healthy pancreas is 1:50. During the hyperinsulinemia period, the circulating IAPP level also rises and the serum IAPP/insulin ratio changes (Herndon et al., 2014). Islet amyloidosis (IA) is associated with significant β-cell loss in pancreatic islets and is a usually unchanged feature of feline diabetes and type 2 diabetes mellitus in humans (Hay et al., 2015; Jotha- Mattos, 2021). About 80-95% of diabetic cats suffer from type 2 diabetes mellitus (Davidson, 2015). One hypothesis regarding type 2 diabetes is that loss of function in β-cells is due to amyloid deposition that causes β-cell degradation. Many new researchs have been made in understanding the pathogenesis of islet amyloidosis in cats and humans, with the determination of the amyloid precursor protein. This previously unknown condition originates from a hormone called islet amyloid polypeptide (IAPP), also known as amyline (Nelson & Reusch 2014). Islet amyloid polypeptide (IAPP) is normally produced in β-cells, stored with insulin in the secretory sacs, and released into the circulation with insulin. IAPP levels are high in insulin-resistant obese cats (Nelson & Reusch 2014). It has been shown that in cats, islet amyloid originates from islet amyloid polypeptide (IAPP), as in humans. The fibrillar forms of islet amyloid polypeptides have been shown to be cytotoxic in vitro and induce cell death (apoptosis). In this way, a potential pathogenic link between islet amyloidosis and β-cell loss in feline diabetes mellitus was determined (Hay et al., 2015; Jotha- Mattos, 2021). Amyloid, which accumulates in the islet cells of the pancreas in cats, humans and primates (monkeys), is in the amyloidogenic amino acid structure of IAPP. Islet amyloid deposition was not detected in hamsters, rats and mice. Islet amyloidosis can occur in up to 90% of cats with diabetes and similarly in people with type 2 diabetes. Humans and cats with type 2 diabetes have an average of 50% β-cell loss from islet amyloidosis (Hay et al., 2015; Jotha- Mattos, 2021). However, islet amyloidosis is also a common finding in nondiabetic cats. In a recent study,

amyloid deposition was observed in 56% of diabetic cats and 42% of control cats. It is not known why amyloid deposition develops in some but not all diabetic cats. Therefore, it is not known whether amyloidiosis is the cause or the result of the disease (Nelson & Reusch 2014). Amyloid is toxic to beta cells and results in cell death. Therefore, insulin secretion decreases. If amyloid deposition persists (persistent obesity), islet cell destruction continues and eventually diabetes mellitus develops (Zoran, 2005).

1.3.2. Insulin Resistance

Insulin resistance is characterized by decreased response of tissues to insulin despite secreted insulin. Insulin resistance is defined as decreased sensitivity to insulin. In the beginning, an attempt is made to compensate for insulin resistance with excessive insulin secretion. However, the long duration of this situation with stress negatively affects β-cells and contributes to the development of hyperglycemia and glucose intolerance (Scott-Moncrieff, 2010; Verbrugghe et al, 2012; Carrera-Boada & Martinez-Moreno, 2013). In many conditions or diseases, insulin resistance can develop. Individual sensitivity in insulin resistance can be explained by low insulin sensitive glucose (GLUT). GLUT is the name of the transporters that allow glucose to be transported into the cell. Insulin resistance is an important component in the pathogenesis of feline type 2 diabetes (Gottlieb & Rand 2018, Clark & Hoenig, 2021). Diabetic recovery can be achieved with the resolution of insulin resistance in cats with type 2 diabetes in whom appropriate glycemic control is achieved in a short time. However, most diabetic cats can be controlled with 1-3 U dose (<1 U/kg) insulin. In cats requiring an insulin dose of more than 6 U (>1.5 U/kg) to achieve appropriate glycemic control, if persistent hyperglycemia is still observed despite this high dose, then it should be considered as insulin resistant (Scott-Moncrieff, 2010)

1.3.3.Obesity

The lifestyles of domestic and wild cats vary considerably. With the domestication, the transition from the open environment to the closed environment resulted in a decrease in physical activity. Because cats do not need to hunt for nutrition and therefore do not need much time. This change in the lifestyle of cats has also caused a change in their eating habits. Cats that were fed low-carb and high-protein while hunting after domestication, began to be fed relatively high-carb commercial diets. For this reason, obesity in domestic cats has started to be seen recently. There has been an increase in the incidence of obesity-related insulin resistance and type 2 diabetes mellitus (Verbrugghe et al, 2012)

It has been demonstrated that obesity is the most important risk factor for the development of insulin resistance (Bloom & Rand, 2014). In cases of weight gain, the glucose transport molecule GLUT4 decreases in muscle and adipose tissue, and at the same time, the amount of intra and extra myocellular lipids increases. GLUT is responsible for the transport of extracellular glucose into the cell. Cats fed a diet of 50% carbohydrate-derived starts gain weight in a short time, which has adverse effects on postprandial glucose and insulin concentrations. However, obesity-related changes in glucose hemostasis can be improved by weight loss (Hoelmkjaer & Bjornvad, 2014; Clark & Hoenig, 2021).

1.3.3.1.Conditions Associated with Obesity in Cats

Obesity is associated with many diseases that are included in the endocrine disorders of cats. Weight gain significantly reduces insulin sensitivity, and each kilogram increase in body weight reduces insulin sensitivity by 30%. In adult cats with a 44% increase in body weight, insulin sensitivity decreases by approximately 50% (Hoenig, 2014). In addition, the risk of developing diabetes was 2 times higher in overweight cats and, 4 times higher in obese cats compared to normal weight cats. Overweight and obesity are risk factors for urinary tract infections, hepatic lipidosis, dermatological diseases and oral cavity diseases.

2. CLINICAL FINDINGS

2.1. Symptoms of non-ketoacidic diabetes mellitus

Symptoms of diabetes mellitus are hyperglycemia, glycosuria, polyuria, polydipsia, polyphagia, weight loss, anorexia, lethargy, muscle weakness, cataracts in dogs, neuropathy in cats. Moreover, the classic clinical findings of diabetes mellitus are polyuria and polydipsia (PU/PD), lethargy, weight loss, and polyphagia. Less commonly, weakness, neuropathy (plantigrade posture), depression, and anorexia may occur (especially in ketoacidosis). In chronic hyperglycemia caused by insulin deficiency, using of glucose is impaired. In addition, increased hepatic gluconeogenesis and glycogenolysis contribute to hyperglycemia. Decreased peripheral use of glucose causes its accumulation in the blood. As a result of excessive accumulation of glucose in the blood, the renal threshold is exceeded and osmotic diuresis begins. The renal threshold for glucose is 180 mg/dL in dogs and 280 mg/dL in cats. Dehydration increases with diuresis and polydipsia begins to appear as a compensator. In cases where glucose metabolism is impaired, such as insulin deficiency or insulin resistance, the hypothalamic center is constantly stimulated, and calorie loss, polyphagia, weight loss and lethargy are observed due to glycosuria. In addition, insulin deficiency

contributes to weight loss and muscle atrophy by causing protein catabolism (Elliot, 2005; Aytuğ, 2011; Greco, 2018).

Diabetes predisposes to some eye disorders. The most common of eye disorders are glaucoma (eye pressure) and cataract (clouding of the lens of the eye). Cataracts usually develop more in dogs because of uncontrollable diabetes. This problem is less common in cats. In the case of hyperglycemia, aldose reductase converts glucose to sorbitol. Sorbitol can then be oxidized to fructose with the aid of sorbitol dehydrogenase. However, this reaction develops slowly and the sorbitol concentration rises to high levels inside the cell. A high sorbitol concentration causes the water in the aqueous humor to leak into the lens via osmotic pressure, resulting in excessive hydration of the lens. This situation may cause anatomical deterioration of the lens and cataract development in diabetic patients if the disease cannot be controlled (Ritcher et al., 2002)

One of the most common chronic complications in diabetic cats is diabetic neuropathy, occurring in approximately 10% of patients. However, diabetic neuropathy in dogs is an uncommon clinical finding. As clinical findings, patients have problems with standing on the ground (plantigrade posture), such as weakness in the hind legs, decreased ability to jump, standing on the soles of the paws, and muscle atrophy. Abnormalities in sensory nerves are not as severe as in motor nerves. The front legs tend to be less severely affected than the hind legs. Although axonal degeneration and demyelination are thought to be associated with persistent hyperglycemia, the exact cause of diabetic neuropathy is unknown (Reusch, 2012; Carrera-Boada & Martinez-Moreno, 2013).

Animals with diabetes are susceptible to bacterial and fungal infections. As a result of this sensitivity, frequently recurring chronic diseases such as cystitis, prostatitis, bronchopnomonia and dermatitis are diagnosed by veterinarian. The increased susceptibility to these diseases is due to impaired chemotactic, phagocytic, and decreased neutrophil function. The formation of the lumen and wall of the urinary bladder is due to disruption by Proteus spp., Aerobacter aerogenes, Escherichia coli, glucose-fermenting bacteria (Başoğlu & Sevinç, 2004)

2.2. Symptoms of ketoacid diabetes mellitus and hyperosmolar hyperglycemic diabetes mellitus

Diabetic ketoacidosis (DKA) is a complex disease process in humans and animals with diabetes mellitus. This process occurs in about 12-37% of cats with diabetes. Diabetic ketoacidosis causes severe illness from dehydration, hyperosmolarity, electrolyte abnormalities, and acidemia. Patients gives anamnesis that they have previously been diagnosed with diabetes mellitus, or

that they have seen signs of diabetes mellitus such as polyuria, polydipsia, polyphagia, weight loss. Although diabetic ketoacidosis is not common in dogs, rapid-onset blindness from diabetic cataract in dogs and plantigrate posture in cats from peripheral neuropathy may occur (Crenshaw & Peterson 1996; Kerl, 2001)

Weak body structure, muscle weakness, lethargy, dehydration, and hypothermia is the symptom of physical examination of diabetic ketoacidosis. Hepatomegaly and icterus may be diagnosed in some dogs and cats with diabetic ketoacidosis. However, these findings are rare. Other clinical findings in dogs and cats include cataract (dog) and ketone odor, mild renomegaly or plantigrade posture (cats) (Kerl, 2001)). Crenshaw et al. (1996), in a study on diabetic cats, reported that decreased activity, anorexia, weakness, and vomiting were common in cats with ketoacidosis compared to nonketotic cats.

Hepatic glycogenolysis and glucogenesis result in the production of endogenous glucose. Glucose produced from the liver is released into the circulation. Because cellular glucose uptake is prevented due to insufficient insulin activity. In the absence of insulin, cellular glucose demand stimulates release of glucagon from the alpha cells of the pancreas, exacerbating hyperglycemia. Hyperglycemia has many harmful effects such as hyperosmolality (hyperosmolality) and osmotic diuresis. The hyperosmolality causes dilutional hyponatremia and increased osmotic diuresis. Fluid and electrolyte loss is exacerbated (Behrend et al., 2018; Gottlieb & Rand, 2018).

Glucagon facilitates ketogenesis in hepatocytes (instead of triglyceride production in the hepatocellular cytoplasm, ketone bodies is produced). Glucagon increases mitochondrial uptake of free fatty acids by decreasing hepatic malonyl coenzyme A concentration and increasing hepatic carnitine (carnitine) level. Morover, more free fatty acids (FFA) are allowed to enter the mitochondria for the production of ketone bodies. Malonyle coenzyme A normally inhibits the oxidation of fatty acids to free fatty acids in the hepatic cytoplasm. With the decrease of this enzyme level, result in that free fatty acids facilitate the entry into mitochondria. Malonyle Co enzyme A inhibits carnitine palmitotransferase-I (carnitine palmitotransferase I; CPT-I) enzyme activity. CPT-1 is required for the transport of non-esterified fatty acids to the mitochondria for the production of ketone bodies. Mitochondrial FFA either enter the citric acid cycle for energy production or enter the formation of ketone bodies (acetoacetic acid and β-hydroxybutyric acid). Failure to inhibit lipolysis due to insulin deficiency results in more FFA for ketone formation. Lack of suitable substrate for the citric acid cycle impairs the ability of FFA in mitochondria to be converted into energy via the citric acid cycle. The result of this is excessive ketoacid production in a short

time and the body cannot metabolize it properly. Thus, hyperketonemia occurs. Ketoacids are released into the systemic circulation. Some of the acetoacetic acid is converted to acetone which is a volatile fatty acid that is eliminated by the lungs. This acid causes ketone odor and can be detected in some animals with diabetic ketoacidosis. Because ketoacids are strong acids and this occurs systemic acidosis. Since ketoacids are excreted from kidney, urinary electrolyte loss is facilitated and osmotic diuresis caused by hyperglycemia worsens (Kerner & Hoppel, 2000; Prakash, 2018).

3. DIAGNOSTIC AND LABORATORY FINDINGS

Diabetes mellitus is usually diagnosed based on the determination of persistent fasting hyperglycemia and glycosuria with clinical findings. Normal blood glucose concentration in cats and dogs is between 75-120 mg/dl. However, while the renal threshold of glucose is 180 mg/dL in dogs and 280 mg/dL in cats (Gottlieb & Rand, 2018; Greco 2018). In cats and dogs with diabetes mellitus, persistent hyperglycemia, hypercholesterolemia, increased liver enzyme activities (Alkaline Phosphatase (ALP), Alanine aminotransferaz (ALT), Aspartate Aminotransferase (AST), neutrophilic leukocytosis, proteinuria, increased urine density and glycosuria are detected (Crenshaw & Peterson 1996; Gottlieb & Rand, 2018; Greco, 2018). Other laboratory findings of ketoacid diabetes mellitus are metabolic acidosis, azotemia, hyponatremia, hypo or hyperkalemia, hypochloremia, hypomagnesemia, hypophosphataemia, hyperlipazemia, ketonemia, ketonuria, bacteriuria, hematuria, and pyuria (presence of inflammatory cells in the urine (leukocytes) (Kerl, 2001; Greco, 2018).

It is necessary to make a differantial diagnosis of stress hyperglycemia (and glycosuria) from diabetes before starting treatment. In healty cats, hormones and epinephrine result in transient hyperglycemia and glycosuria. Stress can cause severe hyperglycemia (288 mg/dl; >16 mmol/l) and usually resolves within a few hours. Therefore, before diagnosing diabetes mellitus, it is important to collect urine samples for urinary tract infections, determination of glycosuria and ketonuria. Stress hyperglycemia, intravenous dextrose infusion, and renal proximal tubular defect or damage should be considered in the differential diagnosis of diabetes mellitus in the presence of glycosuria. In order to avoid stress hyperglycemia in cats, it is recommended to collect samples at home (Aytuğ, 2011; Greco, 2018).

3.1. Blood Insulin and C-Peptide Level

Determination of blood insulin and C-Peptide levels is of great importance, especially in the diagnosis of diabetes and some types of hypoglycemia. Determination of blood insulin level is very important in terms of evaluating the functions of pancreatic β-cells. For this purpose, serum insulin level and C-Peptide level in serum and urine can be determined by radioimmunological methods. Beta cells release C-Peptide and pro-insulin into the circulation together with the hormone insulin. This precursor, called proinsulin, produced and released from β-cells, is formed as a result of combining the A and B chain of insulin with a polypeptide called C-Peptide. That's why the C-Peptide is also called the "connective peptide". With the separation of the C-Peptide from pro-insulin, insulin hormone is formed. Determining the serum C-Peptide level has some advantages over the determination of the serum insulin level. After insulin is secreted and given to the circulation, half of it is taken up by the liver and the remainder by other cells of the body as needed. In contrast, C-Peptide has no hormonal function and remains in the circulation longer. Therefore, the serum insulin level may be misleading in the evaluation of β-cell function. Determining the C-Peptide level provides a correct evaluation of β-cell function. 10% of the circulating C-Peptide is taken up by the liver and its half-life is two times longer than insulin. Determining the level of C-Peptide is very important to evaluate the function of beta cells. Whether the insulin secretory capacity of the pancreas is impaired or not can be determined by measuring the C-Peptide level (Hoenig et al., 2006)

3.2. Serum Fructosamine concentration and Glycosed Hemoglobin (HbA1c)

The definitive diagnosis of diabetes mellitus should not be made only by determining hyperglycemia and glycosuria. Therefore, glycosylated proteins such as fructosamine and glycosylated hemoglobin are frequently used markers in the diagnosis, monitoring and long-term assessment of glycemic status in cats with type 2 diabetes mellitus. Fructosamine and HbA1c consists of irreversible and non-enzymatic binding of glucose to hemoglobin and amino acids. The serum fructosamine and glycosylated hemoglobin concentration is directly proportional to the serum glucose concentration. Serum fructosamine is the glycosylated form of serum proteins such as albumin and is directly related to serum glucose concentration. However, the fructosamine concentration does not reflect instantaneous changes in blood glucose concentration (1-3 weeks). According to one study, 1 g/kg of 50% dextrose given intravenously to 17 cats did not cause an increase in serum fructosamine concentration (Reusch et al., 1993; Lutz et al,

1995). Therefore, fructosamine and HbA1C are helpful in distinguishing between stress-induced hyperglycemia and diabetes mellitus-induced hyperglycemia in cats. Fructosamine is total glycated serum proteins that can be measured calorimetrically ((Reusch et al., 1993; Lutz et al, 1995). Glycosed hemoglobin, part of glycated hemoglobin A1c (HbA1c), is specifically a product of glucose and hemoglobin and is measured by chromatography. In cats, glycosylated hemoglobin alone is rarely used to diagnose diabetes mellitus (Reusch et al., 1993; Lutz et al, 1995).

Albumin has a shorter half-life than hemoglobin. Whereas glycosylated hemoglobin concentration indicates a longer glycemic state (6-8 weeks). Serum fructosamine concentration gives shorter duration (1-3 weeks) changes of blood glucose concentration than glycosylated hemoglobin. Fructosamine and glycosylated hemoglobin levels are affected by changes in serum protein and hemoglobin concentration (Reusch et al., 1993; Lutz et al, 1995, Reusch & Harberer 2001). Fructosamine concentration is generally preferred because it can be measured more easily and quickly in practice. This parameter is also recommended in diabetic cats for the assessment of chronic hyperglycemia after diagnosis. Compared with the measurement of blood glucose concentration, the major advantage of determining the fructosamine concentration. Fructosamine is not affected by stress hyperglycemia and pre-postprandial hyperglycemia, which cause short-term changes in blood glucose concentration (Reusch & Harberer 2001, Slingerland et al, 2009).

4. TREATMENT

4.1. Oral Hypoglycemic Drugs

Oral hypoglycemics can be used in cats that still have the ability to secrete and synthesize endogenous insulin. These medicines work by slowing the absorption of glucose from intestine or by increasing the sensitivity of tissues to insulin. If beta cells in the pancreas are partially functional, the effect of oral hypoglycemics can be very good. Oral hypoglycemics used in cats are sulfonylureas (glipizide), α-glucosidase inhibitors (acarbose). Also, incretin hormones, biguanide, thiazolinidinediones can use (Palm, 2013; Zoran, 2013; Greco, 2018).

The most commonly used oral hypoglycemic drug in diabetic cats is glipizide from the sulfonylurea group. Sulfonylurea group has another derivatives such as glyburide and glimepiride. The main mechanism of action of sulfonylurea group is to stimulate insulin secretion from β cells. In addition, they inhibit hepatic gluconeogenesis by increasing tissue sensitivity to circulating insulin. It increases using of hepatic glucose and decreases hepatic insulin extraction. In addition to the positive effect of sulfonylureas, such as increasing insulin secretion, they also

have a negative effect, such as increasing pancreatic amyloid accumulation. Currently, glipizide is the only agent proven to be used for treatment in cats (Sparkers et al., 2015). Glipizide treatment should be started at 2.5 mg/cat twice daily with food. This dose can be increased up to 5 mg/cat if necessary (Sparkers et al., 2015).

Metformin, a biguanide, is a potent inhibitor of hepatic glucose production. Mechanism of action is ability to alter intestinal glucose metabolism. The dose of metformin is 10-50 mg/cat orally once a day (Palm, 2013).

Troglitazone (Rezulin, Resulin, Romozin) is an antidiabetic and anti-inflammatory drug. It is a thiazolidinedione that inhibits hepatic glucose output by slowing down gluconeogenesis and glycogenolysis in human diabetes (Palm, 2013).

α-Glycosidase Inhibitors such as acarbose and miglitol are an alpha-glucosidase inhibitor that delays the enzymatic breakdown of starches into glucose in the small intestine. Acarbose inhibits the hydrolysis of complex starch by blocking pancheastic amylase and inhibits the increase in postprandial blood glucose concentration. It also impairs glucose absorption from the intestines by reducing fiber digestion. In this way, they reduce glucose uptake with food. It exerts such an effect by suppressing the enzymes that bind to the membranes of intestinal brush-tipped structures in the intestines and absorb monosaccharides (Palm, 2013).

4.2. New Treatment Approaches to Feline Diabetes

Recently, incretins have been used in the treatment of type 2 diabetes. Incretin-based therapies have revolutionized the treatment of human diabetes, with the use of safer and more convenient long-acting drugs, replacing insulin therapy. Incretin hormones are defined as hormones released from the intestines after food intake. These hormones are called glucagon-like peptide-1 (GLP-I) and glucose-dependent insulinotropic peptide (GIP). GLP-I is secreted from L-cells located in the ileum and colon, while GIP is secreted from K-cells located in the upper parts of the intestine. GLP-1 delays gastric emptying and increases the feeling of satiety. GLP-I increases insulin secretion in the pancreas and suppresses glucagon secretion during hyperglycemia as a glucose-binding pathway. It also protects beta cells from oxidative and toxic injury and promotes the expansion of beta cell mass. It has been revealed that GLP-I-derived drugs are as effective as insulin, cause weight loss and improve glycemic control in people with type 2 diabetes. Incretin has been shown to be effective in cats as well, although not as effectively as in other species. Incretin increases insulin secretion by 70% in humans and 30% in cats (Gilor et al, 2016)

Lispro insulin is a genetically engineered human insulin analogue. This new insulin is formed by reversing the sequence of proline at position B28 and lysine at position B29. It reduces the formation of insulin dimers and hexamers. This change provides much faster absorption and causes the effect to start immediately. This situation; It reduces glucose entry into cells, suppresses endogenous glucose production, reduces free fatty acids and glycerol levels. It has been reported in studies (Sears et al 2012, Malerba et al 2018) that it acts much faster than other insulins. It has been reported to be used successfully especially in diabetic ketoacidosis. (Malerba et al. 2018)

Cabergoline; very sensitive to dopamine 2 receptors in monkeys and rats; It is a dopamine receptor antagonist. It is good in many features. One of these effects requires less dosing with its long duration. It is well tolerated. It increases sensitivity to insulin. Licensed products are available for cats as well as humans and dogs. In the study, the use of cabergoline reduced the need for insulin in diabetic remission in three cats. (Scudder et al. 2020, Micelli et al. 2021)

4.3. Dietary Choice in Cats with Diabetes

In the diet of cats, protein and amino acids are required daily. Because cats are true carnivores and cannot suppress urea cycles or transaminases, which other species can do in a state of starvation. Because the use of dietary carbohydrates is ineffective. Cats are deficient in salivary amylase and also have low intestinal amylase and disaccharidase concentrations. Since they do not have the fructokinase enzyme, they cannot use fructose sugar metabolically. In cats, the activity of hepatic enzymes such as glucokinase, which is involved in the storage of postprandial glucose as glycogen, is significantly lower. Therefore, large amounts of postparandial glucose have less ability to be stored. Because of this situation, Cats do not need glucose (Gilor et al, 2016).

In foods containing high protein and low carbohydrate, the cat's energy need is provided from protein and it reduces postprandial hyperglycemia with its low carbohydrate ratio. Diabetic cat food should be high protein and low carbohydrate. The point to be considered in the nutrition of diabetic cats is to reduce the amount of food given and increase the frequency of feeding. In this case, the risk of hypoglycemia is reduced and better glycemic control is achieved throughout the day (Ford & Lynch, 2013).

4.4. Insulin Therapy

In cats, the blood glucose concentration should be in between 72-120 mg/dl, ie 4-6.7 mmol/L. The primary goal of insulin therapy is to minimize the findings associated with diabetes mellitus. With insulin therapy, it is essential to always

keep the blood glucose level below 14 mmol/L (252 mg/dl) and to avoid clinically important hypoglycemia. However, it is aimed to keep the blood glucose level below 11 mmol/l (198 mg/dl) by following the patient very seriously. At the same time, patients' desire to drink water, frequency of urination and weight are recorded (Herrtage, 2009; Ford & Lynch, 2013).

In pharmacodynamic studies in healthy cats, it was determined that insulin glargine and insulin detemir showed activity over 24 hours. In addition, there are alternative studies showing that the duration of clinical activity varies between 10-16 hours. However, it is stated that in some cats, blood glucose levels decrease significantly with long-acting insulin analogues, while more limited decreases are observed in some cats. Insulin glargine, insulin detemir, and Protamine Zing insulin (PZI) have longer-lasting clinical effects in cats than lente insulin. Therefore, better glycemic control is likely to be achieved if used twice daily (Salesov et al., 2018)

Recommended insulin injection sites are scruff (neck), side of chest, side of belly, and flank (Ford & Lynch, 2013).

The starting dose of intermediate or long-acting insulin preparations for uncomplicated nonketotic cats is usually 0.25-0.5 IU/kg. The dose can usually be rounded to the lowest unit (≤ 2 IU/cat, every 12 hours). However, if the glucose concentration exceeds 20 mmol/L (360 mg/dl), the highest dose (0.5 U/kg) can be given. Dose changes should generally be made every 5-7 days. Rapid increases in insulin doses are a common cause of hypoglycemia and can lead to hyperglycemia and poor control (Nelson & Reusch 2014).

5. PROBLEMS ENCOUNTERED DURING INSULIN USE

5.1. Insulin overdose and Somogy effect

The somogy effect is also called post-hypoglycemic hyperglycemia. This phenomenon is defined as hyperglycemia that returns during hypoglycemia, mainly due to hypersecretion of anti-insulin hormones such as epinephrine and glucagon. Cats are more prone to Somogyi phenomenon compared to dogs. It is the response of hormones such as epinephrine and glucagon triggered by insulin, especially due to an excess of insulin dose in the evening. High insulin dose causes hypoglycemia during sleep. Therefore, epinephrine and glucagon hormones released and this situation increases blood glucose level until morning. In the morning measurements, hyperglycemia is determined. Owners of patients give the anamnesis that glycemic control is good and sometimes bad on some days. In this case, the Somogy effect should always be suspected. Patients may increase their insulin dose if they are not informed in advance of the somogy effect. In this case, hypoglycemia due to high insulin dose may be observed in

cats (Reusch, 2013). Diagnosis requires documentation of hypoglycemia or a very rapid decrease in blood glucose concentration following hyperglycemia (blood glucose concentration >17 mmol/l, 300 mg/dl) within 12 hour period. Cats are more prone to the development of the Somogyi effect than dogs (Tarkosova et al., 2016)

5.2. Hypoglycemia

Hypoglycemia (BG< 2 mmol/L; 54 mg/dl) is a life-threatening biochemical abnormality that needs immediate increase to blood glucose. It is more common in diabetic cats than dogs. Owners of patients should be warned to pay attention to signs of hypoglycemia such as seizures, lying down, anorexia, tremors, vomiting, ataxia and lethargy. In such a case, the first application to be made at home is to apply honey or glucose to the mucous membranes. Ideally, owners should have dextrose gels at home for use in case of hypoglycemia (Rand, 2013)

Severe hypoglycemia is common in cats administered high-dose insulin (>6 U/cat). In such a case, parenteral glucose administration is required in clinics. For this purpose, 50% dextrose solution is initially diluted by half and given intravenously 2-4 ml slowly over 5-10 minutes. In case of euglycemia (return of glucose to normal), clinical findings improve rapidly. The blood glucose concentration is then monitored, after which treatment is continued with 5% dextrose solution to maintain euglycemia. Insulin antagonists such as corticosteroids or glucagon can be used as a continuous infusion when necessary (Rand, 2007)

6. CONCLUSİON

Diabetes mellitus in cats is more common than in the past due to the increase in living indoors in recent years. Knowing the cause of this process is a very important criterion for the success of the treatment. It is very important to make a full differential diagnosis and to use advanced diagnostic methods for the diagnosis of diabetes mellitus. In the treatment, the choice of insulin, its dose and frequency of administration, and the use of drugs such as oral hypoglycemics, as well as the content of nutrition and formula, have an important role in the success of the treatment. We believe that our article will lead to better understanding of this difficult process, accurate diagnosis and successful treatment.

7. REFERENCES

1. Aytuğ, N. (2011). Köpek ve Kedilerin İç Hastalıkları Klinik El Kitabı. 1st Ed., 370-378. F. Özsan Matbaacılık Ltd. Sti. Bursa/Türkiye.
2. Basoglu, A., & Sevinç, M. (2004) Evcil Hayvanlarda Metabolik ve Endokrin Hastalıklar. 1. Baskı. Konya, Bahcıvanlar Basım Sanayi. 263-304. 2004.
3. Behrend, E., Holford, A., Lathan, P., Rucinsky, R., & Schulman, R. (2018) 2018 AAHA diabetes management guidelines for dogs and cats. *Journal of the American Animal Hospital Association*, *54*(1), 1-21.
4. Bloom, A.C., & Rand, J. (2014). Feline Diabetes Mellitus Clinical use of long –acting glargine and detemir. Journal of Feline Medicine and Surgery. 16, 205-215.
5. Carrera-Boada, A.C., & Martinez-Moreno, M.J. (2013). Pathophysiology of diabetes mellitus type 2: beyond the duo "insulin resistance secretion deficit. Nutr Hosp,. 28(Supl.2): 78-87.
6. Clark, M., & Hoeni, M. (2021). Feline comorbidities: Pathophysiology and management of the obese diabetic cat. *Journal of Feline Medicine and Surgery*, *23*(7), 639-648.
7. Crenshaw, L.K., & Peterson, E.M. (1996) Pretreatment clinical and laboratory evaluation of cats with diabetes mellitus: 104 cases (1992-1994). JAVMA, 209, 5, 943-949.
8. Davison, J.L. (2015). Diabetes mellitus and pancreatitis- cause or effect ? Journal of Small Animal Practice. 56, 50-59.
9. Elliot, D.A. (2005). Feline diabetes mellitus – an update. North American Veterinary Conference. 8-12 Jan 2005, Orlando Florida USA.
10. Ford, S.L., & Lynch, H. (2013). Practical use of home blood glucose monitoring in feline diabetics. Vet Clin Small Animal; 43 : 283-301.
11. Gilor, C., Rudinsky, J.A., & Hall, J.M. (2016) New Approaches to Feline Diabetes Mellitus: Glukagon-like peptide-1 analogs. Journal of Feline Medicine and Surgery, 18, 733-743.
12. Gottlieb, S., & Rand, J. (2018). Managing feline diabetes: current perspectives. *Veterinary Medicine: Research and Reports*, *9*, 33. 2018.
13. Greco DS. Diabetes mellitus in animals: diagnosis and treatment of diabetes mellitus in dogs and cats. In *Nutritional and Therapeutic Interventions for Diabetes and Metabolic Syndrome* (pp. 507-517). Academic Press.
14. Hay, D.L., Chen, S., Lutz, T.A., Parkes, D.G., & Roth, J.D. (2015). Amylin: pharmacology, physiology, and clinical potential. *Pharmacological reviews*, *67*(3), 564-600.

15. Herndon, M.A., Breshears, A.M., & McFarlane, D. (2014). Oxidative Modification, Inflammation and Amyloid in the Normal and Diabetic Cat Pancreas. J. Comp. Path. 151, 352-362.
16. Herrtage, M.E. (2009). New strategies in management of feline diabetes mellitus . 34th World Small Animal Veterinary Congress , 2009, Sao Paulo Brazil.
17. Hoelmkjaer, M.K., & Bjornvad, R.C. (2014). Management of obesity in cats. Veterinary Medicine: Research and Reports, 5: 97-107. 2014
18. Hoenig, M., Thomaseth, K., Brandao, J., Waldron, M., & Ferguson, D.C. (2006). Assessment and mathematical modeling of glucose turnover and insulin sensitivity in lean and obese cats. *Domestic animal endocrinology*, *31*(4), 373-389.
19. Hoenig, M. (2014). Comparative Aspects of Human, Canine, and Feline Obesity and Factors Predicting Progression to Diabetes. Vet. Sci., 1: 121-135.
20. Jotha-Mattos, L., Vieira, A.B., da SM Castelo, M., Queiroz, A.D.M., de Souza. H.J.M., de Alencar, N.X., & Lima, L.M.T.R. (2021) Amyloidogenesis of feline amylin and plasma levels in cats with diabetes mellitus or pancreatitis. *Domestic Animal Endocrinology*, *74*, 106532.
21. Kerl, E.M. (2001) Diabetic Ketoacidosis: Pathophysiology and Clinical and laboratory Presentation, Small Animal/Exotics Compendium, 23, 3, 220-229.
22. Kerner, J., & Hoppel, C. (2000). Fatty acid import into mitochondria. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1486*(1), 1-17.
23. Kleinert, M., Clemmensen, C., Hofmann, S.M., Moore, M.C., Renner, S., Woods, S.C., & Tschöp, M.H. (2018). Animal models of obesity and diabetes mellitus. *Nature Reviews Endocrinology*, *14*(3), 140-162.
24. Lutz, T.A., Rand, J.S., & Ryan, E. (1995). Fructosamine concentrations in hyperglycemic cats. *The Canadian Veterinary Journal*, *36*(3), 155.
25. Malerba E., Mazzarino M., Del Baldo F., Corradini S., Carotenuto G., Giunti M., & Fracassi F. (2019). Use of lispro insulin for treatment of diabetic ketoacidosis in cats. *Journal of feline medicine and surgery*, *21*(2), 115-123.
26. Miceli DD., Vidal PN., Pompili GA., Castillo VA., Soler Arias EA., & Niessen S.J. (2021). Diabetes mellitus remission in three cats with hypersomatotropism after cabergoline treatment. *Journal of Feline Medicine and Surgery Open Reports*, *7*(1),

27. Nelson, R.W., & Reusch, C.E. (2014). Animal models of disease: classification and etiology of diabetes in dogs and cats. *Journal of Endocrinology*, *222*(3), T1-T9.
28. O'neill, D.G., Gostelow, R., Orme, C., Church, D.B., Niessen, S.J.M., Verheyen, K., & Brodbelt, D.C. (2016). Epidemiology of diabetes mellitus among 193,435 cats attending primary-care veterinary practices in England. *Journal of Veterinary Internal Medicine*, *30*(4), 964-972.
29. Osto, M., Zini, E., Reusch, E.C., & Lutz, A.T. (2013). Diabetes from human to cats. General and Comparative Endocrinology, 182, 48-53.
30. Öhlund, M., Fall, T., Holst, S.B., Hansson-Hamlin, H., Bonnett, B., & Egenvall, A. (2015). Incidence of Diabetes Mellitus in Insured Swedish Cats in Relation to Age, Breed and Sex. J Vet Intern, 29: 1342-1347.
31. Palm, C.A. (2013). Oral hypoglycemics in cats with diabetes mellitus. Vet Clin Small Animal; 43: 407-415.
32. Pérez-López, L., Boronat, M., Melián, C., Saavedra, P., Brito-Casillas, Y., & Wägner, A.M. (2019). Assessment of the association between diabetes mellitus and chronic kidney disease in adult cats. *Journal of veterinary internal medicine*, *33*(5), 1921-1925.
33. Prakash, S. (2018). Beta (β)-Oxidation of Fatty Acid and its associated Disorders. *Int. J*, *5*(1), 158-172.
34. Rand, J.S. (2007). Adventages in management of feline diabetes mellitus. North American Veterinary Conference. 13-27 Jan 2007, Orlando Florida USA.
35. Rand, J.S. (2013). Diabetic Ketoacidozis and hyperosmolar hyperglicemic state in cats. Vet Clin Small Animal; 43: 367-379.
36. Reusch, C.E., & Haberer, B. (2001). Evaluation of fructosamine in dogs and cats with hypo-or hyperproteinaemia, azotaemia, hyperlipidaemia and hyperbilirubinaemia. *Veterinary Record*, *148*(12), 370-376.
37. Reusch, C.E., Liehs, M.R., Hoyer, M., & Vochezer, R. (1993). Fructosamine: a new parameter for diagnosis and metabolic control in diabetic dogs and cats. *Journal of Veterinary Internal Medicine*, *7*(3), 177-182.
38. Reusch, C.E. (2012). Diagnosis and management of feline diabetes mellitus . Southern European Veterinary Conference and Congreso Nacional de AVEPA. 18-21 Oct 2012, Barcelona Spain.
39. Reusch, C.E. (2013). New incretin hormonal therapies in humans relevant to diabetic cats. Vet Clin Small Animal; 43 : 417-433.

40. Richter, M., Guscetti, F., & Spiess, B. (2002). Aldose reductase activity and glucose-related opacities in incubated lenses from dogs and cats. *American journal of veterinary research*, *63*(11), 1591-1597.
41. Salesov, E., Zini, E., Riederer, A., Lutz, T.A., & Reusch, C.E. (2018). Comparison of the pharmacodynamics of protamine zinc insulin and insulin degludec and validation of the continuous glucose monitoring system iPro2 in healthy cats. *Research in veterinary science*, *118*, 79-85.
42. Scott-Moncrieff, C.J. (2010). Insulin Resistance in Cats, *Veterinary Clinics of North America: Small Animal Practice,* Volume 40, Issue 2, 241-257.
43. Scudder, CJ., Hazuchova K., Gostelow R., Church, DB., Forcada Y., Fowkes RC., & Niessen SJ. (2021). Pilot study assessing the use of cabergoline for the treatment of cats with hypersomatotropism and diabetes mellitus. *Journal of Feline Medicine and Surgery*, *23*(2), 131-137.
44. Sears KW, Drobatz KJ and Hess RS. Use of lispro insulin for treatment of diabetic ketoacidosis in dogs. J Vet Emerg Crit Care 2012; 22: 211–218.
45. Slingerland, I.L., Fazılova, V.V., Plantinga, A.E., Kooistra, S.H., & Beynen, C.A. (2009). Indoor confinement and physical inactivity rather than the proportion of dry food are risk factors in the development of feline type 2 diabetes mellitus. The Veterinary Journal 179, 247-253.
46. Sparkes, H.A., Cannon, M., Church, D., Fleeman, L., Harvey, A., Hoenig. M., Peterson, E.M., Reusch, E.C., Taylor. S., & Rosenberg, D. (2015). ISFM Consensus Guidelines on the Practical Management of Diabetes Mellitus in Cats. Journal of Feline Medicine and Surgery, 17, 235-250.
47. Tarkosova, D., Story, M.M., Rand, S.J., & Svoboda, M. (2016). Feline obesity-prevalence, risk factors, pathogenesis, associated conditions and assessment: a review. Veterinarni Medicina, 61, 6: 295-307.
48. Turgut, K., & Ok, M. (2001). Kedi ve Köpek Gastroenterolojisi. 1. Baskı. Konya, Bahcıvanlar Basım Sanayi, 547-579. 2001.
49. Verbrugghe, A., Hesta, M., Daminet, S., & Janssens, J.P.G. (2012). Nutritional Modulation of Insulin Resistance in the True Carnivorous Cat: A Review. Critical Reviews in Food Science and Nutrition, 52: 172-182.
50. Zoran, D.L., & Rand, J.S. (2013). The role of diet in prevention and management of feline diabetes. Vet Clin Small Animal; 43: 233-243.
51. Zoran, D.L. (2005) Management of diabetes mellitus. North American Veterinary Conference. 8-12 Jan 2005, Orlando Florida USA.

Effects Of Pregnancy Yoga

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Introductıon

Although pregnancy is a physiological event, it is a miraculous event in which many physical and psychological changes occur. Pregnant women may encounter problems such as fatigue, low back pain, varicose veins, edema, insomnia, nausea, vomiting, hemorrhoids, headache, feeling of fear and mood disorders. Yoga practice can be used for pregnant women to spend their pregnancy and postpartum periods more comfortably. Because it is known that yoga has many benefits during pregnancy, childbirth and postpartum period.Yoga is a mind-body practice that includes deep breathing, posture, and meditation. Although there is not much literature on yoga during pregnancy, it continues to increase. It is stated that yoga regulates the nervous system and physiological functioning of the body, provides physical fitness and improves psychological well-being. Studies have also shown that yoga provides benefits during pregnancy in conditions such as anxiety, depression, low back pain and sleep disorders, and positively affects mother-infant attachment, birth and postpartum process. However, there are few studies that have found improvement in the outcomes of high-risk pregnancies. Positive outcomes, applicability, and affordability of yoga during pregnancy, birth and postpartum period are stated in studies. In this chapter; It is aimed to present the effects of yoga practice in pregnant women on physiological, psychological and birth in line with the literature. Being aware of the positive effects on pregnancy, birth and postpartum processes will make a significant contribution to the better health outcomes of the mother and baby by enabling the use of yoga practices. It is thought that health personnel working in this field, especially midwives/nurses, intensify their training and studies in this field for pregnant women, and new researches will be of great benefit.

Physiological and Psychological Effects of Pregnancy Yoga

Yoga practice during pregnancy has many physiological and psychological positive effects on mothers. In the study of Chen et al (2017), it was observed that yoga during pregnancy reduced salivary cortisol levels. Kumar et al. (2016) reported in a randomized controlled study that yoga practice 3 times a week for 8 weeks improved low back pain and disability due to low back pain better than topical gel and heat methods. In a study evaluating the effect of yoga on salivary amylase levels and sleep parameters, it was found that it significantly reduced salivary α-amylase levels and significantly extended nighttime sleep time (Hayasa and Shimada, 2018). Hayasa and Shimada (2018) conducted a prospective study with 91 pregnant women to determine the effect of yoga on instantaneous changes in the nervous system and how maternal yoga affects stress and sleep. Salivary α-amylase levels were significantly reduced during all

assessment periods in the yoga group and immediately after yoga. Akarsu and Ratfisch (2018) examined the psychosocial health and prenatal attachment levels of 63 women at 14-16 gestational weeks by practicing yoga twice a week for two months. After the application, the psychosocial health and prenatal attachment levels of the yoga group were found to be significantly higher than the control group. Sharma (2018) conducted a study on 20 women to determine the psychological effects of prenatal yoga practice on pregnant women, and it was found that yoga exercises were beneficial in relieving anxiety, depression and stress during pregnancy.

Novelia et al (2018) performed 90 minutes of yoga twice a week on 30 pregnant women in their third trimester to determine the effects of yoga on anxiety levels in pregnant women. After the application, the anxiety level of the yoga group was found to be significantly lower than the control group. It has been concluded that yoga reduces the anxiety levels of pregnant women in the third trimester. Yuluanti et al (2018) conducted a randomized controlled study on 102 women to determine the effect of prenatal yoga practice on anxiety and depression, and it was determined that the level of anxiety and depression in the yoga group was lower two weeks and four weeks after the yoga practice compared to the control group. Prenatal yoga practice has been shown to reduce anxiety and depression during pregnancy. Hamdiah et al. (2017) examined the effects of prenatal yoga practices on anxiety, blood pressure and fetal heart. It is a three-group study. A randomized controlled trial was conducted on 49 primigravida women at 13-33 weeks of gestation. 4 yoga sessions (60 min once a week) yoga -8 yoga sessions (60 min once a week) yoga is standard obstetric care. It was determined that anxiety and systolic blood pressure rates decreased significantly in the group participating in 8 sessions of yoga practice, while fetal heart rate increased. It was concluded that prenatal yoga practice in primigravidas has significant effects on anxiety level, systolic blood pressure and fetal heart rate.

Newham et al. (2014) in order to determine the effect of pregnancy yoga on maternal anxiety. In a randomized controlled study on 51 women in their trimesters, 8 weeks of yoga practice was applied to the experimental group. Anxiety, depression, and cortisol levels were looked at. It has been concluded that prenatal yoga is beneficial in reducing the anxiety and depressive symptoms of women about childbirth. Abolghasem et al (2014) In order to determine the effects of pregnancy yoga on pregnancy characteristics and anxiety, 8 weeks, 90 minutes twice a week. Yoga and standard obstetric care were provided. Anxiety levels were found to be significantly reduced in the yoga group.

Bershadsky et al. (2014) conducted a study to determine the effect of prenatal yoga on cortisol and depressive symptoms, and it was determined that cortisol

levels in the yoga group were lower on yoga days compared to normal activity days. was found to be somewhat reduced.

In the study conducted by Satyapriya et al. in 2009 on perceived stress during pregnancy, yoga and deep relaxation technique were compared with standard prenatal exercises; At the end of the study, a significant decrease was observed in the yoga group, while stress increased by 6.60% in the control group. In another study by Chen et al., prenatal yoga and routine prenatal care were compared and their effects on stress were examined; It was determined that the stress levels of the pregnant women in the yoga group decreased significantly compared to the control group. In a randomized controlled study, it was found that yoga positively affected psychosocial well-being, and in another review and other studies, pregnancy yoga increased the quality of life of pregnant women and reduced stress. Depression is common during pregnancy, affecting 10-49% of women. In the study conducted by Khalajzadeh et al., it was determined that anxiety was lower in the experimental group after yoga applied to 2nd and 3rd trimester pregnant women for 8 weeks, 2 days a week and 60 minutes, and it was reported that yoga is an effective method to reduce anxiety during pregnancy. In another study, the effects of yoga and social support control group on prenatal depression and anxiety were examined, and at the end of the study, improvement was observed in both groups. In a systematic review, it was stated that yoga reduces pain and stress and is more effective than standard prenatal exercises and walking. As a result of the study conducted by Battle et al., it was seen that yoga significantly reduced the severity of depression and was a viable method. In the study of Davis et al., yoga therapy and standard treatment were compared and their effects on depression and anxiety were examined; It was found that both groups significantly reduced the symptoms of depression and anxiety, but when the groups were compared among themselves, no significant difference was observed. In the study in which prenatal hatha yoga was compared with standard care, depression symptoms were evaluated during pregnancy and postpartum period; Yoga has been found to improve mood at the moment. Studies have shown that prenatal attachment level is higher in pregnant women who exercise and do relaxation exercises.

Effects of Yoga on Childbirth

Birth pain is known to be unique to each mother and is a complex and multifaceted event. The methods recommended for labor pain are divided into two as pharmacological and non-pharmacological, and while they are preferred, they are primarily reliable, simple, accessible and do not harm the mother and baby physiology. In this context, studies have been conducted to examine the

effects of yoga practice, which includes breathing relief, exercise and deep relaxation, on childbirth. In the study investigating the effect of yoga on labor pain; It was determined that pregnant women who applied yoga program felt higher maternal comfort and suffered less pain during and up to 2 hours after birth compared to the control group, and there was a significant decrease in the total duration of labor. In addition, it was stated that women in the yoga group had significantly less labor pain. In another randomized controlled study, antenatal yoga program was compared with the control group. When the cervical dilatation reached 3-4 cm and 2 hours after birth, the pain felt in the experimental group was found to be significantly less. In addition, the delivery period was found to be shorter in the experimental group. In the study conducted by Narendran et al., the group that took yoga exercises, breathing techniques and meditation and the control group that walked for 30 minutes twice a day were evaluated. It was observed that the number of people with a birth weight of 2500 g or higher in the yoga group was significantly higher than the control group, and the rates of preterm birth, intrauterine growth retardation and pregnancy-induced hypertension were significantly lower. The effects of yoga on the postpartum period In the process where some situations experienced during pregnancy return to the past and some situations develop, the increased estrogen, progesterone and cortisone levels in the endocrine system decrease to normal levels, while the increase in prolactin level creates a difficult life period for the mother to transition to new roles and responsibilities. While many women easily adapt to the physiological, psychological and social changes that occur during this period, they may experience different emotional problems in women who have difficulty in adapting and cannot. When we look at the literature on the postpartum effects of yoga, it is seen that there are fewer studies related to childbirth compared to pregnancy. In the study of Ko et al; While there was a significant decrease in body weight, body fat ratio and fat mass in depression, no significant difference was observed in fatigue symptoms. In the study conducted by Buttner et al., the effects of yoga on postpartum depression were examined; Depression symptoms were significantly lower, anxiety measures decreased, and the improvement in well-being and quality of life was significantly faster in women in the yoga group.

Conclusion

It is stated that yoga is effective in improving physiological and psychological symptoms and modulating the autonomic nervous system, especially the sympathetic nervous system. In addition, it has been observed that yoga is effective in preventing both stress and stress-related diseases. It was determined that yoga significantly reduced anxiety and depression scores. Similarly, in other

studies evaluating the effectiveness of yoga in psychological symptoms, it is stated that yoga reduces stress, anxiety and depressive symptoms compared to standard antenatal care. It is stated that yoga increases the oxygen level in the body, increases blood flow and prevents hypertension, thanks to breath awareness. However, more studies with a high level of evidence are needed.

As a result, pregnancy yoga helps to protect the physical, mental and spiritual integrity of the pregnant by positively affecting the physical and psychological changes experienced during pregnancy.

References

1. Abolghasemi, S., Azizi, S., Fard, M. A., Behzadi, S., & Bakhtjo, E. (2014). The effect of yoga approaches on pregnant women anxiety. *Kuwait Chapter of Arabian Journal of Business and Management Review*, *3*(11), 206-211.
2. Akarsu, R.H., & Rathfisch, G. (2018). The effect of pregnancy yoga on the pregnant's psychosocial health and prenatal attachment. *IJTK*, *17*(4), 732-740.
3. Alkan E., Aslantekin Özçoban F. Yoganın Gebelik, Doğum ve Doğum Sonuçları Üzerine Etkisi. *Smyrna Tıp Dergisi 2017:65*
4. Battle CL, Uebelacker LA, Magee SR et al. Potential for prenatal yoga to serve as an intervention to treat depression during pregnancy. Womens Health Issues 2015;25(2):134-41.
5. Bershadsky, S., Trumpfheller, L., Kimble, H.B., Pipaloff, D., & Yim, I.S. (2014). The effect of prenatal Hatha yoga on affect, cortisol and depressive symptoms. *Complementary Therapies in Clinical Practice*, *20*(2), 106-113.
6. Buttner MM, Brock RL, O'Hara MW, Stuart S. Efficacy of yoga for depressed postpartum women: A randomized controlled trial. Complement Ther Clin Pract 2015;21(2):94-100.
7. Chen PJ, Yang L, Chou CC et al. Effects of prenatal yoga on women's stress and immune function across pregnancy: A randomized controlled trial. Complement Ther Med 2017;31:109-17.
8. Esencan Y, Tuğba., Şimşek Ç. Nursing care during the postpartum period. Zeynep Kamil Tıp Bülteni 2017;48(4):131-4.
9. Höbek-Akarsu, R., ve Rathfisch, G. (2019). Gebelik yogasının fizyolojik ve psikolojik etkileri: Bir sistematik derleme. Spor Bilimleri Araştırmaları Dergisi, 4(1), 43-53.
10. Hamdiah, H., Suwondo, A., Hardjanti, T. S., Soejoenoes, A., & Anwar, M. C. (2017). Effect of prenatal yoga on anxiety, blood pressure, and fetal heart rate in primigravida mothers. Belitung Nursing Journal, 3(3), 246-254.
11. Hayase, M., & Shimada, M. (2018). Effects of maternity yoga on the autonomic nervous system during pregnancy. Journal of Obstetrics and Gynaecology Research, 44(10), 1887-1895.
12. Ko YL, Yang CL, Fang CL et al. Community-based postpartum exercise program. J Clin Nurs 2013;22(15-16):2122-31.

13. Kumar, P. B., Shetty, H., Biliangady, H., & Kumar, D. (2016). Efficacy of yoga on low back pain & disability in primi gravidas. International Journal of Physiotherapy, 3(2), 182-185.
14. Narendran S, Nagarathna R, Gunasheela S, Nagendra HR. Efficacy of yoga in pregnant women with abnormal doppler study of umbilical and uterine arteries. Journal of the Indian Medical Association 2005;103(1):12-4.
15. Newham, J.J., Wittkowski, A., Hurley, J., Aplin, J. D., & Westwood, M. (2014). Effects of antenatal yoga on maternal anxiety and depression: A Randomized controlled trial. Depression and Anxiety, 31(8), 631-640
16. Novelia, S., Sitanggang, T. W., & Yulianti, A. (2018). Effects of yoga relaxation on anxiety levels among pregnant women. *Nurse Media Journal of Nursing*, *8*(2), 86-95.
17. Sharma, A., Sharma R., & Noheria, S. (2018). Effects of prenatal yogic exercises on psychological parameters of pregnant women during 2 nd and 3 rd trimester. Asian Resonance, 7(4), 248-250.
18. Sharma, R., Sharma, A., & Noheria, S. (2018). Prenatal yoga and it's effect on blood O2 saturation level of pregnant women. Remarking An Analisation, 3(8), 112- 113.
19. Sun YC, Hung YC, Chang Y, Kuo SC. Effects of a prenatal yoga programme on the discomforts of pregnancy and maternal childbirth self-efficacy in Taiwan. Midwifery 2010;26(6):e31-6.
20. Taneja, D. K. (2014). Yoga and health. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine, 39(2), 68-72. Doi: 10.4103/0970-0218.132716.
21. Youngwanichsetha S, Phumdoung S, Ingkathawornwong T. The effects of mindfulness eating and yoga exercise on blood sugar levels of pregnant women with gestational diabetes mellitus. Appl Nurs Res 2014;27(4):227-30.
22. Yulianti, I., Respati, S. H., & Sudiyanto, A. (2018). The effect of prenatal yoga on anxiety and depression in Kudus, Central Java. Journal of Maternal and Child Health, 3(2),100-104. Doi: 10.26911/thejmch.2018.03.02.02.
23. Wren, A.A., Wright, M.A., Carson, J.W., & Keefe, F.J. (2011). Yoga for persistent pain: New findings and directions for an ancient practice. *Pain*, *152*(3), 477–480. Doi: 10.1016/j.pain.2010.11.017.

Abutment Types Used in Implant Supported Prostheses

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INTRODUCTION

Today, implant-supported prosthetic restorations have become a common treatment option in the treatment of lost teeth. In order for implant-supported prosthetic restorations to be considered successful, the abutment material to be used in the preparation of the implant-supported prosthesis must be biologically and mechanically superior, as well as meeting aesthetic expectations after the implant is properly osteointegrated. Dental implant is prosthetic device made of alloplastic material implanted into the oral tissues beneath the mucosal and/or periosteal layer and on or within the bone to provide retention and support for a fixed or removable dental prosthesis; a substance that is placed into and/or on the jaw bone to support a fixed or removable dental prosthesis.[1,2]

Biomechanical factors play a substantial role in implant success or failure. In order to achieve optimized biomechanical conditions for implant-supported prostheses, conscientious consideration of the biomechanical factors that influence prosthesis success is essential. The abutments which is a connection component that is intermediate between the implant and the restoration have an important key effect on achieving the success. The dental implant abutment is the supplemental component of a dental implant that is used to support and/ or retain any fixed or removable dental prosthesis.[1]

According to the dictionary of prosthetic terms, abutment; tooth, part of the tooth and prosthesis; It is the part that serves to support and protect the prosthesis. The part of the implant that opens to the oral environment is called the abutment. It is used to provide retention, ideal exit profile and support for implant-supported prostheses. Currently, there are three types of dental implant abutments: screw-retained, attachment-retained, and cement-retained.[3] Cement retained implant abutments are used more than others.[4]

It is the prosthesis made on the implant that makes an implant functional and completes it. An implant placed in the jawbone not only provides support to the prosthesis, but also maintains the continuity and health of the remaining tissues.[5]

DENTAL IMPLANT COMPONENTS

Misch and Misch created a general language for intraosseous implants in 1992. The language they created is presented according to the placement chronology of the restoration. While formulating the terminology, they referenced the 5 most commonly used implant systems in the United States. Accordingly, the components that make up a dental implant are as follows:[6,8]

- Prosthetic screw
- Abutment

- Transfer coping
  - Direct transfer coping
  - Indirect transfer coping
- Prosthetic coping
- Analogue
- Abutment analog
- Implant body analog
- Implant body

Dental Implant Abutment

The implant component that contributes to the retention of the prosthesis that supports fixed or removable implant prostheses is called abutment.[7]

There are many abutment types produced by different companies for the determined treatment plan and the type of prosthesis to be applied.[8,9]

In an article published in 2000, it was reported that there were 1300 implants and 1500 abutment types with different diameters, lengths, sizes, surface properties and connection types prepared from different materials in dentistry.[10]

I-Classification of Abutments by Connection Type with Implant

At the interface between the abutment and the implant, there is a platform where the abutment can sit on the implant and provide physical retention against incoming axial forces. On these platforms, parts called external hexagons have been added to enable the implant to resist rotational forces. These units can be on the platform as well as inside, in which case they are named with names such as internal hexagon, internal groove (groove), morse taper according to their geometric shapes.[11]

When examined clinically, it is not possible to say that one of the internal and external hexagonal structures is superior to the other. Because both systems are used successfully all over the world.[12-14]

However, it is possible to talk about the advantages and disadvantages of these two systems compared to each other.[14]

1-External hexagon system

Advantages of the external hexagon system

a) It is suitable for implant surgery performed in two stages.
b) It has anti-rotational mechanism
c) It is compatible with different implant systems.
d) Top parts can be changed easily

Disadvantages of the external hexagon system[14]

a) Depending on the size of the hexine, micromovement may occur.

b) It shows less resistance to lateral and rotational forces because the center of rotation is higher. As a result, the probability of microleakage increases.

2- Internal hexagon system

Advantages of the internal hexagon system[14]

a) Abutment implant connection is very easy.

b) Better stability and anti-rotation ability are obtained with the large contact surface in the connection area.

c) Suitable for one-stage implant surgery

d) Stress distribution is fairly balanced

e) Lateral loading shows high resistance as the center of rotation is closer to the marginal bone.

Disadvantages of the internal hexagon system

a) The junction of the lateral walls of the implant is thinning.

b) There are difficulties in solving the angle problems between implants.

II-Classification of Abutments by Use

1- Temporary Implant Abutments

Temporary abutments are produced from titanium or plastic by the manufacturers in accordance with the construction of temporary restorations. In addition to being used as they are produced, they can be personalized to create the appropriate tooth and gingival contour, especially in the aesthetic region. These abutments help the dentist to provide the appropriate exit profile and aesthetics before the permanent restoration, to prevent the mistakes to be made before the permanent restoration and to prevent patient dissatisfaction. Temporary abutments can be subdivided into healing abutments, impression abutments, and metal or plastic abutments used in temporary restorations.[3,10,15]

2-Permanent Implant Abutment

Permanent implant abutments are the type of abutment used to support the final restoration to be made. Permanent abutments can also be examined according to the design of the implant-abutment connection, the material used, the production method, and the type of restoration to be made.[3,15]

III- Classification of Abutments by Prosthesis Retention Type

Cemented or screw-retained systems can be used for the retention of the implant-supported fixed prosthetic superstructure. While making this choice, interocclusal distance, periodontal tissues, occlusion, aesthetic and economic factors are taken into consideration.[16,17]

1- Cemented Abutment

In the cement-retaining system, the prosthetic superstructure is fixed to the abutment with the help of cement. Cemented abutments are preferred especially in the anterior region where aesthetics is at the forefront. However, it is also preferred to ensure correct positioning of implants that are not placed at the appropriate angle, as well as in cases where easier control of occlusion is desired without a screw hole, for example in narrow diameter crowns.[18]

The most important advantages of cement-bonded implant supported suprastructures are passive fit, ease of application, aesthetics, low cost, less screw loosening and breakage.[17,19,20]

The most important disadvantage of cemented abutments is that they need to be removed by cutting as a result of any loosening of the abutment screw after cementation. In addition, there is a risk of residual cement overflowing from the margins under the gingiva.[21,22]

2- Screw Abutment

In screw-connected systems, the prosthetic superstructure is fixed to the abutment with screws. Screw-retained implant-supported prostheses are preferred to provide the necessary retention in cases where the interocclusal distance is not sufficient.[16,18]

In screw holder systems, abutments with occlusal screws or transversal screws are generally used. In screw holder systems, it is recommended to check the screw periodically. The most important disadvantage of the system is the difficulty of obtaining a passively fitting superstructure prosthesis. In addition, problems such as screw loosening, screw breakage and screw loss may be encountered.[17,21,22]

3-Abutment with Attachment Retainer

It is a type of abutment used in the production of overdenture type removable prostheses using a limited number of implants. There are different types on the market such as ball head, O-ring, gold or titanium clips. In recent years, locator abutments have been frequently used in the construction of overdenture prostheses and it has been seen that they have replaced ball-head abutments.[8,23]

IV- Classification of Abutments According to Production Types

If abutments are supplied by implant companies as fabricated, they are called standard/stock abutments, and if they are produced personally in the laboratory, they are called custom abutments.[24,25]

1- Standard (stock) Abutment

Stock abutments are fabricated abutments, usually made of titanium or zirconia, by the manufacturer. Stock abutments are used and modified by the dentist or technician to provide a suitable exit profile and adequate retention for the fixed prosthetic restoration to be prepared. They are routinely used in the construction of implant-supported restorations. These abutments can be produced from titanium or noble metal alloys using CAD/CAM devices, as well as from zirconium or alumina materials.[26,27]

The most important advantage of standard abutments is their low cost because they can be produced prefabricated. Another advantage of standard abutments is that they can be easily prepared intraorally by the dentist or extraorally by the technician. Production of prefabricated abutments as cylindrical is one of the main disadvantages.[28]

2-Custom Abutment

Custom abutments are preferred in cases where there is insufficient interocclusal distance, angulation problems of the implant, and where the original cross-sections of teeth and soft tissue need to be imitated to provide an ideal exit profile. In order to obtain a personal abutment, it is necessary to measure the implant platform at the implant or tissue level by means of impression pieces. These abutments are more costly than prefabricated abutments.[29]

However, custom abutments are superior to stock abutments in terms of providing ideal crown contour, aesthetics and soft tissue support. They are preferred when standard abutments are insufficient.[26]

Conventional methods or CAD-CAM systems can be used for custom abutment production. Zirconia or titanium blocks are generally preferred for abutment production using the CAD-CAM system.[30,31]

V- Classification of Abutments by Material Type

For a successful implant treatment, after the implant is properly osteointegrated, the abutment material to be used must meet some biological, mechanical and aesthetic requirements.[32,33]

1- Titanium Abutment

Today, titanium abutments are the most preferred abutment material due to their resistance to distortion, excellent material stability, mechanical and biological advantages proven by long-term clinical studies for implant-supported prostheses.[34]

The fact that the abutments produced by the manufacturers as prefabricated are mostly made of titanium alloy, their affordable costs, and the practicality of

clinical and laboratory stages have a significant effect on this popularity of titanium abutments.[35]

Due to its superior mechanical properties, titanium alloy is considered as the first abutment option by dentists, especially for implants in the molar region. However, in cases where titanium abutments have thin gingiva and the implant is placed towards the buccal, the reflected gray color of the surrounding mucosa limits the use of these abutments.[36]

2-Ceramic Abutment

With the increase in aesthetic expectations of patients in dentistry, dentists have turned to the use of all-ceramic restorations. However, the use of tooth-colored esthetic ceramic abutments is required in order for all-ceramic restorations to be applied in a way that provides appropriate esthetics on implant prostheses.[37]

Ceramic abutments are used especially in cases where the gingiva is thin and transparent, in cases with high smile lines and in cases where full ceramic restorations are required due to aesthetic needs. Since the fracture resistance of ceramic abutments is not as high as metal abutments, they are recommended to be used only in the anterior region and in the construction of single tooth restorations.[38]

3-Zirconia Abutment

Zirconia is increasingly used in dentistry due to its positive properties such as low thermal conductivity, aesthetics, millability, biocompatibility, high bending strength and fracture strength.[39]

Zirconia abutments are available to offer an alternative to the aesthetic disadvantages of titanium abutments. In patients with superficial implant placement and thin gingival thickness, the metal color of the titanium abutment reflected from the gingiva may adversely affect the aesthetics. In such cases, it has been stated that more aesthetic results can be obtained with the use of zirconia abutments.[40,41]

Studies have reported that zirconia has less plaque accumulation compared to titanium, and that no complications related to fracture were experienced in zirconia abutments in the short term in clinical follow-ups.[42-44]

4-Polyetheretherketone(PEEK) Abutment

With its semi-crystalline structure, PEEK material is a strong thermoplastic material. PEEK material, which has high temperature resistance and good mechanical and electrical properties, also resists hydrolysis. Thanks to these

features, PEEK has been widely used in the automotive, aircraft, chemical and electronic industries for nearly three decades.[45]

PEEK is biocompatible, its hardness and durability are very good. It has an aesthetic advantage due to its white color. They can be easily prepared and modified by the dentist at the chairside. PEEK material is an excellent alternative to titanium in medical orthopedic applications. It is used in implant healing abutment, temporary abutment, removable prosthesis, implant supported bar or orthodontic bite rods in dentistry.[46]

VI- Classification of Abutments by Number of Pieces

1- One-piece Abutment

One-piece abutments, including the implant abutment connection, can be produced entirely with materials such as ceramic, zirconium or titanium.[47]

Titanium has been used as a one-piece abutment material for many years with its superior mechanical properties and long-term success proven by clinical studies.[34]

However, in restorations where titanium abutments are used, the implant should be placed below the level of the alveolar crest to ensure proper aesthetics. Intensely sintered alumina has been introduced as a new abutment material, especially for the search for aesthetic material in the anterior region.[48]

However, in the following years, polycrystalline zirconia abutments, partially stabilized with yttrium, have been introduced to the market against the low mechanical resistance of this material and the high risk of fracture. When the zirconium material was examined, it was determined that it showed high biocompatibility and local and systemic side effects were not encountered in the studies.[49,50]

However, in zirconium abutments placed on titanium implants, wear and fatigue may occur in the implant body and implant screw due to the difference in hardness and elastic modulus between titanium and zirconium.[51,52]

2-Two Piece Abutment (Hybrid Abutment)

Due to the mechanical disadvantages of one-piece ceramic abutments used for aesthetic purposes, the idea of hybrid abutment has been suggested as a result of the studies on combining the durability of titanium and the aesthetic properties of ceramic materials. Hybrid abutment is obtained by gluing a Ti-base and Ti-base individually produced in a CAD/CAM device with a resin cement, which is screwed to the implant.[53]

Hybrid abutments have improved mechanical properties compared to single-piece ceramic abutments. Therefore they have higher fracture resistance.[54]

CONCLUSION

Dental implants are accepted as a valuable option for the restoration of missing teeth and various edentulous sites. Abutments play a central role in the functional and aesthetic aspects of implant treatment. Choosing the right abutment is critical to produce mechanically stable and aesthetically pleasing prosthetic restorations.Many different abutment options are offered to the market by the manufacturers, and this diversity makes it difficult for the dentist to choose. Knowledge of abutment selection for implant-supported fixed prosthetic restorations can help facilitate the dentist's choice of abutment, which is a challenging decision in clinical practice.

REFERENCES

1.Ferro, Keith J. et al. The Glossary of Prosthodontic Terms Ninth Edition Editorial Staff The Journal of Prosthetic Dentistry. 2017;Vol.117 Issue 5S.

2. Moilanen, Pauliina, Jenni Hjerppe, Lippo Lassila, and Timo Närhi. 2018. "Fracture Strength And Precision Of Fit Of Implant Retained Monolithic Zirconia Crowns." Journal of Oral Implantology aaid-joi-D-17-00249.

3.Misch, C.E., 2005, Dental Implant Prosthetics, Elsevier Mosby, St.Louis Missouri, 0-3230195-2.

4.Squier, R. S., J. R. Agar, J. P. Duncan, and T. D. Taylor. "Retentiveness of Dental Cements Used with Metallic Implant Components." The International Journal of Oral & Maxillofacial Implants 2001;16(6):793–98.

5. Mathew, Merin, Kamalakanth Shenoy, and KS Ravishankar. "Flexural Strength of Hydrogen Plasma-Treated Polypropylene Fiber-Reinforced Polymethyl Methacrylate Denture Base Material." The Journal of Indian Prosthodontic Society 2018;18(3):257.

6. Misch, C.E., 1999. Contemporary Implant Dentistry St. Louis. Mosby 2. Baskı.

7. The glossary of prosthodontic terms. J Prosthent Dent. 2005;94 (1):10-92.

8. Misch CE, Misch CM. Generic terminology for endoosseous implant prosthodontics. J Prosthet Dent. 1992;68(5):809-12

9-Abrahamsson I, Berglundh T, Glantz PO, Lindhe J. The mucosal attachment at different abutments: An experimental study in dogs.Journal of clinical periodontology.1998;25(9):721-7.

10. Binon Paul P. *Implants and Components: Entering the New Millennium The International Journal of Oral & Maxillofacial Implants*. 2000;15(1):76-94. [PubMed[]

11.CE M. Dental implant Prosthetics. St. Louis Missouri:Mosby;2005. 32-41. p.

12. Aboyoussef H, Weiner S, Ehrenberg D. Effect of an antirotation resistance form on screw loosening for single implant-supported crowns. The Journal of Prosthetic Dentistry.

13. Cibirka RM, Nelson SK, Lang BR, Rueggeberg FA. Examination of the implant abutment interface after fatigue testing. The Journal of prosthetic dentistry. 2001;85(3):268-75.

14. Maeda Y, Satoh T, Sogo M. In vitro differences of stress concentrations for internal and external hex implant-abutment connections: a short communication. Journal of Oral Rehabilitation. 2006;33(1):75-8.

15. Krennmair G, Seemann R, Weinländer M, Wegscheider W, Piehslinger E. Implant prosthodontic rehabilitation of anterior partial edentulism: a clinical review. Int J Oral Maxillofac Implants. 2011;26(1):1043-50. [PubMed]

16. Lee A, Okayasu K, Wang HL. Screw-versus cement-retained implant restoration: current concepts. Implant Dent. 2010;19(1):8-15. [Crossref] [PubMed])
17. Hebel KS, Gajjar RC. Cement-retained versus screw-retained implant restorations: achieving optimal occlusion and esthetics in implant dentistry. J Prosthet Dent. 1997;77(1):28-35. [Crossref])
18. Wismeijer D, Bragger U, Evans C, Kapos T, Kelly JR, Millen C, et al. Consensus statements and recommended clinical procedures regarding restorative materials and techniques for implant dentistry. Int J Oral Maxillofac Implants. 2014;29 Supple:137-40.
19. Wittneben JG, Joda T, Weber HP, Brägger U. Screw retained vs. cement retained implantsupported fixed dental prosthesis. Periodontol 2000. 2017;73(1):141-51. [Crossref] [PubMed]
20. Nogueira LB, Moura CD, Francischone CE, Valente VS, Alencar SM, Moura WL, et al. Fracture strength of implant-supported ceramic crowns with customized zirconia abutments: a screw retained vs. cement retained. J Prosthodont. 2016;25(1):49-53. [Crossref] [PubMed]
21. Davarpanah M MH, Clinical manuel of implant dentistry. London:Qiuintesence;2004.
22. Palmer RM SB, Howe LC, Palmer PJ. Implants in clinical dentistry. London: Martin Dunitz Ltd;2005.
23. Büttel AE, Bühler NM, Marinello CP. Locator or ball attachment: a guide for clinical decision making. Schweizer Monatsschrift fur Zahnmedizin. Revue mensuelle suisse d'odonto-stomatokogie-Rivista mensile svizzera di odontologia e stomotologia. 2009;119(9):901-18.
24. Karunagaran S, Paprocki G, Wicks r, Markose S. A review of implant abutments-abutment classification to aid prosthetic selection. The Journal of the Tennessee Dental Association.2013;93(2):18-23;quiz -4.
25. Gehrke P, Johannson D, Fischer C, Stawarczyk B,Beuer F. In vitro fatigue and fracture resistance of one-and two-piece CAD/CAM zirconia implant abutments. İnternational Journal of Oral&Maxillofacial Implants. 2015;30(3).
26. Priest G. Virtual-designed and computer-milled implant abutments. Journal of Oral and Maxillofacial Surgery. 2005;63(9):22-32.
27. Fuster-Torres MA. Albalat-Estela S, Alcaniz-Raya M, Penarrocha-Diago M. CAD/CAM dental systems in implant dentistry:update. Med Oral Patol Oral Cir Bucal 2009;14(3):E141-5.
28. Öztürk Ö. Kişiye özel Dental İmplant Abutmentleri. Atatürk Üniversitesi Diş Hekimliği Fakültesi Dergisi.2004; 26(15):73-9.

29. Grossmann Y, Pasciuta M, Finger IM. A novel technique using a coded healing abutment for the fabrication of a CAD/CAM titanium abutment for an implant-supported restoration. J Prosthet Dent. 2006;95(3):258-61. [Crossref] [PubMed]
30. Grossmann Y, Madjar D. Prosthetic treatment for severely misaligned implants: a clinical report. J Prosthet Dent. 2002;88(11):259-62. [Crossref] [PubMed]
31. Marchack CB, Vidjak FM, Futatsuki V. A simplified technique to fabricate a custom milled abutment. J Prosthet Dent. 2007;98(7):416-7. [Crossref]
32. Blatz MB, Bergler M, Holst S, Block MS. Zirconia abutments for single-tooth implants-rationale and clinical guidelines. Journal of oral and maxillofacial surgery. 2009;67(11):74-81.
33. Gomes A-L, Montero J. Zirconia implant abutments: a review. Med Oral Patol Oral Cir Bucal. 2011;16(1):e50-5.
34. Zembic A, Sailer I, Jung RE, Hammerle CHF. Randomized-controllled clinical trial of customized zirconia and titanium implant abutments for single-tooth implants in canine and posterior regions: 3-year results. Clinical oral implants research. 2009;20(8):802-8.
35. Kohal RJ, Att W, Bachle M, Butz F. Ceramic abutments and ceramic oral implants. An update. Periodontology 2000.2008;47(1):224-43.)
36. Jung RE, Sailer I, Hammerle C, Attin T, Schmidlin P. In vitro color changes of soft tissues caused by restorative materials. International Journal of Periodontics and Restorative Dentistry. 2007;27(3):251.
37. Yildirim M, Edelhoff D, Hanisch O, Spiekermann H. Ceramic abutments-a new era in achieving optimal esthetics in implant dentistry. International Journal of Periodontics &Restorative Dentistry. 2000;20(2)
38. Henriksson K, Jemt T. Evaluation of custom-made procera ceramic abutments for single-implant tooth replacement: a prospective 1-year follow-up study. International Journal of Prosthodontics. 2003;16(6).
39. Adatia ND, Bayne SC, Cooper LF, Thompson JY. Fracture resistance of yttria-stabilized zirconia dental implant abutments. J Prosthodont. 2009;18(1):17-22. [Crossref] [PubMed]).
40. Piconi C., Maccauro G. Zirconia as a ceramic biomaterial. Biomaterials. 1999;20(1):1-25
41. Denry I, Kelly JR. State of the art of zirconia for dental applications. Dental materials. 2008;24(3):299-307.
42. Linkevicius T, Apse P. Influence of abutment material on stability of peri-implant tissues: a systematic review. Int J Oral Maxillofac Implants. 2008;23(3):449-56. [PubMed]

43. Andreiotelli M, Wenz HJ, Kohal RJ. Are ceramic implants a viable alternative to titanium implants? A systematic literature review. Clin Oral Implants Res. 2009;20 Suppl 4:32-47. [Crossref] [PubMed]
44. Rimondini L, Cerroni L, Carrassi A, Torricelli P. Bacterial colonization of zirconia ceramic surfaces: an in vitro and in vivo study. Int J Oral Maxillofac Implants. 2002;17(6):793-8. [PubMed]
45. Neumann EAF, Villar CC, França FMG. Fracture resistance of abutment screws made of titanium, polyetheretherketone, and carbon fiber-reinforced polyetheretherketone.
46. Stawarczyk B, Beuer F, Wimmer T, Jahn D, Sener B, Roos M, et al. Polyetheretherketone a suitable material for fixed dental prostheses? Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2013;101(7):1209-16.
47. Conejo J, Kobayashi T, Anadioti E, Blatz MB. Performance of CAD7CAM monolithic ceramic implant-supported restorations bonded to titanium inserts: A systematic review. Eur J Oral Implantol. 2007;10(Suppl 1):139-46.
48. Prestipipino V, Ingber A. Esthetic high-strength implant abutments. Part II. Journal of Esthetic and Restorative Dentistry. 1993;5(2):63-8.)
49. Ichikawa Y, Akagawa Y, Nikai H, Tsuru H. Tissue compatibility and stability of a new zirconia ceramic in vivo. The Jornal of prosthetic dentistry. 1992;68(2):322-6)
50. Covacci V, Bruzzese N, Maccauro G, Andreassi C, Ricci G, Picon C, et al. In vitro evaluation of the mutagenic and carcinogenic power of high purity zirconia ceramic. Biomaterials. 1999;20(4):371-6)
51. Egusa H, Ko N, Shimazu T, Yatani H. Suspected association of an allergic reaction with titanium dental implants: a clinical report. The Journal of prosthetic dentistry. 2008;100(5):344-7
52. Kanbara T, Yajima Y, Yoshinari M. Wear behavior of tetragonal zirconia polycrystal versus titanium and titanium alloy. Biomedical Materials. 2011;6(2):021001.)
53. Guilherme NM, Chung K-H, Flinn BD, Zheng C, Raigrodski AJ. Assessment of reliability of CAD-CAM tooth-colored implant custom abutments. The Journal of prosthetic dentistry. 2016;116(2):206-13
54. Sailer I, Philipp A, Zembic A, Pjetursson BE, Hammerle CH, Zwahlen M. A systematic review of the performance of ceramic and metal implant abutments supporting fixed implant reconstructions. Clinical Oral Implants Research. 2009,20:4-31.

A Brief Overview Of Elderly Neglect And Abuse: Recommendations to Care Provider

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INTRODUCTION

The World Health Organization (WHO), which defines aging as the decrease or loss of the ability to adapt to environmental factors, it defines abuse as; Defines it as "a single or repeated act or lack of appropriate action that causes harm or distress to an older person, occurring in any relationship where there is an expectation of trust" (World Health Organization, y.y.; Yeşil, Taşci ve Öztunç, 2016). Neglect is when individuals who are responsible for taking care of the elderly person, such as institution employees, family members or private caregivers, do not meet the daily needs of the elderly individual for self-care, medical needs, physiological needs, the elderly individual cannot receive the services and care they need due to physical weakness, mental disorder or inadequacy in mental functions, or considered to be insufficient. While neglect can be caused by the caregiver due to lack of knowledge, it can also be caused by behavioral difficulties of older adults (Cooper, Selwood ve Livingston, 2008; Gökçe Kutsal ve diğerleri, 2022). Neglect is also the conscious or unconscious inability to meet the social, physical and emotional needs of the elderly (Yeşil ve diğerleri, 2016).

Life expectancy has increased due to developments in health services (Bond ve Butler, 2013). This prolongation results in more people who are more susceptible to chronic and disabling diseases, cognitive impairment, and even more dependent on long-term care and institutional care needs. Due to the nature of their clinical and social conditions, the elderly in the last stage of human life are more vulnerable. It constitutes a significant part of the population exposed to the risk of neglect and abuse (Andela, Truchot ve Huguenotte, 2021). The elderly who are abused have a higher mortality rate than the elderly who are not abused (Bond ve Butler, 2013). Elder abuse is common worldwide, estimated to affect one in six elderly people in the community, according to a recent meta-analysis (Yon, Mikton, Gassoumis ve Wilber, 2017).

1. Risk Factors of Neglect and Abuse

The population at risk is quite common. Despite the morbidity, mortality and significant costs for society caused by Neglect and Abuse, it seems that it is not sufficiently recognized and reported (Bond ve Butler, 2013).

Elderly neglect and abuse does not have a specific target group and target location. Individuals of all races, cultures and socioeconomic groups have been victims. It can occur in a nursing home, hospital or even at home. Older women and those aged >85 years and older are more likely to be victims. Elderly people who are less able to report abuse or defend themselves are at increased risk of neglect and abuse (Bond ve Butler, 2013).

Table 1. Neglect and Abuse Risk Factors (Bond ve Butler, 2013)

• Decreased physical health, such as needing more help in activities of daily living
• Dementia and cognitive impairment
• Female gender
• Previous exposure
• Increasing age
• Social Isolation
• Victim or caregiver with mental health or substance abuse problems

4. Types of Abuse

Abuse; There are types such as financial, psychological, physical (Simone, Wettstein, Senn, Rosemann ve Hasler, 2016) emotional, sexual abuse, age-based advocacy (Gökçe Kutsal ve diğerleri, 2022). It was found that the most common types of abuse are financial and psychological, and multiple victimization occurs in more than a third of the cases (Fraga Dominguez, Storey ve Glorney, 2019). Neglect is also recognized as a form of elder abuse(Gökçe Kutsal ve diğerleri, 2022).

3. Signs of Neglect and Abuse

Health workers should be able to recognize the signs of neglect and abuse. Inconsistency between statements made by the patient and caregiver, absence of a caregiver with intellectual disability, inconsistency of the history and laboratory or radiology findings, and suspicious explanations for injury are generally signs of neglect and abuse (Bond ve Butler, 2013).

Situations such as the lack of necessary skills or knowledge, the lack of awareness of the types of support available, or the inability to provide care because they are sick increase the risk of neglect. Lack of adequate food, basic hygiene, shelter, water or appropriate clothing, failure to check medications regularly, denial of access or assistance to essential devices such as walkers, canes, glasses, wheelchairs, delays in treatment, frequent doctor changes, diaper or bedding the lack of medical care (pressure sores, foley catheters, colostomy) are signs of neglect (Altendorf, Draper, Wijeratne, Schreiber ve Kanareck, 2020; Yaffe ve Tazkarji, 2012).

Financial abuse is the illegal or improper use of an elderly person's property or assets (Bond ve Butler, 2013; Simone ve diğerleri, 2016).

Psychological abuse is to cause emotional pain or distress by displaying behaviors such as verbal aggression, insults, threats, intimidation, and refusal to communicate (Simone ve diğerleri, 2016). Unexplained changes in behavior such as depression, withdrawal, changes in mental status, isolation from family members and friends, controlling, demeaning, seeming overly concerned about spending money, or

appearing verbally or physically aggressive to the patient are signs of psychological abuse (Bond ve Butler, 2013).

Physical abuse is the use of physical force that causes bodily injury, physical pain, or dysfunction. Unexplained fractures, wounds, lacerations, burns, delay in seeking medical attention after injury, and unexplained sexually transmitted diseases are a sign of physical abuse (Bond ve Butler, 2013; Simone ve diğerleri, 2016).

Unconstitutional abuse means violation of constitutionally guaranteed human rights. For example, claims that result in theft of identity documents, coercion, or denial of rights (Simone ve diğerleri, 2016).

5. Factors affecting the recognition and reporting of abuse and neglect in the elderly

Factors originating from care providers and the elderly themselves are included. Care providers; There are factors such as lack of education about elder neglect and abuse, not knowing or not being used to the necessity of reporting and reporting, seeing it as a symptom of old age and being afraid of complications that may occur after reporting it (Bond ve Butler, 2013; Carmona-Torres ve diğerleri, 2017; Evans, Hunold, Rosen ve Platts-Mills, 2017).

Factors arising from the elderly themselves; There are factors such as unawareness of neglect and abuse, fear of further abuse, fear of abandonment, concern that the caregiver will be harmed, deterioration in self-confidence and a sense of deserved abuse, denial, thinking that it is a burden, and health status (Bond ve Butler, 2013; Carmona-Torres ve diğerleri, 2017; Evans ve diğerleri, 2017). Likewise, older individuals may be reluctant to report abuse by their families or relatives (Yeşil ve diğerleri, 2016).

6. Consequences of Neglect and Abuse and Suggestions for Solution

Elderly neglect and/or abuse is among the preventable problems. If it is not prevented or not known, it can cause undesirable consequences, including diseases and deaths. For this reason, it is important to take the necessary measures to prevent these situations from ever happening, and if they do, early diagnosis and treatment and rehabilitation are important (Gökçe Kutsal ve diğerleri, 2022).

In addition to physical injuries, there are serious consequences for individuals and society such as long-term psychological consequences, use of emergency rooms, hospitalization, disability or life-threatening illness, increased risk of staying in a nursing home, and death (Evans ve diğerleri, 2017; Rosen ve diğerleri, 2016; World Health Organization, y.y.).

Elderly victims of neglect and abuse have a three times higher risk of death than non-victims. It causes an additional burden of approximately $5.3 billion on the

country's annual health expenditures, leading to an increase in direct medical costs (Dong, 2015).

Looking at the results, diagnosing, reporting and preventing elder abuse is one of the public health priorities (Rosen ve diğerleri, 2016).

In this direction, there are a wide range of responsibilities from family members to health workers, from educational institutions to policy-making institutions. It is very important to plan trainings for students from all levels of education (Primary, Secondary Education, University), to produce and implement policies on aging, to provide opportunities for the active participation of the elderly in society and to provide services on healthy aging and living (Yeşil ve diğerleri, 2016).

CONCLUSION AND RECOMMENDATIONS

There is not enough data on abuse in terms of elderly people living in the community as well as nursing home populations. More than one risk factor has been identified in the reported cases of elder abuse. Knowledge of risk factors and a multifaceted strategy are required to detect and prevent elder abuse.

Elderly neglect and abuse is still unrecognized and underreported. In-service training efforts for healthcare providers are needed to protect our elderly patients and reduce the incidence of abuse. Healthcare professionals who come into contact with the elderly, who are at risk for neglect and abuse, should recognize the symptoms of neglect and abuse and make evaluations accordingly and have a high awareness. Health professionals, especially physicians, can make a difference in the life of an older person by becoming familiar with reporting requirements and available help resources.

Funds are needed to better understand and develop the field of neglect and abuse of the elderly, to disseminate cost-effective solutions and to raise awareness on the issue. Non-governmental organizations, universities, municipalities and state institutions working on this issue should be supported in terms of financing.

Scientifically measurable and cost-effective solutions should be provided for elder neglect and abuse. Likewise, preventive strategies must be developed in the protective context before it occurs. In this context, developing technology can be taken as reference.

The abuse of the elderly is a multifaceted and complex phenomenon that makes it difficult for decision makers to grasp and take action. It is difficult to assess globally. Therefore, it should be evaluated culturally and handled regionally and nationally. At the same time, global networks should be established, coordination and financing provided.

Based on all these findings, we believe that as in higher education, priority should be given to elder neglect and abuse education in all education levels, and more emphasis should be given to this issue in institutes academically.

REFERENCES

Altendorf, A., Draper, B., Wijeratne, C., Schreiber, J. ve Kanareck, D. (2020). Neglect of older people: Touching on forensic and pathophysiological aspects. *Gerontologist*, *60*(6), E449–E465. doi:10.1093/geront/gnz084

Andela, M., Truchot, D. ve Huguenotte, V. (2021). Work Environment and Elderly Abuse in Nursing Homes: The Mediating Role of Burnout. *Journal of Interpersonal Violence*, *36*(11–12), 5709–5729. doi:10.1177/0886260518803606

Bond, M. C. ve Butler, K. H. (2013). Elder Abuse and Neglect. Definitions, Epidemiology, and Approaches to Emergency Department Screening. *Clinics in Geriatric Medicine*, *29*(1), 257–273. doi:10.1016/j.cger.2012.09.004

Carmona-Torres, J. M., Carvalhal-Silva, R. M., Viera-Mendes, M. H., Recio-Andrade, B., Goergen, T. ve Rodríguez-Borrego, M. A. (2017). Maus-tratos no ambiente familiar contra idosos nas ilhas dos açores. *Revista Latino-Americana de Enfermagem*, *25*. doi:10.1590/1518-8345.1871.2932

Cooper, C., Selwood, A. ve Livingston, G. (2008). The prevalence of elder abuse and neglect: A systematic review. *Age and Ageing*, *37*(2), 151–160. doi:10.1093/ageing/afm194

Dong, X. Q. (2015). Elder Abuse: Systematic Review and Implications for Practice. *J Am Geriatr Soc*, *63*(6), 1214–1238. doi:10.1111/jgs.13454

Evans, C. S., Hunold, K. M., Rosen, T. ve Platts-Mills, T. F. (2017). Diagnosis of Elder Abuse in US Emergency Departments HHS Public Access. *J Am Geriatr Soc*, *65*(1), 91–97. doi:10.1111/jgs.14480

Fraga Dominguez, S., Storey, J. E. ve Glorney, E. (2019). Characterizing Elder Abuse in the UK: A Description of Cases Reported to a National Helpline. *Journal of Applied Gerontology*, *2022*(0), 1–12. doi:10.1177/07334648221109513

Gökçe Kutsal, Y., Aslan, D., Aydos, T. R., Kut, A., Erberk Özen, N., Saygun, M., … Karahan, S. (2022). Hekimlerin Yaşlı İhmal ve İstismarı Konusundaki Farkındalığına Yönelik Kısa Bir Değerlendirme. *STED / Sürekli Tıp Eğitimi Dergisi*, *31*(2), 0–2. doi:10.17942/sted.1098957

Rosen, T., Bloemen, E. M., Lofaso, V. M., Clark, S., Flomenbaum, N. E. ve Lachs, M. S. (2016). Emergency Department Presentations for Injuries in Older Adults Independently Known to be Victims of Elder Abuse. *The Journal of emergency medicine*, *50*(3), 518–526. doi:10.1016/J.JEMERMED.2015.10.037

Simone, L., Wettstein, A., Senn, O., Rosemann, T. ve Hasler, S. (2016). Types of abuse and risk factors associated with elder abuse. *Swiss medical weekly*,

146(January), w14273. doi:10.4414/smw.2016.14273

World Health Organization. (y.y.). Abuse of older people. 3 Temmuz 2022 tarihinde https://www.who.int/news-room/fact-sheets/detail/abuse-of-older-people adresinden erişildi.

Yaffe, M. J. ve Tazkarji, B. (2012). Understanding elder abuse in family practice. *Canadian family physician Medecin de famille canadien*, *58*(12), 1336–40, e695-8. http://www.ncbi.nlm.nih.gov/pubmed/23242889%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3520657 adresinden erişildi.

Yeşil, P., Taşci, S. ve Öztunç, G. (2016). Yaşlı Istismar Ve Ihmali. *Journal of Duzce University Health Sciences Institute*, *6*(2), 128–134.

Yon, Y., Mikton, C. R., Gassoumis, Z. D. ve Wilber, K. H. (2017). Elder abuse prevalence in community settings: a systematic review and meta-analysis. *The Lancet Global Health*, *5*(2), e147–e156. doi:10.1016/S2214-109X(17)30006-2

Depression-like Behaviors in Diabetic Animal Studies Induced by Streptozotocin

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Diabetes mellitus (DM) is a metabolic disorder which is displayed hyperglycemia and defects of insulin secretion or its action (American Diabetes Association, 2010). The world diabetes prevalence in estimated to be 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (Saaedi et al, 2019). Depression and the risk of developing depression in patients with diabetes are higher than in the nondiabetic population (Robinson et al, 2018). The picture of depression seen in diabetes is frequently examined with different animal models in preclinical studies. In this chapter, the model most commonly used to induce diabetes and the behavior of animals in the most commonly used depression test are analyzed.

Streptozotocin

Streptozotocin (STZ) administration damages pancreatic beta cells (Eleazu et al, 2013). With this feature, High dose STZ creates a type 1 diabetes model, especially with certain doses (Furman, 2015). In some animal species, high-dose STZ application is used to create a prediabetes Picture (Zborowski etal , 2021). Lower dose STZ administration is used to create a model of type 2 diabetes when combined with different dietary regimens (Luo et al, 2016). It is also used as a neuropathic pain model due to diabetes (Ali et al, 2015). STZ cannot cross the blood brain barrier (Reynolds, 1982). However, as a result of its application into the brain, it damages some special anatomical regions.

For this reason, it is also used as a sporadic Alzheimer's model with intracerebral vascular application (Chen et al, 2014). In this chapter, studies that induced a type 1 diabetes model with STZ are investigated.

Forced Swimming Test

It was firstly designed by Porsolt et al in 1977 (Porsolt et al, 1977). It is the most commonly used animal depression test (Yankelevitch-Yahav et al, 2015). After a certain period of time, floating behavior (immobile) like a piece of wood is observed in rats or mice forced to swim. While this behavior coincides with depression, total activity time is accepted as a behavioral parameter that increases with antidepressant activity. In 1995, the forced swimming test was modified by Detke and Lucki and the water height was increased. Some basic behavior was observed in the experiment after the tail and hind limbs were raised to a height where they could not feel or touch the bottom of the water (Detke and Lucki, 1995).

Table 1: Behavior patterns in the forced swim test

Behavior patern	Describtion	Mean
İmmobility (floating)	Total immobility time (The period when 3 or 4 limbs are immobile)	An increase in this period indicates an increase in depression-like behaviors.
Mobility time	Total mobility time (the period when at least 2 limbs are mobile)	An increase in this period indicates an increase in antidepression-like behaviors.
Latency	Time until the first inactivity time elapses	The shortening this period is considered depressive like behavior.
Swimming	Horizontal movements in water.	The increase in this behavior is considered as antidepressant activity. It is associated with the serotonergic system.
Climbing	Vertical movements in water.	The increase in this behavior is considered as antidepressant activity. It is associated with the noradrenergic system.
Head shake	Head shaking behavior	adaptive behavior
Dive	Diving behavior	adaptive behavior

Tablo 2. Animal Studies 2000-2016

Study	STZ dose	glycemic level	Animal	Behavior parameters	Result
Gomez and Barros, 2000	60 mg/kg i.p	glucosuria	adult male Wistar rats	İmmobility ↑Time, Latency time ↓, Climbing ↓, Head shakes ↔	DLB↑
Caletti et al, 2012	60 mg/kg i.p	≥ 200 mg/dL	Adult Male Wistar rats	Floating ↔, Freezing ↑, Swimming↓* Climbing↓* Dive x head shakes↓	DLB ↑, ↔
Haider et al, 2013	60 mg/kg i.p	polydipsia, polyuria and polyphagia,	Adult male Albino Wistar rat	İmmobility ↑Time, Climbing Number↓	DLB↑
Wayhs et al, 2013	60 mg/kg i.p.	≥ 250 mg/dL	Adult Male Wistar rats	İmmobility ↑Time, Latency time ↓	DLB↑
El-Marasy et al, 2014	52,5 mg/kg i.p	≥ 250 mg/dL	Adult Male Wistar rats	İmmobility ↑Time	DLB↑
Elbatsh, 2015	60 mg/kg i.p	≥ 200 mg/dL	Adult male albino Wistar rats	İmmobility ↑Time, Latency time ↓	DLB↑
Gupta et al, 2014	200 mg/kg i.p	≥ 200 mg/dL	Swiss Albino male mice	İmmobility ↑Time	DLB↑
Nadeem et al, 2015	60 mg/kg	>200 mg/dL	? male albino Wistar rats	İmmobility time↑, Climbing↔, Swimming ↓	DLB↑
Fatemi Tabatabaei et al, 2016	60 mg/kg	>300 mg/dl	Adult female Wistar rats	İmmobility time↔, Swimming time ↔	DLB ↔
Zhou et al, 2016	150 mg/kg	16.7 mmol/L	Adult male ICR mice	İmmobility Time ↑	DLB↑
Wang et al, 2016	60 mg/kg	≥11.1 mmol/L (equal to 200 mg/dL)	Adult Male Kunming (KM) male mice	İmmobility Time ↑	DLB↑

Table 3. Animal Studies 2017-2022

Farajpour et al, 2017	200 mg/kg	>300 mg/dl	Adult Male albino mice	İmmobility Time ↑	DLB↑
Rebolledo-Solleiro and Fernández-Guasti, 2018	50 mg/kg	>250 mg/dL	Adult female rat (proestrus/estrus (P/E) vs. metestrus/diestruS M/D),Adult male wistar rats	İmmobility time↑(male, P/E), ↔(M/D) Climbing↔(all) Swimming ↓ (male, P/E)	DLB↑ (male, p/e), ↔ (M/D)
Şahin et al, 2019	50 mg/kg i.p	≥ 300 mg/dL	Adult male Wistar albino rats	İmmobility ↑Time, Latency time ↓	DLB↑
Rahmani et al, 2018	60 mg/kg i.p.	>300 mg/dl	Adult male Wistar rats	İmmobility Time↑	DLB↑
Mbiantcha et al 2019	200 mg/kg i.p	≥ 300 mg/dL	Adult Female, male mice	İmmobility Time↑	DLB↑
Bambi et al, 2020	200 mg/kg i.p	>250 mg/dL	Adult male Swiss mice	İmmobility Time↑	DLB↑
Djuichou Nguemnang et al, 2020	200 mg/kg i.p.	≥ 300 mg/dL	Young Swiss strain mice (females and males)	İmmobility Time↑	DLB↑
Liu et al, 2020	50 mg/kg i.p	16.7 mmol/L	Adult male Sprague-Dawley	İmmobility Time ↑, Climbing time ↓, Swimming time↔	DLB↑
ALmohaimeed et al, 2021	60 mg/kg i.p	≥ 250 mg/dL	Young male Sprague–Dawley rat	İmmobility Time ↑	DLB↑
Tsafack et al, 2021	200 mg/kg i.p	≥ 300 mg/dL	Adult female and male mice (*Mus musculus*, Swiss)	İmmobility Time↑	DLB↑
Kotagale et al, 2021	100 mg/kg i.p	≥ 250 mg/dL	Adult Male Swiss albino mice	İmmobility Time↑	DLB↑

In studies with STZ, a diabetes model was created after a single dose administration. A type of diabetes mellitus was induced in mice with far higher doses (100-200 mg/kg) (See Table 2-3). There is also a mouse study in which a diabetes model was created with a relatively low dose (Wang et al, 2016). An increase in blood glucose levels (at least 200-300 mg/dL) was accepted as a condition for acceptance of diabetes. In rats, a single dose diabetes model was induced with relatively lower doses (50-60 mg/kg) than in mice (See Table 2-3). Confirmation was similarly done with hyperglycemia (at least 200-300 mg/dL) (See Table 2-3). Some studies accepted that model confirmation were glycosuria, polydipsia, polyuria and polyphagia in diabetic rat model (Gomez and Barros, 2000; Haider et al, 2013).

Total immobility time (floating time) was evaluated in all studies as depression like behavior (See Table 2-3). The general consensus is that this period is increased in diabetic rats or mice. This indicates that diabetic animals exhibit depression-like behavior in the forced swimming test. In some studies, it was observed that depression-like behaviors did not alter or partially changed

compared to naitive subjects (Caletti et al, 2012; Fatemi Tabatabaei et al, 2016). The latency time, the shortening of which is considered a sign of depression, is limited in the number of studies examined. In these studies, it has been shown that this period is shortened and depression-like behaviors increase (Gomez and Barros, 2000; Elbatsh, 2015; Şahin et al, 2019; Wayhs et al, 2010).

It has been shown that swimming behavior related to the serotonergic system is negatively reduced in diabetic animals (Caletti et al, 2012; Nadeem et al, 2015; Rebolledo-Solleiro and Fernández-Guasti, 2018). This behavior pattern was not affected in some other studies (Fatemi Tabatabaei et al, 2016; Liu et al, 2020). Further, it was observed that the climbing behavior associated with the noradrenergic system, which is another indicator of antidepressant activity, decreased in the diabetic animals (Caletti et al, 2012; Gomez and Barros, 2000; Haider et al, 2013; Liu et al, 2020). Climbing behavior pattern was not affected in some studies (Nadeem et al, 2015; Rebolledo-Solleiro and Fernández-Guasti, 2018).

It consists of 2 stages: a 15-minute training swim on the first day, and a 5-minute test swim after 24 hours, in which depression is evaluated in rat forced swimming test. Gomez et al showed that depression-like behaviors increased in both training swimming (15-minute) and swimming (5-minute) in which depression was evaluated (Gomez and Barros, 2000).

Head shake, which is an adaptive behavior, has also been shown to be reduced in diabetic animals (Caletti et al, 2012).

In the study conducted in diabetic female rats, while depressive behavior immobility time increased in the proestrus/estrus (P/E) phase of the menstrual cycle, it did not change in the metestrus/diestru phase. While swimming behavior related to the serotonergic system was negatively affected and decreased in proestrus and estrus stages, climbing behavior was not affected at any stage (Rebolledo-Solleiro and Fernández-Guasti, 2018).

Depression behaviors have increased in diabetic animals generally. Different authors explain this situation through different pathophysiological pathways. The general consensus of the studies in terms of behavioral physiology will enable further studies to be carried out more easily.

The diabetes model created with STZ also creates a neuropathic pain model. It should be investigated whether the increased depressive behaviors are due to diabetes or neuropathic pain due to diabetes, or a combination of both. In further studies, it can be combined with behavioral experiments testing neuropathic pain in diabetic animals.

Although further studies are needed, the forced swimming test is extremely convenient to examine depression behaviors in the diabetes model induced by STZ.

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References

1. Ali, G., Subhan, F., Abbas, M., Zeb, J., Shahid, M., & Sewell, R. D. (2015). A streptozotocin-induced diabetic neuropathic pain model for static or dynamic mechanical allodynia and vulvodynia: validation using topical and systemic gabapentin. Naunyn-schmiedeberg's Archives of Pharmacology, 388(11), 1129-1140.
2. Almohaimeed, H. M., Mohammedsaleh, Z. M., Batawi, A. H., Balgoon, M. J., Ramadan, O. I., Baz, H. A., ... & Ayuob, N. N. (2021). Synergistic Anti-inflammatory and Neuroprotective Effects of Cinnamomum cassia and Zingiber officinale Alleviate Diabetes-Induced Hippocampal Changes in Male Albino Rats: Structural and Molecular Evidence. *Frontiers in Cell and Developmental Biology*, *9*.
3. American Diabetes Association. (2010). Diagnosis and classification of diabetes mellitus. *Diabetes care*, *33*(Supplement_1), S62-S69.
4. Bampi, S. R., Casaril, A. M., Domingues, M., de Andrade Lourenço, D., Pesarico, A. P., Vieira, B., ... & Savegnago, L. (2020). Depression-like behavior, hyperglycemia, oxidative stress, and neuroinflammation presented in diabetic mice are reversed by the administration of 1-methyl-3-(phenylselanyl)-1H-indole. Journal of Psychiatric Research, 120, 91-102
5. Caletti, G., Olguins, D. B., Pedrollo, E. F., Barros, H. M., & Gomez, R. (2012). Antidepressant effect of taurine in diabetic rats. Amino Acids, 43(4), 1525-1533.
6. Chen, Y., Liang, Z., Tian, Z., Blanchard, J., Dai, C. L., Chalbot, S., ... & Gong, C. X. (2014). Intracerebroventricular streptozotocin exacerbates Alzheimer-like changes of 3xTg-AD mice. Molecular neurobiology, 49(1), 547-562.
7. Detke, M. J., & Lucki, I. (1995). Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. Behavioural brain research, 73(1-2), 43-46.
8. Djuichou Nguemnang, S. F., Tsafack, E. G., Mbiantcha, M., Ateufack, G., Yousseu Nana, W., Atsamo, A. D., ... & Ben Besong, E. (2020). Antihypernociceptive, anxiolytic, and antidepressant properties of aqueous and ethanol extracts of Dissotis thollonii Cogn.(Melastomataceae) in mice. Advances in Pharmacological and Pharmaceutical Sciences, 2020.
9. ElBatsh, M. M. (2015). Antidepressant-like effect of simvastatin in diabetic rats. *Canadian Journal of Physiology and Pharmacology*, *93*(8), 649-656.
10. El-Marasy, S. A., Abdallah, H. M., El-Shenawy, S. M., El-Khatib, A. S., El-Shabrawy, O. A., & Kenawy, S. A. (2014). Anti-depressant effect of

hesperidin in diabetic rats. *Canadian journal of physiology and pharmacology*, *92*(11), 945-952.
11. Eleazu, C. O., Eleazu, K. C., Chukwuma, S., & Essien, U. N. (2013). Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. *Journal of diabetes & metabolic disorders*, *12*(1), 1-7.
12. Farajpour, R., Sadigh-Eteghad, S., Ahmadian, N., Farzipour, M., Mahmoudi, J., & Majdi, A. (2017). Chronic administration of Rosa canina hydro-alcoholic extract attenuates depressive-like behavior and recognition memory impairment in diabetic mice: a possible role of oxidative stress. *Medical principles and practice*, *26*(3), 245-250.
13. Furman, B. L. (2015). Streptozotocin-induced diabetic models in mice and rats. *Current protocols in pharmacology*, *70*(1), 5-47
14. Gomez, R., & Barros, H. M. (2000). Ethopharmacology of the antidepressant effect of clonazepam in diabetic rats. *Pharmacology Biochemistry and Behavior*, *66*(2), 329-335.
15. Gupta, D., Radhakrishnan, M., & Kurhe, Y. (2014). Ondansetron, a 5HT3 receptor antagonist reverses depression and anxiety-like behavior in streptozotocin-induced diabetic mice: possible implication of serotonergic system. *European journal of pharmacology*, *744*, 59-66.
16. Haider, S., Ahmed, S., Tabassum, S., Memon, Z., Ikram, M., & Haleem, D. J. (2013). Streptozotocin-induced insulin deficiency leads to development of behavioral deficits in rats. Acta Neurologica Belgica, 113(1), 35-41.
17. Kotagale, N., Rahangdale, S., Borkar, A., Singh, K., Ikhar, A., Takale, N., ... & Taksande, B. (2021). Possible involvement of agmatine in neuropharmacological actions of metformin in diabetic mice. European Journal of Pharmacology, 907, 174255.
18. Liu, H. Y., Wei, H. J., Wu, L., Liu, S. M., Tang, Y. Y., Zou, W., ... & Tang, X. Q. (2020). BDNF-TrkB pathway mediates antidepressant-like roles of H2S in diabetic rats via promoting hippocampal autophagy. Clinical and Experimental Pharmacology and Physiology, 47(2), 302-312.
19. Luo, C., Ke, Y., Yuan, Y., Zhao, M., Wang, F., Zhang, Y., & Bu, S. (2016). A novel herbal treatment reduces depressive-like behaviors and increases brain-derived neurotrophic factor levels in the brain of type 2 diabetic rats. Neuropsychiatric Disease and Treatment, 12, 3051.
20. Mbiantcha, M., Khalid, R., Dawe, A., Mehreen, A., Atsamo, D. A., Ateufack, G., ... & Izhar, A. (2019). Antihypernociceptive and neuroprotective effects of Combretin A and Combretin B on

streptozotocin-induced diabetic neuropathy in mice. Naunyn-Schmiedeberg's Archives of Pharmacology, 392(6), 697-713.
21. Nadeem, R. I., Ahmed, H. I., & El-Denshary, E. E. D. S. (2015). Effect of imipramine, paroxetine, and lithium carbonate on neurobehavioral changes of streptozotocin in rats: impact on glycogen synthase kinase-3 and blood glucose level. Neurochemical research, 40(9), 1810-1818.
22. Porsolt, R. D., Le Pichon, M., & Jalfre, M. L. (1977). Depression: a new animal model sensitive to antidepressant treatments. Nature, 266(5604), 730-732.
23. Rahmani, G., Farajdokht, F., Mohaddes, G., Babri, S., Ebrahimi, V., & Ebrahimi, H. (2020). Garlic (Allium sativum) improves anxiety-and depressive-related behaviors and brain oxidative stress in diabetic rats. Archives of physiology and biochemistry, 126(2), 95-100.
24. Rebolledo-Solleiro, D., & Fernández-Guasti, A. (2018). Influence of sex and estrous cycle on blood glucose levels, body weight gain, and depressive-like behavior in streptozotocin-induced diabetic rats. Physiology & behavior, 194, 560-567.
25. Reynolds, J. E. (1982). Martindale: the extra pharmacopoeia. London, UK; The Pharmaceutical Press.
26. Robinson, D. J., Coons, M., Haensel, H., Vallis, M., & Yale, J. F. (2018). Diabetes and mental health. Canadian journal of diabetes, 42, S130-S141.
27. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., ... & IDF Diabetes Atlas Committee. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes research and clinical practice, 157, 107843.
28. Şahin, T. D., Göçmez, S. S., Eraldemir, F. C., & Utkan, T. (2019). Anxiolytic-like and antidepressant-like effects of resveratrol in streptozotocin-induced diabetic rats. Archives of Neuropsychiatry, 56(2), 144.
29. Tabatabaei, S. R. F., Rashno, M., Ghaderi, S., & Askaripour, M. (2016). The aqueous extract of Portulaca oleracea ameliorates neurobehavioral dysfunction and hyperglycemia related to streptozotocin-diabetes induced in ovariectomized rats. Iranian journal of pharmaceutical research: IJPR, 15(2), 561.
30. Tsafack, E. G., Mbiantcha, M., Ateufack, G., Djuichou Nguemnang, S. F., Nana Yousseu, W., Atsamo, A. D., ... & Ben Besong, E. (2021). Antihypernociceptive and Neuroprotective Effects of the Aqueous and Methanol Stem-Bark Extracts of Nauclea pobeguinii (Rubiaceae) on STZ-

Induced Diabetic Neuropathic Pain. Evidence-Based Complementary and Alternative Medicine, 2021.
31. Wang, J., Duan, P., Cui, Y., Li, Q., & Shi, Y. (2016). Geniposide alleviates depression-like behavior via enhancing BDNF expression in hippocampus of streptozotocin-evoked mice. Metabolic brain disease, 31(5), 1113-1122.
32. Yankelevitch-Yahav, R., Franko, M., Huly, A., & Doron, R. (2015). The forced swim test as a model of depressive-like behavior. JoVE (Journal of Visualized Experiments), (97), e52587.
33. Zborowski, V. A., Heck, S. O., Marques, L. S., Bastos, N. K., & Nogueira, C. W. (2021). Memory impairment and depressive-like phenotype are accompanied by downregulation of hippocampal insulin and BDNF signaling pathways in prediabetic mice. *Physiology & Behavior*, *237*, 113346
34. Zhou, X. Y., Zhang, F., Hu, X. T., Chen, J., Tang, R. X., Zheng, K. Y., & Song, Y. J. (2017). Depression can be prevented by astaxanthin through inhibition of hippocampal inflammation in diabetic mice. Brain research, 1657, 262-268.

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Anxiety-like Behaviors in Diabetic Animal Studies Induced by Streptozotocin

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According to WHO, The estimated total number of people living with anxiety disorders is approximately 264 million, with a worldwide prevalence of 3.6% (WHO, 2017). Anxiety disorders are also quite high in the diabetic patient population (Lloyd et al, 2000). In this book chapter, anxiety-like behaviors were analyzed in rodents for which the stz induced type 1 diabetes model.

Elevated Plus Maze (EPM)

It was defined as a y-shaped form by Montegomery in 1955. The system, which includes two open arms in Y-shaped form, is based on the anxiety created by the new environment and open high arms (Montgomery, 1955). It was later modified by Handley and Mithani in 1984, and by Pellow et al. in 1985, and consisted of two closed and two open arms. (Handley and Mithani, 1984; Pellow et al, 1985). In summary, height and the new environment are sources of anxiety. Depending on the decrease in anxiety like behavior (ALB), the time spent in open arms, which are risky areas, and the number of entries increase (Çalışkan et al, 2017). It is the most frequently preferred anxiety test to induce natural and unconditional anxiety in rodents (Korte and De Boer, 2003). therefore, the ALB of diabetic subjects was analyzed in the EPM.

Table 1: Behavior patterns in the elevated plus maze

Behavior patern	Describtion	Mean
Open arm time	In the test setup, it is the risky area exposed to height. Time spent in this field.	An decrease in this period indicates an increase in ALB
Open arm entrance	This behavior pattern is the number of times it enters the risky area.	An decrease in this frequency indicates an increase in ALB
Closed arm time	Closed arms are the safety zone in the test setup. Time spent in this field.	An increase in this period indicates an increase in anxiety-like behaviors.
Closed arm entrance	Closed arms are the safety zone in the test setup. Time spent in this field. This patern describes enterance numbers.	An increase in this period indicates an increase in ALB
Total arm entrance	This behavior pattern is the number of times it enters the risky and safety area.	Locomotor activity.
Head dipping	this behavior is the behavior of looking down in the risk zone (open arm).	An decrease in this frequency indicates an increase in ALB
Strech- attend posture	It is a posture triggered by stress. The animal becomes parallel to the ground.	An increase in this frequency indicates an increase in ALB
Distal open arm time	In the test setup, it is the risky area exposed to height. Time spent in this field.	An decrease in this period indicates an increase in ALB
Freezing time	It ıs the behavıor of ınactıon for a perıod of tıme.	An increase in this period indicates an increase in ALB

Table 2: 1997-2017 Studies

Researcher	STZ dose	glycemic level	Animal	Behavior parameters	Result
Thorré et al, 1997	65 mg/kg i.p	glucosuria	Adult Male wistar rats	Open arm time (%)↔ , open arm entries(%)↓, closed arm entries↓, TAE ↓	ALB ↑, ↔
Ramanathan et al, 1998	50 mg/kg i.p	GOD/POD method	? Charles Foster strain albino rats, of either sex	Open arm time↓, closed arm time ↑, closed arm entries ↔, open arm entries↔	ALB↑
Comin et al, 2010	35 mg/kg i.p	>250 mg/dL	Adult male Wistar rats	Open arm time (%)↔, open arm entries (%)↔, closed arm entries↓, Stretch-attend posture↑	ALB↑
Gupta et al, 2014	200 mg/kg i.p.	≥200 mg/dL	Swiss albino adult mice (female/male)	Open arm time↓, Open arm entries (%)↓,TAE↔	ALB↑
Fatemi Tabatabaei et al, 2016	60 mg/kg	>300 mg/dl	female Wistar rats	Open arm time↔, Open arm entries (%)↔,total arm entries ↓	ALB ↔
Pipkin et al, 2017	45 mg/kg i.p	≥ 250 mg/dL (≥ 650 mg/dL excluded study)	Adult Male Wistar rats	Closed arm time ↔	ALB ↔

Table 3: 2019-2020 Studies

Researcher	STZ dose	glycemic level	Animal	Behavior parameters	Result
Çalışkan et al ,2019	50 mg/kg i.p	≥ 300 mg/dL	Adult Female Wistar rats	Open arm time↔, distal open arm time↔, open arm entries↔, head dipping↓, Stretch-attend posture↑, freezing time↑	ALB↔, ↑
Mbiantcha et al, 2019	200 mg/kg i.p	≥ 300 mg/dL	Adult Female, male mice	Open arm time↓Closed arm time↑	ALB↑
Şahin et al, 2019	50 mg/kg i.p	≥ 300 mg/dL	Adult male Wistar albino rats	Open arm time↓, open arm entries (%)↓	ALB↑
Yuan et al, 2019	150 mg/kg i.p	?	Adult male mice of C57BL/6J	Open arm time↓, open arm entries ↓	ALB↑
Gharaati et al, 2020	60 mg/kg i.p	>150 mg/dL	Adult male Wistar rats	Open arm time↓, open arm entries (%)↔	ALB↑
Calışkan et al, 2020	50 mg/kg i.p	≥ 300 mg/dL	Adult Male Sprague-Dawley rats	Open arm time↔, distal open arm time↔, open arm entries↔, head dipping↔, Stretch-attend posture↑	ALB↔,↑

Table 4: 2020-2021 Studies

Djuichou Nguemnang et al, 2020	200 mg/kg i.p.	≥ 300 mg/dL	Young Swiss strain mice (females and males)	Open arm time↓,closed arm time ↑	ALB↑
Liu et al, 2020	50 mg/kg i.p	16.7 mmol/L	Adult male Sprague-Dawley	Open arm time↓,Open arm entries (%)↓,TAE↔	ALB↑
Rahmani et al, 2020	60 mg/kg i.p.	>300 mg/dl	Adult male Wistar rats	Open arm time (%)↓, open arm entries (%)↓	ALB↑
Sevastre-Berghian et al, 2020	30 mg/kg i.p.	≥ 250 mg/dL	Adult male Wistar rats	TAE↔, total distance travelled↔, ,Closed arm entries ↔	ALB↔
Jiang et al, 2021	55mg/kg i.p	>16.7 mmol/L	Adult male Sprague-Dawley	Open arm time↓,Open arm entries (%)↓,total armentries↔	ALB↑
Kotagale et al, 2021	100 mg/kg i.p	≥ 250 mg/dL	Adult Male Swiss albino mice	Open arm time (%) ↓,Open arm entries(%) ↓,TAE (%)↔	ALB↑
Tsafack et al, 2021	200 mg/kg i.p	≥ 300 mg/dL	Adult female and male mice (*Mus musculus*, Swiss)	Open arm time↓,Closed arm time↑	ALB↑

In studies with STZ, a diabetes model was induced after a single dose administration (See Table 2,3,4). A type of diabetes mellitus was induced in mice with far higher doses (100-200 mg/kg) (See Table 2,3,4). In rats, a single dose diabetes model was induced with relatively lower doses (30-60 mg/kg) than in mice (See Table 2,3,4). An increase in blood glucose levels (at least 150-300 mg/dL) was accepted as a condition for acceptance of diabetes. (See Table 2-3).

Some studies accepted that model confirmation were glycosuria, GOD/POD method in diabetic rat model (Thorré et al, 1997; Ramanathan et al, 1998).

Open field time (time/%) was evaluated in all studies for anxiety-like behavior (See Table 2-3-4). The general consensus is that this period is shortened in diabetic rats or mice. This indicates point out that diabetic animals displayed anxiety-like behavior in the elevated plus test In some of the studies presented, the time spent in risk areas (open arm time, open arm entrance) did not change (Comin et al, 2010; Çalışkan et al ,2019; Çalışkan et al ,2020; Fatemi Tabatabaei et al, 2016 Thorré et al, 1997). In some studies, the time spent in the closed arm or closed arm entrance did not change (Pipkin et al, 2017; Ramanathan et al, 1998; Sevastre-Berghian et al, 2020).

Strech- attend postüre (stress related postüre) and freezing time increased dramatically in diabetic subjects (Comin et al, 2010; (Çalışkan et al, 2019; Çalışkan et al, 2020). As for head dipping behavior, adversely was affected by diabetes and reduced (Çalışkan et al, 2019; Çalışkan et al, 2020).

The general picture is that diabetes increases anxiety-like behaviors. This provides an opportunity to investigate the pathophysiology of diabetes-anxiety. some authors point out that thirst and physiological modifications affect anxiety-like behavior, especially in animals with diabetes (Rebolledo-Solleiro et al, 2013).

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References

1. Almohaimeed, H. M., Mohammedsaleh, Z. M., Batawi, A. H., Balgoon, M. J., Ramadan, O. I., Baz, H. A., ... & Ayuob, N. N. (2021). Synergistic Anti-inflammatory and Neuroprotective Effects of Cinnamomum cassia and Zingiber officinale Alleviate Diabetes-Induced Hippocampal Changes in Male Albino Rats: Structural and Molecular Evidence. *Frontiers in Cell and Developmental Biology*, *9*.
2. Comin, D., Gazarini, L., Zanoni, J. N., Milani, H., & de Oliveira, R. M. W. (2010). Vitamin E improves learning performance and changes the expression of nitric oxide-producing neurons in the brains of diabetic rats. *Behavioural Brain Research*, *210*(1), 38-45.
3. Çalışkan, H., AKAT F., & ZALOĞLU, N. Şartsız Hayvan Anksiyete Testleri. Ankara Sağlık Hizmetleri Dergisi, 16(1), 35-40.
4. Caliskan, H., Akat, F., Tatar, Y., Zaloglu, N., Dursun, A. D., Bastug, M., & Ficicilar, H. (2019). Effects of exercise training on anxiety in diabetic rats. *Behavioural brain research*, *376*, 112084.
5. Çalışkan, H., Akat, F., Ömercioğlu, G., Baştuğ, G., Fıçıcılar, H., & Baştuğ, M. (2020). Aerobic exercise has an anxiolytic effect on streptozotocin-induced diabetic rats.
6. Djuichou Nguemnang, S. F., Tsafack, E. G., Mbiantcha, M., Ateufack, G., Yousseu Nana, W., Atsamo, A. D., ... & Ben Besong, E. (2020). Antihypernociceptive, anxiolytic, and antidepressant properties of aqueous and ethanol extracts of Dissotis thollonii Cogn.(Melastomataceae) in mice. Advances in Pharmacological and Pharmaceutical Sciences, 2020.
7. Gharaati, M. E., Nahavandi, A., Mojarad, T. B., & Roghani, M. (2020). Diabetic Encephalopathy Affecting Mitochondria and Axonal Transport Proteins. Basic and Clinical Neuroscience, 11(6), 781.
8. Gupta, D., Radhakrishnan, M., & Kurhe, Y. (2014). Insulin reverses anxiety-like behavior evoked by streptozotocin-induced diabetes in mice. *Metabolic brain disease*, *29*(3), 737-746.
9. Handley, S. L., & Mithani, S. (1984). Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn-Schmiedeberg's archives of pharmacology, 327(1), 1-5.
10. Korte, S. M., & De Boer, S. F. (2003). A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. European journal of pharmacology, 463(1-3), 163-175.
11. Kotagale, N., Rahangdale, S., Borkar, A., Singh, K., Ikhar, A., Takale, N., ... & Taksande, B. (2021). Possible involvement of agmatine in

neuropharmacological actions of metformin in diabetic mice. European Journal of Pharmacology, 907, 174255.
12. Jiang, W., Tang, Y. Y., Zhu, W. W., Li, C., Zhang, P., Li, R. Q., ... & Tang, X. Q. (2021). PI3K/AKT pathway mediates the antidepressant-and anxiolytic-like roles of hydrogen sulfide in streptozotocin-induced diabetic rats via promoting hippocampal neurogenesis. Neurotoxicology, 85, 201-208.
13. Liu, H. Y., Wei, H. J., Wu, L., Liu, S. M., Tang, Y. Y., Zou, W., ... & Tang, X. Q. (2020). BDNF-TrkB pathway mediates antidepressant-like roles of H2S in diabetic rats via promoting hippocampal autophagy. Clinical and Experimental Pharmacology and Physiology, 47(2), 302-312.
14. Lloyd, C. E., Dyer, P. H., & Barnett, A. H. (2000). Prevalence of symptoms of depression and anxiety in a diabetes clinic population. Diabetic medicine, 17(3), 198-202.
15. Mbiantcha, M., Khalid, R., Dawe, A., Mehreen, A., Atsamo, D. A., Ateufack, G., ... & Izhar, A. (2019). Antihypernociceptive and neuroprotective effects of Combretin A and Combretin B on streptozotocin-induced diabetic neuropathy in mice. Naunyn-Schmiedeberg's Archives of Pharmacology, 392(6), 697-713.
16. Montgomery, K. C. (1955). The relation between fear induced by novel stimulation and exploratory drive. Journal of comparative and physiological psychology, 48(4), 254.
17. Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of neuroscience methods, 14(3), 149-167.
18. Pipkin, J. A., Cruz, B., Flores, R. J., Hinojosa, C. A., Carcoba, L. M., Ibarra, M., ... & O'Dell, L. E. (2017). Both nicotine reward and withdrawal are enhanced in a rodent model of diabetes. Psychopharmacology, 234(9), 1615-1622.
19. Rahmani, G., Farajdokht, F., Mohaddes, G., Babri, S., Ebrahimi, V., & Ebrahimi, H. (2020). Garlic (Allium sativum) improves anxiety-and depressive-related behaviors and brain oxidative stress in diabetic rats. Archives of physiology and biochemistry, 126(2), 95-100.
20. Ramanathan, M., Jaiswal, A. K., & Bhattacharya, S. K. (1998). Differential effects of diazepam on anxiety in streptozotocin induced diabetic and non-diabetic rats. Psychopharmacology, 135(4), 361-367.
21. Rebolledo-Solleiro, D., Crespo-Ramírez, M., Roldán-Roldán, G., Hiriart, M., & de la Mora, M. P. (2013). Role of thirst and visual barriers in the differential behavior displayed by streptozotocin-treated rats in the

elevated plus-maze and the open field test. Physiology & behavior, 120, 130-135.
22. Sevastre-Berghian, A. C., Ielciu, I., Mitre, A. O., Filip, G. A., Oniga, I., Vlase, L., ... & Hanganu, D. (2020). Targeting oxidative stress reduction and inhibition of HDAC1, MECP2, and NF-kB pathways in rats with experimentally induced hyperglycemia by administration of Thymus marshallianus Willd. extracts. Frontiers in Pharmacology, 11, 581470.
23. Şahin, T. D., Göçmez, S. S., Eraldemir, F. C., & Utkan, T. (2019). Anxiolytic-like and antidepressant-like effects of resveratrol in streptozotocin-induced diabetic rats. Archives of Neuropsychiatry, 56(2), 144.
24. Tabatabaei, S. R. F., Rashno, M., Ghaderi, S., & Askaripour, M. (2016). The aqueous extract of Portulaca oleracea ameliorates neurobehavioral dysfunction and hyperglycemia related to streptozotocin-diabetes induced in ovariectomized rats. Iranian journal of pharmaceutical research: IJPR, 15(2), 561.
25. Thorré, K., Chaouloff, F., Sarre, S., Meeusen, R., Ebinger, G., & Michotte, Y. (1997). Differential effects of restraint stress on hippocampal 5-HT metabolism and extracellular levels of 5-HT in streptozotocin-diabetic rats. Brain research, 772(1-2), 209-216.
26. Tsafack, E. G., Mbiantcha, M., Ateufack, G., Djuichou Nguemnang, S. F., Nana Yousseu, W., Atsamo, A. D., ... & Ben Besong, E. (2021). Antihypernociceptive and Neuroprotective Effects of the Aqueous and Methanol Stem-Bark Extracts of Nauclea pobeguinii (Rubiaceae) on STZ-Induced Diabetic Neuropathic Pain. Evidence-Based Complementary and Alternative Medicine, 2021.
27. Yuan, P., Zhang, J., Li, L., & Song, Z. (2019). Fluoxetine attenuated anxiety-like behaviors in streptozotocin-induced diabetic mice by mitigating the inflammation. Mediators of Inflammation, 2019.
28. World Health Organization. (2017). Depression and other common mental disorders: global health estimates (No. WHO/MSD/MER/2017.2). World Health Organization.

Symptoms Of Peripheral Arterial Diseases and Their Management

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Peripheral arterial diseases are the name given to the group of diseases that include the iliac, femoral, popliteal and tibial arteries. If one or more of these arteries become narrowed or blocked, serious circulation problems develop in the legs and feet. Peripheral arterial diseases are one of the types of cardiovascular diseases (Novo, 2002; Kumar, Abbas, Fausto, and Mitchell, 2007; Norgren and et al., 2007; Gandi and et al, 2011). Atherosclerosis is the most important cause. In fact, atherosclerosis is a natural process that occurs with aging in all vessels of the body. However, a number of factors accelerate this natural process, causing narrowing and occlusion of some arteries at an early age. Fat particles, cholesterol, calcium and some other substances and some blood cells accumulate in the artery wall, causing a hardening called atherosclerotic plaque (Kumar, Abbas, Fausto, and Mitchell, 2007). These plaques may increase their volume over time, causing the vessel to narrow and completely occlude over time, or by forming clots on the irregular surface above them, causing the vessel to suddenly completely occlude. In this case, the blood that needs to pass through the clogged artery and feed the tissues cannot reach the target tissue (Kumar, Abbas, Fausto, and Mitchell, 2007; Bonaca, Hamburg and Creager, 2021)

Since the tissues cannot get enough oxygen, some problems and complaints occur. When the vessel reaches a certain diameter reduction (usually more than 50%) due to the narrowing of the vessel due to atherosclerosis, it begins to give symptoms (symptom) according to the area fed by the vessel (Kumar, Abbas, Fausto, and Mitchell, 2007; Zipes, Libby, Bonow, Braunwald, 2008). The most frequently used students in peripheral arterial patients are “intermittent arterial patients”. When walking a certain distance, patients experience cramp-like pain in the hips, thighs, and legs. The pain becomes so severe that the patient has to stop. After resting for a while, the pain goes away and he continues to walk again. However, after a certain period of time, pain occurs again and the patient has to rest again. The shorter this pain-free walking distance, the more serious the condition. The reason for the pain is the increase in the need for oxygen in the leg muscles with effort (walking), but due to the narrowing of the vein, the blood cannot be delivered to that area as much as necessary. The leg is also cold because it does not get enough blood. In the advanced stages, there is a decrease in leg hair and thinning due to the melting of the muscles. An advanced stage of the disease is that the pain is also at rest. Patients cannot sleep because of the pain and they try to relieve their pain by hanging their legs off the bed. The last stage is the stage in which the wounds on the feet begin to open. Generally, sores occur on the fingertips and heel, which are most exposed to pressure.

Wound healing does not occur because these areas cannot be folded sufficiently (Ferreira and Macedo, 2010; Song and et al., 2019).

There are 5 P rules defined for acute arterial occlusion.

Pain; There is a diffuse pain. It is a type that develops suddenly and peaks rapidly. It leaves the affected extremity weak, forces the patient to sit down, and may even cause him to fall to the ground. Rarely, however, the pain may be mild or absent. Pallor (whitening in colour), Pulselessness (disappearance of pulses), Paresthesia; Before the deep sense and pressure sensations, the light sense, two-point discrimination, vibration and proprioception senses are lost. Paralysis; The important thing in the examination is to examine the movements of the intrinsic muscles of the foot for the purpose of looking for motor deficits, especially in distal obstructions, since the foot movements are made by the muscles located more proximal. In addition to these, decreased capillary filling, presence of cold extremities and decreased venous filling guide the diagnosis. Another important key point is to always compare the findings with the other extremity. Pain over time is helpful in the differential diagnosis of embolism and thrombosis in acute arterial occlusions. In thrombotic conditions, the pain subsides with the passing of the first wave of vasospasm and resolves with the presence of collateral or becomes chronic ischemic pain. In Chronic Arterial Insufficiency, the pain is in the form of ischemic resting pain or claudication. Cladication ('claudicatio' means limb in Latin). Varies depending on the level and extent of the disease (Hirsch, Criqui, Treat-Jacobson, et al., 2001;Ferreira and Macedo, 2010).

Leg claudication: It usually presents as cramp-like calf pain, can be caused by exercise and goes away with rest. In the presence of collaterals in elderly patients, resting pain and calcification may be absent due to low physical activity. Another point to be considered in elderly patients is to distinguish them from non-vascular pain, which is characterized by an exaggerated neuromuscular response to stretching, especially at night Hirsch, Criqui, Treat-Jacobson, et al., 2001;Ferreira and Macedo, 2010).

Hip/thigh claudication: It is seen in disease with aortoiliac distribution. It is in the form of a painful discomfort that brings weakness rather than muscle pain in the form of cramps, and in this respect, it is similar to the pain of osteoarthritis caused by exercise. Differential diagnosis from OA can be made when the pain in OA occurs with varying degrees of exercise, does not improve significantly with rest, and the variable level of pain is affected by weather conditions and physical activity. Similarly, pain can be described in lumbar

paraspinal compression. However, there is a feeling of numbness even when standing without moving with compression (as a result of increased lumbar lordosis), it does not improve with rest, numbness can also affect the perineum. An important point in the differential diagnosis of peripheral vascular disease with bilateral aortoiliac distribution is its accompanying impotence in men. (). In venous claudication, the cause of pain is increased venous pressure. It is caused by swollen, tense venous vessels. Pain occurs because adequate response is not achieved with activity, but does not go away with rest Hirsch, Criqui, Treat-Jacobson, et al., 2001;Ferreira and Macedo, 2010).

Foot Claudication: Isolated state is very rare, it is usually associated with calf claudication. Thromboangiitis Obliterans is seen rather than atherosclerotic disease because of its tendency to keep more distal, it is characterized by feeling of wood on standing and being cold at night Hirsch, Criqui, Treat-Jacobson, et al., 2001;Ferreira and Macedo, 2010).

Ischemic Resting Pain: It is in the form of pain that is disturbing at night, involving the foot in the distal of the tarsal bones or localized to the ischemic ulcer or gangrene area.

It wakes the patients from sleep, the patient rubs the foot, walks (gravity relaxes the patient by increasing the distal circulation) or takes painkillers. The differential diagnosis of this pain at night is pain due to arthritis. However, pain in arthritis occurs at variable times, while ischemic pain occurs when the patient lies down at any time. Venous Pain is not characteristic pain. It is usually easily recognized by the presence of varicose veins and accompanied by swollen extremities. It mostly occurs due to valvular insufficiency and is exacerbated by standing. There is numbness, burning, stinging sensation on varicosity.

In deep venous insufficiency, there is a widespread feeling of heaviness and fatigue in the extremity. Although venous pain usually improves with elevation, deep venous insufficiency may initially increase pain with elevation. In the presence of concomitant thrombosis, tenderness is present along the affected vein tracing. Arterial disease must be ruled out in the presence of pain in patients with chronic venous insufficiency (simply varicose veins) and no previous complaints of pain. Two more diseases should be kept in mind in the differential diagnosis for painful extremity. Neuritis is especially present in patients with diabetes and is accompanied by paresthesia and accompanying trophic skin changes. In Reflex Sympathetic Dystrophy, the affected extremity is warm and wet at first, but over time it becomes cold, dry and accompanied by trophic changes. When approaching the painful extremity (and any vascular disease) it is necessary to carefully palpate the patient's pulses.

Femoral pulses: The patient should be externally rotated from the hip and viewed from ½-2 finger width lateral to the pubic tubercle.

Popliteal pulses: The knees should be partially flexed and looked at. If it is easily palpable, the presence of a fusiform popliteal aneurysm should be kept in mind.

Posterior Tibial pulses: They should be viewed from the groove behind the medial malleolus.

Dorsalis Pedis pulses: It should be observed between the first and second metatarsal bones.

However, 10% of normal individuals do not have a dorsalis pedis pulse, therefore, in cases where it cannot be obtained, the lateral tibial artery (terminal branch of the peroneal artery) pulse should be sought first, just medial to the lateral malleolus. While palpating the pulses, the presence of a murmur should be investigated in the area where the pulse is weaker than normal and more proximal.

Critical Ischemia Symptoms: There is atrophy of the calf muscles and may cause asymmetry. There is a decrease in hair growth, especially on the dorsal aspect of the foot and toes. There is thickening of the toenails due to the slowing of nail growth. Atrophy occurs in the skin and its appendages and subcutaneous tissue, resulting in a shiny, desquamated and skeletal appearance. After the pressure applied to the finger pulp, a delay in the return of capillary flow is detected. Buerger sign is present (Limited arterial flow and peripheral vascular bed, especially post-capillary venules, become white with elevation as a result of chronic dilatation and turn red when stepped on). Localized whitening and cyanosis due to decreased capillary filling is usually a precursor to ischemic gangrene or ulceration Hirsch, Criqui, Treat-Jacobson, et al., 2001;Ferreira and Macedo, 2010).

Venous Insufficiency Symptoms: Saphenofemoral incompetence or varicose veins appear due to deep or perforating venous incompetence. This results in a brownish edema, mild and rarely seen in the first hours of the day. Stasis dermatitis occurs over prominent varicosities. More advanced ulcerations occur. Thrombosed superficial veins feel like ligaments and are surrounded by localized signs of inflammation such as erythema, skin pigmentation, warmth, swelling, and induration. In the presence of thrombosed deep veins, distal edema, Homans' sign (pain in dorsoflexion), Bancroft sign (pain with anteroposterior compression), and Löwenberg sign (pain when the cuff is inflated over 80 mm Hg around the calf) are accompanied, but these are only present in 1 case of cases. Available in /3 (Doppler is very helpful in diagnosis.

Swollen Limb: High venous pressure is the most common cause of swollen extremity.

It mostly occurs due to insufficient rotation under the effect of gravity when standing in the presence of an incompetent valve. Edema is usually in the ankles and calves, the feet are spared (venous hypertension with incompetent perforating veins is reflected in the superficial veins). In the final stages, it takes the form of an inverted champagne bottle. There may be associated stasis dermatitis. There is brown edema due to destruction of extravasated red blood cells. Increased fibrin in the interstitium causes inflammation and fibrosis in the subcutaneous duct and in advanced stages causes atrophy and trauma and easy injury. It is most commonly localized around the lower 1/3 of the leg and ankle. Lymphedema is diffuse, starting more distally in the toes and progressing upwards. It is spongy, not brownish and does not leave pits, does not show resistance when pressure is applied and easily recovers.

The skin becomes hypertrophied over time. Elephantiasis is seen in the advanced stage (lichenified, thick skin folds hanging over the ankle). Post-thrombotic syndrome is a clinical picture in which stasis dermatitis and ulceration are associated with elephanthiasis. It occurs due to the formation of secondary lymphedema as a result of invasive infection spreading from the skin to the ulcer, obliterating the lymphatics. 'Lipidema' is a chronic swollen leg in women. Caused by the maldistribution of fat in the arms and legs (thick ankles). It is prone to orthostatic edema and gives a blunt pain. It never completely resolves with elevation and diuretics. It is symmetrical and the feet are protected (Hirsch, Criqui, Treat-Jacobson, et al., 2001; Ferreira and Macedo, 2010).

SUGGESTIONS

- Avoid tight clothing and accessories, and loose trousers should be preferred.
- Foot and leg care should be taken care of. They should know daily foot and leg care, care should be taken with warm water and a neutral soap, and should be dried lightly without rubbing.
- With the help of a mirror, the feet and legs should be monitored daily for redness, cuts and blisters, and comfortable shoes should be preferred.
- Feet and legs should be protected from possible traumas and house design should be done accordingly.
- Extremities should be monitored for temperature and color.
- The patient should be informed that she should not cross her/his legs.
- Since standing and sitting for a long time will cause venous stasis, patients should be advised to change positions frequently, walk and develop an exercise program (Bozkurt, 2021; Kayhan and Guner, 2021).

REFERENCES

1. Baykal,Y., Sağlam, K., Yılmaz, A. ve diğ. (2005) Kalp Yetersizliği ve Tedavisi.
2. Bonaca P,M., Hamburg M.N., and Creager A.M., Contemporary Medical Management of Peripheral Artery Disease. Circulation Research. 2021;128:1868–1884 https://doi.org/10.1161/CIRCRESAHA.121.318258
3. Bozkurt K (2021). PERİFERİK ARTER VE VEN HASTALIKLARI ULUSAL TEDAVİ KILAVUZU 2021. Erişim Adresi: https://uvcd.org.tr/wp-content/uploads/2021/04/Ulusal-Tedavi-Klavuzu-2021.pdf.
4. Bozkurt, E., Açıkel, M. (2002). Konjestif Kalp Yetersizliği Tedavisinde Beta Bloker Kullanımı, AÜTD, 34:1-5
5. Çiftci, O., Kavalcı, C. (2014). Ventriküler Aritmi Sendromlarının Acil Serviste Yönetimi. Journal of Clinical and Analytical Medicine. Erişim Adresi: http://www.jcam.com.tr/files/KATD-2835.pdf
6. Çil E, Kimya Y, Heper C, Sunay B. Ve ark., (2002). Multidisipliner Kardiyoloji. Ed. Heper C. Nobel &Güneş Kitapevi. Sf: 323-354.
7. Eagle, Kİ., Baliga RR. (2007). Ed (Keleş, İ). Pratik Kardiyoloji, Genel Kardiyovasküler Hastalıkların Değerlendirilmesi ve Tedavisi, İstanbul Medikal Yayıncılık.
8. Erol, Ç. (2008). İç Hastalıkları, MN Medical & Nobel Tıp kitabevi, 1875-1939.
9. ESC (2010). Atriyal fibrilasyon tedavi kılavuzu, Erişim Tarihi 12.12. 2014, http://www.tkd-online.org/dergi/TKDA_38_80_1_65.pdf
10. ESC, (2009) Senkop tanı ve tedavi kılavuzu (2009 güncellemesi), Türk Kardiyol Derneği. Arş Suppl 8, 2009
11. ESC, (2012). Akut ve Kronik Kalp Yetersizliği Tanı ve Tedavisine Yonelik 2012 ESC Kılavuzu, Türk Kardiyol Dern Arş 2012, Suppl. 3
12. ESC, (2012c). Israrcı ST-segment yükselmesi belirtileri göstermeyen hastalarda Akut Koroner Sendromların (AKS) tedavi kılavuzları, Türk Kardiyol Dern Arş 2011, Supp3
13. Falk, K., Patel, H., Swedberg, K. ve diğ. (2009). Fatigue in patients with chronic heart failure -A burden associated with emotional and symptom distress, European Journal of Cardiovascular Nursing, 8(2): 91-96.
14. Ferreira C.A and Macedo B,F. (2010). A review of simple, non-invasive means of assessing peripheral arterial disease and implications for medical management, Annals of Medicine, 42:2, 115-126, DOI: 10.3109/07853890903521070

15. Filiz, T.M. (2006). Birinci Basamakta Kalp-Damar Hastalığı Olan Hastalara Yaklaşım, Aile Hekimliği, 73-79.
16. Gandhi S, Weinberg I, Margey R, Jaff R,M. (2011). Comprehensive Medical Management of Peripheral Arterial Disease. Progress in Cardiovascular Diseases, 54, 1;,2-13. https://doi.org/10.1016/j.pcad.2011.02.004.
17. Guyton, C.A., Hall, J.E. (2007). Tıbbi Fizyoloji, (H. Çavuşoğlu, B. Çağlayan Yeğen, Çev.). Ankara: Nobel Tıp Kitapevleri.
18. Güneşdoğdu Akpınar, F. (2008). Akut Miyokard İnfarktüslü Hastalarda Ortalama Trombosit Hacmi Ve Kanama Zamanının Değerlendirilmesi, Uzmanlık Tezi İstanbul.
19. Hirsch, A.T, Criqui, MH D Treat-Jacobson, et al. (2001). Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA, 286, pp. 1317-1324.
20. Horasanlı, E. (2014). http://www.tard.org.tr/tark2014/web_sunumlar/e_horasanli.pdf Erişim Tarihi: 26.11.2014
21. Insull, W. (2009).The Pathology of Atherosclerosis: Plaque Development and Plaque Responses to Medical Treatment, The American Journal of Medicine, 122 (1): Supplement 1:S3-S14.
22. Kavradım, T.S., Özer, C.Z. (2013). Kalp Yetersizliği Tanısı Olan Bireylerde Semptom Yönetimi. Türk Kardiyol Dern Kardiyovasküler Hemşirelik Dergisi - Turk Soc Cardiol Turkish Journal of Cardiovascular Nursing;4(6):1-14. Erişim Adresi: http://adminwebcast.tkd.org.tr/Files/Pdf/KVHD-86158%20derleme1-son.pdf
23. Kayhan, A,S., and Güner, E. (2021). Periferik arter hastalığında yaşam tarzı değişikliğinin yönetimi: Neler yapılabilir? SBÜ Hemşirelik Dergisi, 3(2), 115-120. doi: 10.48071/sbuhemsirelik.823885.
24. Kepez, A., Kabakcı, G. (2004). Kalp yetersizliği tedavisi. Hacettepe Tıp Dergisi; 35:69-81. Erişim Adresi: http://www.tip.hacettepe.edu.tr/actamedica/2004/sayi_2/baslik2.pdf
25. Koç, M. (2006). Kronik Kalp Yetersizliğinde Fonksiyonel Kapasite Ve Prognozu Değerlendirmede Serum NT-proBNP Düzeyi, Uzmanlık Tezi, Adana.
26. Kozan, Ö. (2009). Temel Kalp Hastalıkları Atlası, Güneş Tıp Kitapevleri.
27. Kumar, V., Abbas, A.K., Fausto, N., Mitchell R.N. (2007) Robbins Temel Patoloji (Basic Patoloji). Ed. Çevikbaş, U. Copyright Nobel Tıp Kitapevleri Ltd. Şt.

28. Norgren L, W.R. Hiatt, J.A. Dormandy, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II) J Vasc Surg, 45 (Suppl S) (2007), pp. S5-S67
29. Novo S. (2002). Classification, epidemiology, risk factors, and natural history of peripheral arterial disease. Diabetes Obes Metab, 4;pp. S1-S6
30. Song P, Rudan D, Zhu Y, Fowkes FJI, Rahimi K, Fowkes FGR, Rudan I. Global, regional, and national prevalence and risk factors for peripheral artery disease in 2015: an updated systematic review and analysis. Lancet Glob Health. 2019; 7:e1020–e1030.
31. Şahna, E., Deniz, E., Aksulu, H.E. (2006). Miyokardiyal iskemi-reperfüzyon hasarı ve melatonin. Anadolu Kardiyol Derg; 6: 163-8. Erişim Adresi: http://www.anakarder.com/sayilar/38/buyuk/2006-163-168.pdf. Erişim Tarihi: 26.11.2014.
32. Tutkun, T. (2008). Konjestif Kalp Yetersizliğinde Karaciğer Fonksiyon Testlerindeki Değişiklikler, Mortalite ve Hastanede Kalış Süresine Etkisi, Uzmanlık Tezi, İstanbul.
33. Türk Kardiyoloji Derneği. (2007). Kalp Yetersizliği Akut Koroner Sendromlar Hipertansiyon Hemşirelik Bakım Kılavuzu. Erişim Adresi: http://www.tkd.org.tr/~/media/files/tkd/calisma-gruplari/kardiyovaskuler-hemsirelik/tkd_kalp_yetersizligi_hbk.pdf
34. Zipes, D.P., Libby, P., Bonow, R.O., Braunwald, E. (2008). Braunwald Kalp Hastalıkları, Nobel Tıp Kitapevleri, İstanbul.

Hemorrhoids

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Introduction

Hemorrhoids are anatomical structures of the anal canal which contribute to the continence mechanism. Due to various reasons they may become enlarged, engorged and thus symptomatic. The often encountered symptoms are bleeding, mucosal soiling and pruritus mostly during and after defecation but they may also occur spontaneously. A colorectal surgeon must comprehend this clinical entity well since it comprises a significant amount of outpatient clinic admissions and other benign proctological conditions can easily be mistaken as hemorrhoids by both patients and health professionals.

Anatomy

The upper anatomical part of the surgical anal canal is called Anal Transitional Zone (ATZ) where cushion like venous plexuses reside in its subepithelial space, hence the name Corpus Cavernosum Recti (CCR) (Fig. 1). This plexus is not continous and often three distinct cushions (left lateral, right anterior and right posterior in supine position) are prominent, which are called hemorrhoids. These veins tend to be tortuous in nature and with their surrounding smooth muscle fibers and connective tissues, they are also referred as anal glomerula (1). They are anatomical structures. The integrity of these plexuses are maintained with the muscle fibers originating from the Internal Anal Sphincter (IAS) and Conjoined Longitudinal Muscle (CLM). Superior Rectal Artery (SRA) is the main arterial source while the venous drainage is maintained by hemorrhoidal veins.

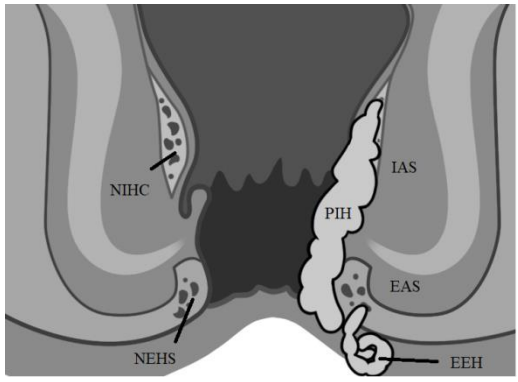

FIGURE 2. The Illustration of Sphincters and Hemorrhoidal Structures. IAS:Internal Anal Sphincter, EAS:External Anal Sphincter, PIH:Prolapsed Internal Hemorrhoid, EEH: Engorged External Hemorrhoid, NIHC:Normal Internal Hemorrhoidal Cushion, NEHS: Normal External Hemorrhoidal Structure

Epidemiology and Etiology

Although it is challenging to assess the true prevalence of the hemorrhoidal disease due to the fact that most of the individuals remain asymptomatic and therefore do not seek medical treatment, the contemporary literature reports a disease burden of 10% among the general population with a slight female predisposition (2).

Variousfactors contribute to the pathogenesis. For this reason it is hard to define a single mechanism to be the underlying cause. However, almost every factor contributing to the development of this disease ultimately result in either impaired venous drainage or destruction of the connective tissue within the cushions (3). In most cases these two main alterations overlap. Studies demonstrated an increased metalloproteinase activity, namely MMP-9, and neovascularization due to overexpression of VEGF and TGF-ß in clinically significant hemorrhoidal cushions (4,5). Additionally, the resting anal canal pressure is found to be elevated compared to healthy individualsin patients with hemorrhoidal disease (6). Ultimately two distinct theories are generally accepted;

1. Hyperplasia Theory: Impaired venous drainage of CCR due to the increased IAS pressure which results in prolapsus of the cushions into the anal canal.

2. Anal Sliding Theory: Submucosal smooth muscle destruction as a result of increased intraabdominal pressure, which again leads to prolapsus. Chronic constipation, chronic cough, pregnancy and numerous other entities can result in increased intraabdominal pressure.

Interestingly, no association between increased hemorrhoidal disease prevalence and liver cirrhosis could be shown, even though cirrhosis clearly increases the intraabdominal pressure (7). Nevertheless, rectal varices develop in most of the cirrhotic patients and can be mistaken as hemorrhoids.

Classification

Hemorrhoids which originate from the ATZ are described as internal hemorrhoids. Occasionally, hemorrhoids may also occur below the dentate line and are referred as external hemorrhoids. Contemporarily, Goligher Classification is still applied to grade internal hemorrhoids (8). According to this classification four distinct grades can be distinguished (Fig. 2);

Grade I: Slightly enlarged cushions with no visible prolapsus.

Grade II: Cushions prolapse and become visible during defecation or straining but reduce spontaneously.

Grade III: Prolapsed cushions which can be reduced manually.

Grade IV: Irreducible cushions.

Despite being routinely applied in clinical setting worldwide, this classification lacks some important aspects of this disease, such as circumferential hemorrhoids, acute thrombosed hemorrhoids, mucosal prolapsus, etc. Some new classifications are proposed to address this issue. Unfortunately, the data in this regard are sparse and thus no optimal classification can be applied yet.

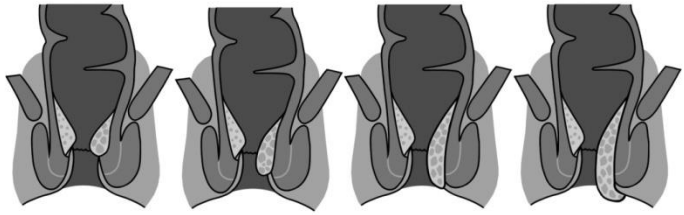

FIGURE 2. Goligher Classification of Hemorrhoids (Grade increases from left to right)

Clinical Presentation and Diagnosis

A hemorrhoidal disease presents itself most commonly with rectal bleeding, prolapsus, itching and pain. Pain is more common in external hemorrhoids. However, when the prolapsed hemorrhoid also includes an external part or it gets thrombosed, pain may still occur. Itching is also very common, but if it is the only symptom the patient describes, the practitioner should focus on pruritus ani rather than hemorrhoidal disease. When pain is the sole symptom with no visible external hemorrhoids, anal fissure should be ruled out, because patients tend to misinterpret skin tags for hemorrhoidal prolapse. When a thrombosis occur within a hemorrhoid cushion, the pain onset is sudden and its course is relentless. Patients with a history of ongoing or intermittent diarrhea should be evaluated for inflammatory bowel diseases. One needs to keep in mind, that if the pain is not accompanied by a visible hemorrhoidal cushion, perianal abscess might be the underlying condition. Other anorectal pathologies such as fistulas, malignancies should also be ruled out.

Nowadays, it is recommended by the european authorities to evaluate the first 15 cm of the rectum with a rectoscopy, in case of a suspicion of hemorrhoidal disease or even if the diagnosis is certain (9). A patient should undergo a complete colonoscopy if any predisposing factors to colorectal malignancies are present, such as increased age, family history, etc.

The physical examination can be performed in both prone-jackknife or left lateral position. A digital rectal examination is a must under any circumstances, except for the acute anal fissures, and the squeeze and resting anal canal pressures must also be evaluated during this examination.

Treatment

The initiation of a treatment only depends on whether the disease is symptomatic or not, regardless of its grade. In general, conservative treatment precedes and is favored over surgical approaches, except for the emergent cases, such as gangrenous hemorrhoids.

Conservative Treatment

First of all, the diet of a patient should be queried before commencing the patient on any medication. If the patients does not consume enough dietary fibre, it becomes very difficult to maintain a healthy bowel homeostasis. Therefore, it is recommended to consume 25 g daily fibre for women and 38 g daily fibre for men (10). The daily water intake should also be queried and if it is deemed insufficient, an adequate water consumption must also be ensured. With these dieatery changes, constipation, the most common problem among patients with hemorrhoidal disease, can be overcome. Patients with chronic constipation may further continue suffering even under a strict fibre diet. In these patients, oral laxatives can be administered for a limited period of time (usually less than a week) (11).

Patients must also be educated about toilet habits. Prolonged defecation time and excessive straining are both associated with increased hemorrhoidal protrusion rates. The conditions related with the Obstructive Defecation Syndrome are beyond the scope of this chapter, however patients can at least be advised f.e. not to bring a mobile phone to toilet, since this kind of habit clearly and inevitably prolongs the toilet stay. Patients should also be encouraged to have sitz baths 3 times a day for 10-15 mins with warm water. Applying warm water relaxes the IAS and thus facilitates venous return which alleviates the symptoms. A detachable shower headcan also be utilized as a proxy for patients who can not perform sitz baths for various reasons.

Both flavonoids (phytonutrients which increase the venous tonus) and calcium dobesilate (a vasoprotective agent) are effective oral therapy options (12, 13). The recommended dose of calcium dobesilate for the acute setting is 500 mg twice a day, and the maintenance dose is 500 mg once a day. Any topical therapy, which results in decreased IAS tonus, alleviates pain or facilitates vasoconstriction, can theoretically be beneficial in treatment. Although no significant harmful effects have been reported in their utilization in hemorrhoidal disease treatment, the data regarding their efficacy in this setting are sparse. We prefer ointments over suppositories, since the latter is harder to maintain in the optimal anatomical position.

Surgical Treatment

Surgical treatments can be put into two main categories; Resection and non-resection techniques. Some of the non-resection techniques can also be performed in outpatient settings and are referred as office-based treatments. They include rubber band ligation, sclerotherapy, infrared coagulation and occasionally laser hemorrhoidoplasty. All of the office-based procedures target internal hemorrhoids, since the anatomical area is significantly less associated with pain. Non-resection techniques mainly aims to facilitate fibrosis around the CCR and thus fixation of the ATZ.

Sclerotherapy

This technique comprises of the injection of a sclerosing agent into the apex of a hemorrhoidal cushion. Various agents can be utilized, however ethoxysclerol or aliminium potassium sulfate and tannic acid (ALTA) are favored over the others (14). It is showed that ALTA is associated with less bleeding than phenol in almond oil (15).

The patient is positioned either in supine or prone-jackknife position and at the apex of each pedicle 1-3 mL sclerosing agent is injected depending on the agent and its concentration. Care should be taken to inject the substance directly to submucosa and avoid IAS, mucosa and CCR. This technique is relatively easy and painless. However, it is associated with high recurrence rates in the long term and repeat attempts are not encoureged due to risk of stricture. This therapy is effective in Grade I-II hemorrhoids.

Infrared Coagulation

This technique provides the same fibrosis and fixation mechanism with infrared energy instead of a sclerosing agent. Patients with low grade hemorrhoidsare candidates for his procedure. A special, portable device is utilized. With the tip of the device, the apex is targeted and 3-4 applications can be done for each hemorrhoid. A recent prospective study favored this technique even over resection procedures (16). However, the data are sparse and the risk of local necrosis limited its use.

Rubber Band Ligation

The principle is that the apex of a hemorrhoid and the underlying rectal mucosa are strangulated with a band. Fibrosis and fixation occur consequently. The hemorrhoid is visualized with a proctoscope. Then the targeted cushion is pulled inside the device either with suction or a grasper and the band is deployed.

Multiple cushions can be addressed at the same time, although it is still advised not to apply bands to more than three cushions in one session (17). The procedure can be repeated at monthly intervals. Rubber band ligation is effective in grade I-II hemorrhoids. Its role in grade III hemorrhoids is still an ongoing debate.

Band slippage and bleedingare common complications. Interestingly, bleeding can also occur 5-7 days after the procedure. Care should be taken, as in all surgical procedures for hemorrhoidal disease, not to overlook the signs of pelvic sepsis, which manifests itself at the early stage with urinary retention, pelvic pain and fever. However, pain is usually attributed to bands which are deployed too close to dentate line. In case of prolonged pain or in the presence of sepsis signs, either an anal examination under general anesthesia or computed tomography (CT) must be performed.

Laser Hemorrhoidoplasty

By using a 980-nm laser diode, the apex of the hemorrhoid is targeted, photo-energy delievered, arterial inflow diminished and consequently, fibrosis and shrinking are achieved (Fig. 3). The intraoperative positioning is the same as the others . The pedicle is grasped gently. Then the probe is inserted inside the pedicle above the dentate line and moved upwards. Three laser shots are made in the apex, two in the middle and the last one in the entrance area. Care should be taken not to damage neither IAS nor mucosa. After targeting each pedicle, we apply cold (mostly an icecube in a glove) for a minute to avoid hematoma.

This method is most effective in grade II hemorrhoids. The advantages are its short operation duration, less pain and low recurrence rates(18). However, despite being evaluated in a few studies, the data are sparse about its usage in grade III hemorrhoids. There is also a lack of information as to whether this technique is superior to other techniques. Therefore, the exact role of this method will be determined by future prospective studies.

FIGURE 3. Neo v Laser 1470 Nm, Diode Laser Machine. G.N.S neoLaser Ltd.©

Doppler-Guided Hemorrhoidal Artery Ligation

A special scope with a doppler device on its tip is inserted into the anal canal(Fig. 4). At the apex of the hemorrhoidal pedicle, its artery is identified and suture ligated. The ceasing of the arterial flow sound confirms a successful intervention. Once the arterial inflow is stopped, the rest of the mechanism is the same as with the laser method. Many surgeons also add a mucopexy by oversewing the pedicle caudally with the same suture till the dentate line and then knotting the first and the last thread together. The result is lifting of the pedicle, hence the name anal lifting.

This technique is associated with low recurrence rates, short operation times, especially in grade II-III hemorrhoids and when a mucopexy is done aswell (19). Its main disadvantage can be pain. The rest of the complications are similar to the other above-mentioned procedures. However, date are sparse about this technique, too. Although there are some qualified trials about this procedure, more prospective trials are required to understand the true value of this method (20).

FIGURE 4. Comepa Angiodin-Procto ADL. Comepa Industries ©

Stapled Hemorrhoidopexy

This is a resection based technique performed by using a type of circular stapler. First the mucosa and submucosa of a circumferential line 4 cm above the dentate line is sutured continuously. Then the anvil of the device is introduced to the proximal of this line. The stapler is closed, with the hemorrhoidal pedicle is partially entrapped within, and fired. The excision of a mucosal doughnut allows lifting of the remaining hemorrhoidal pedicles, thus reduce prolapsus. Care should be taken not to involve rectovaginal septum, as this results in rectovaginal fistulas. The staple line should always be checked after firing the stapler, since bleeding occurs from this line and bleeding is the most common complication of this procedure.

This technique was first developed in 90's to present an alternative to conventional hemorrhodectomy in grade II-III hemorrhoidal diseases. The literature suggests that it should not be undertaken in hemorrhoids which can not be reduced manually or which are thrombosed (21). Although this technique is associated with less pain compared with conventional methods, the recurrence rate is higher andone of the main downsides is that it reduces rectal compliance. Therefore many surgeons avoid using this technique more than once for the same patient. In our surgical practice, we tend to prefer this method less compared with the past, since our experience with other less invasive methods are more satisfying.

Excisional Hemorrhoidectomy

Excisional methods are basically reserved for Grade IV hemorrhoids or emergent situations, such as gangrenous hemorrhoids. They are still the gold standart in hemorrhoidal disease treatment. Recurrence rates are very low. However, they are associated with significant postoperative pain and stricture risk (22).

Except for Whitehead procedure, which is reserved for circumferential hemorrhoids, there are mainly three conventional excisional surgical interventions which are almost identical except for the wound closure at the end of the operation.

Basically, after positioning the patient, a combination of a local anesthetic and epinephrine 1:200,000 is injected in the perianal skin and inside of hemorrhoids. This allows less bleeding during the procedure. The pedicle is grasped. The perianal skin is incised and the hemorrhoidal structure is dissected and lifted off the underlying sphincter structures untill the apex, where the vascular pedicle is reached. We prefer using an electrocautery till this stage, however other options like bipolar devices or simply scissors are also viable.

Once the apex is reached the pedicle is either stitch sutured or sealed with a bipolar device. Care should be taken to leave adequate skin-tissue bridges as the length of perianal skin incision circumfercnce should not exceed the half of the total perianal skin circumferance. Otherwise a postoperative anal stricture is likely to develop.

The above-mentioned steps are common. Once the excision is completed, Milligan-Morgan technique leaves the wound open to secondary healing. Parks technique recommends the closure of wound including the anoderm and Ferguson closes the perianal skin as well. Leaving the wound open exposes the patient to a slightly more stricture risk while suturing the wound may cause slightly more infection. However, these are not great differences and can be omitted. None of these methods are significantly superior to another and we believe it is up to surgeon's preference to tailor them for each patient individually.

Patients should be advised to continue using sitz baths, have daily shower with warm water and keep the area clean with water after defecation. Neither antibiotics nor anal tamponade are not mandated.

Whitehead excision mandates a circumferential incision along the dentate line. All hemorrhoidal structures are excised as well as excessive rectal mucosa. Then the remaining rectal mucosa is sutured to anoderm. This operation is highly effective as it removes all of the pedicles, but it is also associated with serious complications such as strictures, whitehead deformity, gas incontinence, etc (23).

Complications and Their Management

Anal Stenosis

The cause is the excision of too much anoderm. The clinical picture may turn out to be a mild one, which only requires laxatives. However, some patients may undergo dilation or even stricturoplasty (24).

Urinary Retention

This is a common complication of hemorrhoidal interventions, which are usually attributed to peroperative excessive IV fluids, inadequate pain relief and use of anal tamponades (25). The risk also increases if the patient has a urinary retention history such as benign prostate enlargement. To avoid this complication, the above-mentioned risk factors should be eliminated. Some surgeons also prefer using a urinary catheter, but this approach is still an individual practice rather than a scientific recommendation.

Pain

Anoderm is the sensible part of the anal canal. Therefore if an area below the dentate line gets operated on, pain occurs. However, despite some techniques being less related with pain, all surgical procedures can cause postoperative pain. Therefore, a routine commencement on postoperative analgesia is recommended. If the pain persists more than a week despite adequate analgesics or pelvic sepsis signs occur, an examination under general anesthesia must be performed.

Postoperative Hemorrhage

Postoperative bleeding occurs either in the immediate period (within 48 hours) or within postoperative days 7-10. Early bleedings are associated mostly with a technical error, such as overlooked stapler line bleeding. The cause of delayed hemorrhage is not certain, but it can be attributed to necrosis or sepsis of the pedicle apex. These hemorrhages rarely requires early surgical intervention. Mostly an anal tamponade and close monitoring of the patient are sufficient measures. If the bleeding persists or causes hemodynamic instability, readmission to the operation theatre becomes a necessity.

Infection

This is a rare complication of hemorrhoidectomies. If it is suspected, antibiotics should be administered. If an abscess develops, it must be drained. A physician should always take the possibility of a pelvic sepsis and fournier gangrene into account, when evaluating a patient in regard of postoperative infection.

Fecal Incontinence

Hemorrhoidal cushions contribute mostly to gas continence. That means when they are excessively removed, a mild incontinence may occur. But this is usually a rare condition. Another contributor to fecal incontinence is damage to the IAS during surgery. A keypoint in addressing this issue is to evaluate the patient preoperatively in this regard. Especially parous women of advanced age are under risk. One should also keep in mind that stapled hemorrhoidopexy reduces the rectal capacity and thus may cause urge incontinence.

Special Clinical Pictures

Thrombosed External Hemorrhoid

The patient usually describes pain with a sudden onset. It is continous and in some cases very severe. If the patient is compliant and the pain is managable, a

usual conservative therapy as described above should be commenced. If the pain is severe and relentless and the patient too restless, a simple enucleation can be performed. A physician must, however, discriminate an isolated thrombosed external hemorrhoid from a thrombosed prolapsed hemorrhoid and should not attempt this intervention for the latter (26).

Thrombosed Hemorrhoidal Prolapse

A thrombosed hemorrhoidal prolapse usually consists of both an internal and external hemorrhoid. In case of necrosis, an emergent surgery must be performed. In this scenario, it is usually appropriate to excise the whole hemorrhoidal body including the external part. However, an emergent intervention in a hostile edematous environment exposes the patient to a significant risk of postoperative stricture. Therefore even in cases of irreducable and thrombosed hemorrhoids, if there is no necrosis, repositioning with conservative methods should be the line of treatment (27). Nevertheless,an urgent hemorrhoidectomy after a few days of relief can be a definitive treatment choice.

Crohn's Disease

A physician must always prefer conservative treatment options in patients with Crohn's Disease. Patients, who are not under steroid therapy and whose anorectal region is not affected, might be surgical candidates. Again, less invasive techniques should be preferred to excisional therapies.

Immunocompromised Patients

This patient population has an altered wound healing process. As the patients with Crohn's Disease, they are prone to surgical site infections and impaired healing. Conservative treatments must be a physician's first choice, as long as they remain feasible. If a surgery is to be performed, prophylactic antibiotics must be administered.

Pregnancy

Hemorrhoids are common during pregnancy and the symptoms usually resolve after childbirth. Normally, conservative managements with dietary cautions and sitz baths suffice to alleviate symptoms. In cases of strangulated or thrombosed hemorrhoids, a surgical intervention, preferably under local anesthesia, might be performed.

Recurrent Disease

Recurrence should be clearly distinguished from a redundant hemorrhoidal tissue. With the latter, a patient almost never experiences a sufficient relief of symptoms following surgery.

The only way to eliminate the risk of recurrence completely is to remove the CCR completely. This means after every procedure, there is a risk of recurrence. Patients can be commenced on conservative and medical treatment even after recurrence. Nevertheless, if surgery is deemed appropriate, its keypoints are as follows;

1. A ligation based technique is still feasible after a ligation based technique.
2. A ligation based technique is the treatment of choice following a conventional excisional technique, due to increased risk of stricture.
3. One should not perform stapled hemorrhoidopexy in a patient twice.

References

1. Aigner, F., Gruber, H., Conrad, F., et al. (2009). Revised morphology and hemodynamics of the anorectal vascular plexus: impact on the course of hemorrhoidal disease. *International journal of colorectal disease*, *24*(1), 105–113. https://doi.org/10.1007/s00384-008-0572-3
2. Sheikh, P., Régnier, C., Goron, F., et al. (2020). The prevalence, characteristics and treatment of hemorrhoidal disease: results of an international web-based survey. *Journal of comparative effectiveness research*, *9*(17), 1219–1232. https://doi.org/10.2217/cer-2020-0159
3. Lohsiriwat V. (2012). Hemorrhoids: from basic pathophysiology to clinical management. *World journal of gastroenterology*, *18*(17), 2009–2017. https://doi.org/10.3748/wjg.v18.i17.2009
4. Han, W., Wang, Z. J., Zhao, B., et al. (2005). *Zhonghua wei chang wai ke za zhi = Chinese journal of gastrointestinal surgery*, *8*(1), 56–59.
5. Yoon, S. O., Park, S. J., Yun, C. H., et al. (2003). Roles of matrix metalloproteinases in tumor metastasis and angiogenesis. *Journal of biochemistry and molecular biology*, *36*(1), 128–137. https://doi.org/10.5483/bmbrep.2003.36.1.128
6. Sun, W. M., Peck, R. J., Shorthouse, A. J., et al. (1992). Haemorrhoids are associated not with hypertrophy of the internal anal sphincter, but with hypertension of the anal cushions. *The British journal of surgery*, *79*(6), 592–594. https://doi.org/10.1002/bjs.1800790642
7. Al Khalloufi, K., & Laiyemo, A. O. (2015). Management of rectal varices in portal hypertension. *World journal of hepatology*, *7*(30), 2992–2998. https://doi.org/10.4254/wjh.v7.i30.2992
8. Rubbini, M., & Ascanelli, S. (2019). Classification and guidelines of hemorrhoidal disease: Present and future. *World journal of gastrointestinal surgery*, *11*(3), 117–121. https://doi.org/10.4240/wjgs.v11.i3.117
9. Herold, A., Lehur, P.A., Matzel, K.E., O'Connell, P.R. (2017). Hemorrhoids. *Coloproctology* (2nd Ed.). Springer. p. 39.
10. Turner, N. D., & Lupton, J. R. (2011). Dietary fiber. *Advances in nutrition (Bethesda, Md.)*, *2*(2), 151–152. https://doi.org/10.3945/an.110.000281
11. Cengiz, T. B., & Gorgun, E. (2019). Hemorrhoids: A range of treatments. *Cleveland Clinic journal of medicine*, *86*(9), 612–620. https://doi.org/10.3949/ccjm.86a.18079
12. Menteş, B. B., Görgül, A., Tatlicioğlu, E., Ayoğlu, F., & Unal, S. (2001). Efficacy of calcium dobesilate in treating acute attacks of hemorrhoidal

disease. *Diseases of the colon and rectum*, *44*(10), 1489–1495. https://doi.org/10.1007/BF02234604
13. Changazi, S. H., Bhatti, S., Choudary, A., et al. (2020). Calcium Dobesilate Versus Flavonoids for the Treatment of Early Hemorrhoidal Disease: A Randomized Controlled Trial. *Cureus*, *12*(8), e9845. https://doi.org/10.7759/cureus.9845
14. He, A., & Chen, M. (2022). Sclerotherapy in Hemorrhoids. *The Indian journal of surgery*, 1–5. Advance online publication. https://doi.org/10.1007/s12262-022-03414-3
15. Yano, T., & Yano, K. (2015). Comparison of Injection Sclerotherapy Between 5% Phenol in Almond Oil and Aluminum Potassium Sulfate and Tannic Acid for Grade 3 Hemorrhoids. *Annals of coloproctology*, *31*(3), 103–105. https://doi.org/10.3393/ac.2015.31.3.103
16. Nikshoar, M. R., Maleki, Z., & Nemati Honar, B. (2018). The Clinical Efficacy of Infrared Photocoagulation Versus Closed Hemorrhoidectomy in Treatment of Hemorrhoid. *Journal of lasers in medical sciences*, *9*(1), 23–26. https://doi.org/10.15171/jlms.2018.06
17. Chaleoykitti B. (2002). Comparative study between multiple and single rubber band ligation in one session for bleeding internal, hemorrhoids: a prospective study. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet*, *85*(3), 345–350.
18. Lakmal, K., Basnayake, O., Jayarajah, U., et al. (2021). Clinical Outcomes and Effectiveness of Laser Treatment for Hemorrhoids: A Systematic Review. *World journal of surgery*, *45*(4), 1222–1236. https://doi.org/10.1007/s00268-020-05923-2
19. Ratto, C., Campennì, P., Papeo, F., et al. (2017). Transanal hemorrhoidal dearterialization (THD) for hemorrhoidal disease: a single-center study on 1000 consecutive cases and a review of the literature. *Techniques in coloproctology*, *21*(12), 953–962. https://doi.org/10.1007/s10151-017-1726-5
20. Brown, S., Tiernan, J., Biggs, K., et al. (2016). The HubBLe Trial: haemorrhoidal artery ligation (HAL) versus rubber band ligation (RBL) for symptomatic second- and third-degree haemorrhoids: a multicentre randomised controlled trial and health-economic evaluation. *Health technology assessment (Winchester, England)*, *20*(88), 1–150. https://doi.org/10.3310/hta20880
21. Porrett, L. J., Porrett, J. K., & Ho, Y. H. (2015). Documented complications of staple hemorrhoidopexy: a systematic

review. *International surgery*, *100*(1), 44–57. https://doi.org/10.9738/INTSURG-D-13-00173.1
22. Lohsiriwat, V., & Jitmungngan, R. (2022). Strategies to Reduce Post-Hemorrhoidectomy Pain: A Systematic Review. *Medicina (Kaunas, Lithuania)*, *58*(3), 418. https://doi.org/10.3390/medicina58030418
23. Erzurumlu, K., Karabulut, K., Özbalcı, G. S., et al. (2017). The Whitehead operation procedure: Is it a useful technique?. *Turkish journal of surgery*, *33*(3), 190–194. https://doi.org/10.5152/turkjsurg.2017.3483
24. Brisinda, G., Vanella, S., Cadeddu, F., et al. (2009). Surgical treatment of anal stenosis. *World journal of gastroenterology*, *15*(16), 1921–1928. https://doi.org/10.3748/wjg.15.1921
25. Toyonaga, T., Matsushima, M., Sogawa, N., et al. (2006). Postoperative urinary retention after surgery for benign anorectal disease: potential risk factors and strategy for prevention. *International journal of colorectal disease*, *21*(7), 676–682. https://doi.org/10.1007/s00384-005-0077-2
26. Lawrence A, McLaren ER. External Hemorrhoid. [Updated 2021 Aug 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK500009/
27. Hardy, A., & Cohen, C. R. (2014). The acute management of haemorrhoids. *Annals of the Royal College of Surgeons of England*, *96*(7), 508–511. https://doi.org/10.1308/003588414X13946184900967

Ductal Carcinoma In-Situ of The Breast

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INTRODUCTION

Ductal carcinoma in-situ (DCIS) is non-invasive carcinoma of the breast and defined as proliferation of epithelial cells without distrupting the structure of the basement membrane(Cowell et al., 2013).DCIS is a precursor for invasive disease. Studies suggest that 14-53% of untreated DCIS patients may progress to invasive cancer(Erbas vd., 2006). With the broad use of routine breast cancer screening programs, nowadays DCIS can be detected more often.

In 1919 James Ewing classified breast carcinomas and illustrated non-invasive carcinoma. While the illustration clearly showed DCIS he did not use the term. Albert Brodes used the term carcinoma in-situ in 1832(Feinberg et al., 2018).

EPIDEMIOLOGY

One out of every eight women will be diagnosed with breast cancer during her lifetime, and about 20 percent of these breast cancer patients have DCIS(Iatrakis & Zervoudis, 2021).It is expected that more then 50000 new DCIS patients will be diagnosed in the USA in 2022. According to The Surveillance, Epidemiology, and End Results (SEER) data, mean age of DCIS patients ranges from 45 to 63 and majority of the patients are white(Ward et al., 2017). The fact that the median age of breast cancer patients is 61 also indicates that DCIS is the precursor lesion of invasive breast cancer(Rojas & Stuckey, 2016).

With the routine use of screening mammography, there has been a more than 5-fold increase in the incidence of DCIS between 1970 and 2003(Virnig, Tuttle, et al., 2010). Considering most DCIS patients are detected with mammography, DCIS incidence rises at age of 50, which most screening programs start at, and starts to decline after age of 70, which screening programs end.

RISK FACTORS

Risk factors for DCIS are similar to those for invasive breast cancer. Risk factors include history of breast cancer in the first degree relative, older age, increased estrogene exposure (nulliparity, early menarche, late age of first birth, late menopause), obesity and personal history of benign breast disease. In a recent cohort study with 263788 patients, increased physical activity was associated with a reduced risk of DCIS(Peila et al., 2020). Mutations in BRCA-1(Breast cancer-associated) and BRCA-2genes increases risk for DCISas well asinvasive breast cancer.

Incidence of DCIS is strongly related to age and DCIS before age of 40 is rare. Incidence increases after age of 40 and peaks between ages of 65-69 to a peak of 96.7 per 100000(Virnig, Wang, et al., 2010).

HISTOPATHOLOGY

DCIS is the proliferation of malignant epithelial cells within the breast ducts without disturbing the parenchymal structure. It is characteristic that malignant cells do not exceed the basement membrane, and with this feature it is distinguished from invasive breast cancer. DCIS originates from the ductus epithelium in the terminal duct lobular unit(Bragg et al., 2021).

Macroscopy

Most DCIS patients without a palpable mass get diagnosed in routine screening mammography and most of the time these tumors are indistinguishable from healthy tissues with naked eye. Those presented with a palpable mass may be seen as fibrotic masses without distinctive borders unlike most solid tumors do. Such lesions should assessed with specimen radiography to target appropriate areas for histopathologic examination(Fletcher, 2021).

Classification

Traditionally, DCIS classified to 5 subtypes according to histologic growth patterns and nuclear features but in most of the lesions two or more subtypes may coexist. Most common subtypes are comedo and cribriform subtypes(Feig et al., 2019). However coexistence of architectural subtypes results in inefficiency of this classification.

*Comedo type DCIS(Figure1)*named after its apearance characterized with thick walled ducts which exude material with ‘comedon-like’ fashion when compressed. Most distinctive feature of comedo DCIS is atypical pleomorphic cells and intraluminal ‘comedo’ necrosis associated with calcifications(Jaffer & Bleiweiss, 2002). It should be noted that comedo-necrosis and comedo-type are different entities.

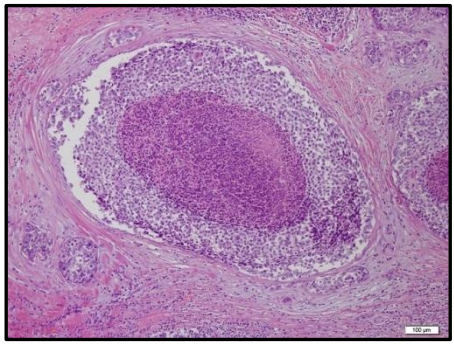

Figure1: *Comedo type DCIS with central necrosis,Haematoxilen-Eosin(HE), 100x (All histopathologic pictures taken from personal archieves of Dr.Onur Şahin with his permission.)*

*Micropapillary type DCIS(Figure2)*named for its papillary extensions outstretched into ductus. Papillae have no fibrovascular cores. Extensions may range from tufts to long processes(Bane, 2013).

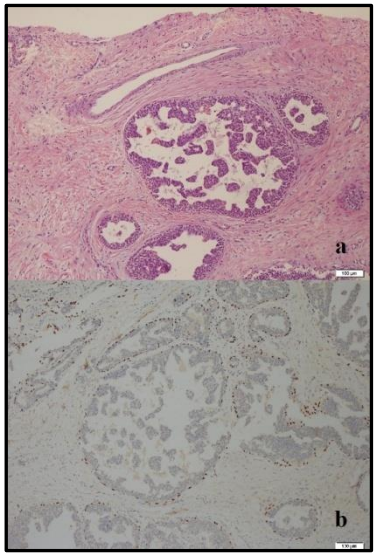

Figure2: *Micropapillary type DCIS.* ***a)****Papillary projections into ductus lacking fibrovasculary cores and club shaped cells with mild pleomorphism, HE, 100x****b)****Myoepitelial cells highlighted with p63 immunstaining, 100x.*

*Cribriform type DCIS(Figure3)*consist of fenestrated patern of intraductal proliferation. Cells show low grade atypia. Proliferation rates are generally low but may present with high-grade atypia and high proliferation rates(Jaffer & Bleiweiss, 2002).

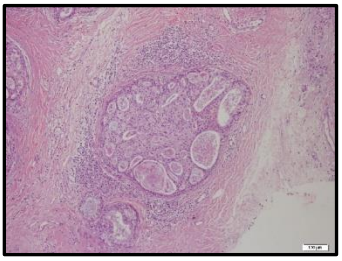

Figure3: *Cribriform type DCIS. Low grade, uniform, round and small nuclei with smooth borders. HE 100x.*

In modern era, DCIS classified according to recurrence and progression to invasive cancer. *Nuclear grade* is one of the important histopathological factors. DCIS lesions are divided into 3 groups according to the cytonuclear grade(The Consensus Conference Committee, 1997). High grade*(Figure4)* lesions tend to recur and progress to invasive breast cancer more often. European Organisation for Research and Treatment of Cancer (EORTC) trial 10853 showed increased recurrence after breast conserving therapy(BCT)(EORTC Breast Cancer Cooperative Group et al., 2006).

Comedo-type necrosis is defined as intraepithelial growth confined in the basal membrane with central necrosis.In the National Surgical Adjuvant Breast Project (NSABP)

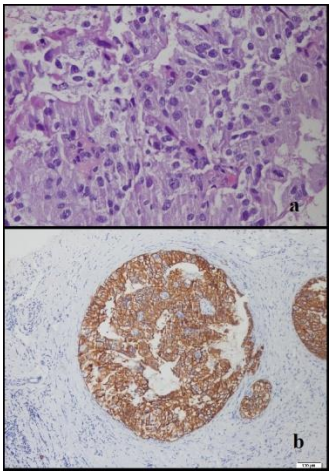

Figure4: *High grade DCIS.* ***a)****Pleomorphic nucleus with prominent necleolus and vesicular chromatin, HE, 400x****b)*** *c-ErbB2 positive immunostainig. Low grade lesions most likely stain positive with ER immunstain and ER staining decreases with higher grade lesions while c-ErbB2 staining increases with higher grade.*

Protocol B-17 trial, comedo-necrosis was found to be the most important factor associated with local recurrence(E. R. Fisher et al., 1999).

With these findings, Silverstein et al. developed Van Nuys Prognostic Index (VNPI)*(Table* **1***)*to determine agressiveness and risk of DCIS recurrence (Poller et al., 1995). Later, in 2003, age was also added to the Van Nuys Prognostic Index as a risk factor and the University of Southern California Prognostic Index (USC/VNPI)*(Table* **1***)*was published by Silverstein (Silverstein, 2003). Patients with USC/VNPI scores of 7-8-9 showed up to 15% recurrence rates in 12 years and patients with scores more then 10 showed even more percents of recurrence.

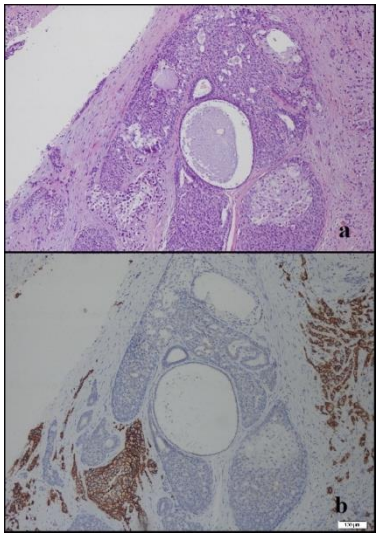

Figure5: *Solid intermediate grade ductal carcinoma in-situ.* ***a)****Sheets of cohesive cells with mild nuclear pleomophism. HE, 100x* ***b)****c-ErbB2 negative staining on ductal carcinoma in-situ (Notice invasive components on the sides of the Picture are stained pozitive with c-ErbB2 immunstainig).*

Table 1 *The USC/Van Nuys Prognostic Index scoring system. One to three points are awarded for each of four different predictors of local breast recurrence*

Score	**1**	**2**	**3**
Size (mm)	≤ 15 mm	16-40 mm	≥40 mm
Margin width (mm)	≥10 mm	1-9 mm	< 1mm
Pathologic classification	Non-high grade *without* necrosis (nuclear grades 1-2)	Non-high grade *with* necrosis (nuclear grades 1-2)	High grade with or without necrosis (nuclear grade 3)
Age	>60	40-60	<40

Molecular Classification

Molecular phenotypes for breast cancer is well established. These molecular phenotypes are identified with expressionsof estrogen receptor(ER) progesterone receptor(PR) and human epidermal growth factor receptor-2 (HER-2): Luminal A, Luminal B, HER-2enriched and basal like. Prognosis and treatment options for invasive breast cancer patients highly depend on these profiles. Prevalence of Luminal A, Luminal B, HER-2 enriched and basal like DCIS patients was found to be 62.5%, 13.2%, 13.6% and 7.7% respectively(Tamimi et al., 2008). In another study, it was reported that DCIS patients with Luminal A phenotype had lower 5-year recurrence rates compared to other phenotypes(Williams et al., 2015).

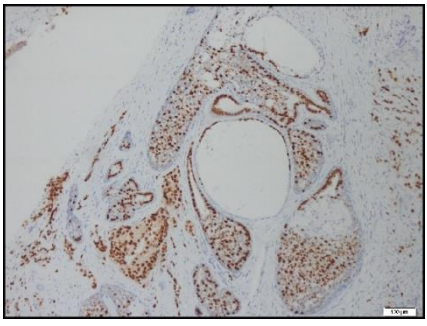

Figure6: İntermediate grade ductal carcinoma in-situ, positive staining for ER immunostain., 100x.

Other Microscopic Features

Ductal carcinoma in-situ is considered as a non-invasive disease because malignant cells does not invade through basement membrane and theoretically does not carry potential to metastatise. In contrast, DCIS with *microinvasion* is defined as invasion of basement membrane with malign cells at one or more area, none of which is more then a dimension of 1mm, thus DCIS with *microinvasion* has the ability to metastatise. Microinvasion in breast histopathology first described by Lagios at 1982(Lagios et al., 1982) and incidence of microinvasive cancer ranges from 0.68% to 2.4%(Hoda et al., 2000).According to the American Joint Committee on Cancer (AJCC) Staging Manual 8th edition, DCIS with microinvasion considered as T1mi and upstages DCIS from stage 0 to stage 1 disease(Kalli et al., 2018).

Multifocality is defined as more then one foci of DCIS separated by at least 5mm of normal tissue(Sikand, 2005). Patients with multifocal disease treated with BCT alone (i.e without adjuvant radiotherapy) showed decreased 10-year disease free survival compared to unifocal DCIS patients treated with BCT alone(Rakovitch et al., 2007).

*Multicentricity*is defined as presence of DCIS in multiple quadrants of the diseased breast with more then 5cm of distance between them.

PRESENTATION

Regardless of their complaints, full examination should be performed for all breast patients, and detailed anamnesis should be taken from all patients. Information such as the age at first menarche and first birth, the number of births, whether there is a family history of breast cancer or other cancer, whether she has received hormone replacement therapy, and whether there is a previous breast biopsy should be included in the anamnesis of every patient.

A detailed examination of both breast and axillary fossa is also important. Any palpable lump or lymph node, discharge from nipple, skin changes and skin retractions should be noted.

Most of the DCIS patients however may not have any complaint or sign. However, a palpable mass can be detected in up to 10% of patients on physical examination(Sundara Rajan et al., 2013).

WORKUP

Mammography

Prior to the introduction of mammographyin routine screening programs, patients with dcis often presented with palpable masses or nipple changes. a smaller number of patients were diagnosed through biopsies performed for other reasons. With the broad use of mammography, the incidence of dcis has increased more than 5 times in the last 30 years. Today, dcis constitutes 25-40% of breast cancers detected by mammography(Harris, 2001, p. 41).

Up to 85-95% of DCIS lesions identified with mammography(Bent et al., 2010). Microcalcifications are the most common mammographic finding of dcis hence, dcis patients constitute 80 percent of breast cancer patients diagnosed with microcalcifications.

There are mainly two types of calcifications associated with DCIS. Poorly differianted lesions with comedo-necrosis mostly associated with branching(linear, rod-like) microcalcifications. In 74% percent of poorly differentiated DCIS lesions microcalcifications were linear(Dinkel et al., 2000). These microcalcifications are coarse granular and tends to follow ductus.

Well differentiated lesions, present themselves in mammograms with fine granular microcalcifications. These lesions mostly associated with cribriform or micropapillary subtypes of DCIS(Holland et al., 1994).

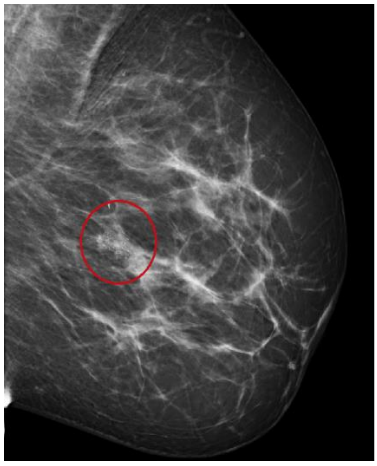

Figure 7: *Mediolateral oblique wiev mammogramphy image showingclustered pleomorphic microcalcifications.*

Ultrasound Imaging

With the advancement of ultrasound (US) device technologies and the increasing experience of imaging personnel, it has become an important

modality in the diagnosis of breast cancer and DCIS. Izomori et al. described US findings for DCIS as cystic or solid mass, ill defined hypoechoic mass, microlobulated mass, duct dilatation and calcifications(Izumori et al., 2010). However, most DCIS lesions diagnosed without forming a solid mass and among these lesions most frequent US finding is hypo-echoic areas in the mammary glands(Watanabe et al., 2017).

The Breast Imaging Reporting and Data System (BI-RADS) is a standardized system for reporting breast pathologies encountered with US, mammography or Magnetic Resonance Imaging(MRI). First edition published by American College of Radiology in 1993 and 5th edition published in 2013 ((Spak et al., 2017)). Criterion for high risk lesion for mammography, US and MRI well defined.

In a study comparing USG and Mammography in terms of specificity and sensitivity in capturing malignant lesions, ultrasound was found to be superior to mammography. the success of mammography increases with a decrease in breast density at a later age. but this study was not designed specifically for DCIS(Devolli-Disha et al., 2009).

Magnetic Resonance Imaging

DCIS lesions may be occult and some lesions may be very hard to detect. The specificity of mammography in detecting malignant lesions may vary in different literature and may decrease, especially in young and high breast density patients. MRI is superior to mammography in the diagnosis of these lesions. It was found that mammography could not detect DCIS lesions in more than 40 percent of patients evaluated together with mammography and MRI, while this rate was 8 percent for MRI (Kuhl et al., 2007).

MRI can also detect additional lesions in the same or opposite breast and may affect surgical plan. In one study, MRI detected additional lesions other then index lesion in 15 percent of the total cohort and 42% of the biopsies performed from these lesions were malignant (Pettit et al., 2009).

BIOPSI

All lesions assessed as high-risk by MRI, US or mammography (i.e BI-RADS 4-5) should be confirmed by preoperative diagnostic biopsy, if possible. Tru-cut biopsy or vacuum-assisted biopsy are the preferred diagnostic methods in DCIS patients. An international consensus conference held in 2009 suggest that US guided percutaneous biopsy of breast lesions should be the gold standard biopsy method if the lesion is eligible and stereotactic biopsy for calcifications not visualized on US(Silverstein et al., 2009).

Ultrasound Guided Biopsy

US guided biopsy can be applied to lesions that are palpable and show structural distortion on mammography, although they cannot be palpated. Multiple core biopsies are taken from the lesion using a 12 or 14 gauge core biopsy gun following a small incision to the skin under sterile conditions and local anesthesia.

Vacuum Assisted Biopsy

Also referred as stereotactic breast biopsy, this procedure uses computed technology to guide a needle into a lesion visible to mammography. With a single insertion of a 3mm diameter needle, 1-2 cm diameter of tissue excision is possible(Heywang-Köbrunner et al., 1998). Patients who had undergone surgical excisional biopsy for high-risk lesions may need another surgical procedure depend on the histopathologycal outcome of the first excision. Using vacuum assisted biopsy allows an accurate preoperative diagnosis and saves patients from morbidiy of a second operation (i.e re-escision, sentinel lymph node biopsy)(Liberman et al., 2001). Excision of all microcalcifications is possible with this method and it is necessary to confirm whether residual microcalcifications remain after the procedure. If all microcalcifications have been excised, a metallic marker clip should be left on the biopsy site.

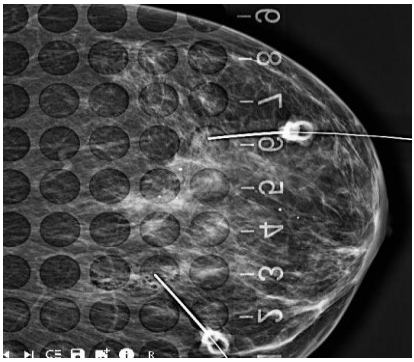

Figure 8:*Mammografi guided (stereotactic) wire localization of microcalcifications, multicentric disease wih two foci localized with 2 guidewires*

Excisional Biopsy

Patients who are not amenable to US guided biopsy or vacuum assisted biopsy, as well as patients who have biopsy results that are disconcordant to imaging should undergo surgical excisional biopsy. This technique is performed with a guidewire or with magnetic seed localization. Post-excision specimen

mammography is essential to comfirm resection of all microcalcifications as well as guidewire or magnetic seed.

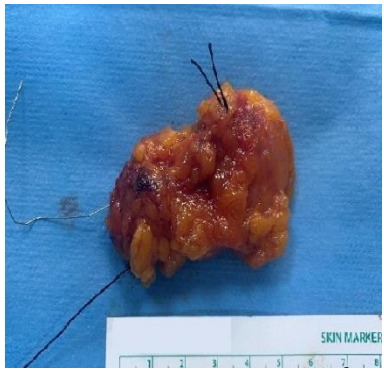

Figure9: *Excisional biopsy specimen of high risk lesion (papillamatous lesion with high suspicion of malignancy). Ultrasound guided wireguide localized lesion laterally. Two short silk suture marking superior and one long silk suture marking lateral of the lesion for orientation.(Taken from personal archieves of Dr.Mustafa Ozbagriacik with his permission.)*

SURGICAL TREATMENT

DCIS is not considered as an invasive lesion but precursor to invasive breast cancer. Due to ethical reasons, prospective studies cannot be conducted on how many of the non-operated DCIS patients will develop invasive cancer in the following years.In a recent retrospective study, it was shown that 36% of non-operated DCIS patients developed invasive cancer in their breasts with DCIS (in the same quadrant with index lesion) in 30-year follow-up(Sanders et al., 2015).Historically DCIS treated with mastectomy. Today treatment options may vary depending on the histologic grade, Er status, extend of disease, access to radiotheraphyand patient age.

Breast Conserving Therapy (Local Excision, Segmental Mastestomy, Lumpectomy)

Since DCIS is frequently seen as multicentric and multifocal, it was treated with mastectomy.In 1982 and 1989, Lagios found that dcis of smaller size (<25mm) is not associated with latent multicentricity, occult invasion or axillary metastases (Lagios et al., 1982, 1989). These informations formed the basis for the treatment of small-scale DCIS lesions with BCT instead of mastectomy.BCT + radiation therapy compared with mastectomy for early stage breast cancer and found to be non-inferior to mastectomy for disease-free survival(DFS) and over-all survival(OS)(B. Fisher et al., 1989). In 1993, the

NSABP-B17 trial compared BCT with BCT +RTin DCIS patients and showed that adding RT to treatment improved the outcome and 5 year disease-free survival(DFS).A recent retrospective cohort study ofDCIS patients using the SEER database showed that survival was best in BCT+RT patients, then in mastectomy patients and worst in BCT-only patients. This study also reveals a paradigm shift towards BCT+RT from mastectomy(Worni et al., 2015).Today, most patients without multicentric, multifocal or high grade lesions treated with BCT + RT.

Indications for BCT are well described in National Comprehensive Cancer Network (NCCN) breast cancer guidelines(Gradishar et al., 2022):

- Achievable cosmetically acceptable resection considering breast and tumor size
- Histologically acceptable tumor-free resection margins (≥2mm).
- No contraindication for radiotherapy
- Unicentric disease (Paient with multifocal disease within a limited area may be considered for BCT).
- Since most DCIS lesions are not palpable and are detected by mammography, it is necessary to mark the masses with appropriate imaging-guided methods before the operation.US-guided wire localization, mammography guided wire localization***(Error! Reference source not found.)*** or Iodine-125 seed localization(Doke et al., 2018).For palpable lesions radiography guided localization techniques are not necessary. After resection of tumor site a specimen radiography should be taken to prove both all microcalcifications and radiographic guide (guidewire or seed) are in the specimen*(Figure10).*

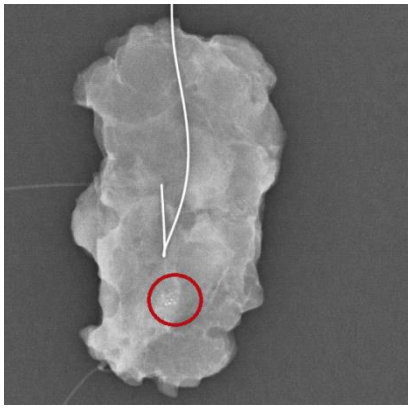

Figure10 *Post-excision spesimen radiography showing microcalcifications (in red circle) and guidewire both in the specimen.*

Achieving tumor free (negative) margins is one of the main goals of BCT. For invasive cancer of breast no-ink on tumor considered as negative margins. However debate on negative margins for DCIS patients yet ongoing. Studies support 1cm negative margin is associated with better DFS in 10 years(Silverstein et al., 1999). Two meta-analysis discussed optimal extent of negative margins. Wang. et al found that 1cm or greater negative margins associated with better outcome(Wang et al., 2012), but in a more recent meta-analysis reported that more then 2mm of negative margin is not associated with better DFS(Marinovich et al., 2016). NCCN guideline for breast cancer suggests negative margins to be greater then 2mm and consideration of re-excision of tumor cavity if the initial negative margin is less then 2mm(National Comprehensive Cancer Network.Breast Cancer (version 4.2022) Retrieved from https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf).Also, Society of Surgical Oncology-American Society for Radiation Oncology-American Society of Clinical Oncology Consensus Guideline (SSO-ASTRO) agreed on a consensus statement. based on a meta-analysis including 7883 patients, SSO-ASTO agrees that negative margins ≥2mm associated with lower local recurrence(Morrow et al., 2016).

To achieve negative margins, frozen section histopathologic work-up to assess margin status should be performed. This assessment requires orientation to specimen which obtained with sutures or surgical clipping***(Error! Reference source not found.)***.After multiple re-excisions, if negative margin (>2mm) is not obtained, mastectomy may be considered.

Mastectomy

After 1980, BCT for breast cancer began to become widespread. First trials comparing BCT vs mastectomy proved similar survivability. However, although the tumor diameter was smaller in the BCT group (larger then 4cm in mastectomy group and smaller than 4 cm in BCT group), the 8-year disease-free survival was higher in the mastectomy group compared to BCT+RT (Silverstein et al., 1992). Later in a meta-analysis Boyoges et. al reported 22.5%, 8.9% and 1.4% local recurrence rates for BCT alone, BCT+RT and mastectomy (Boyages et al., 1999). Approximately 50% of the local recurrences are invasive cancers and associated with cancer related mortality. Concerns about recurrence still lead may still lead surgeons to mastectomy over BCT+RT.

As discussed before mastectomy associated with better DFS compared to BCT+RT, thus RT after mastectomy is rarely necessary. Different then mastectomy performed for an invasive cancer in this aspect, mastectomy for DCIS is performable by using skin sparing mastectomy(SSM) or nipple sparing

mastectomy(NSM) techniques. SSM and NSM allows immediate reconstruction of the breast to achieve better aesthetic outcome and yields better patient satisfaction.

NSM involves enblock resection of breast tissue sparing nipple and skin as an envelope. NSM evaluated for oncologic outcomes with many studies(Benediktsson & Perbeck, 2008; De La Cruz et al., 2015; Galimberti et al., 2018; Sakurai et al., 2013). In a study of 1989 NSM patients (278 in-situ, 1711 invasive), only 11(4%) local recurrences of DCIS patients occurred at 94 months of follow-up(Galimberti et al., 2018).Oncoplastic Breast Consortium consensus conference held in 2018 agrees that NSM can be performed for any tumor size that does not involve the skin or nipple areola complex (NAC) independent of axillary status(Weber et al., 2018).

SSM compromizes protecting of nipple areola complex and allows en-bloc resection of breast tissue and nipple areola complex, leaving most of the skin to allow reconstruction.A meta-analysis reported that recurrence rates were between 2 and 20 percent in studies evaluating recurrence after SSM. However tumor stages vary among these studies, and the study that reported 20 percent recurrence rate consistedof T2b and T3 lesions while others consisted mostly of T0 to T2 lesions(Greenway et al., 2005).

Conventional mastectomy performed through an eliptic Stewart incision, SSM performed through a circum-areolar incision and NSM performed through periareolar, lateral radial, vertical radial or inframammary fold incisions.

Axillary Surgery Traditionally DCIS was treated with modified radical mastectomy with axillary dissection. Results from BCT+RT showed equivalent results and low recurrence, thus lead axillary lymph node dissection(ALND) and mastectomy to be centers of focus of questions. The Joint Center for Radiation Therapy in Boston reported 40 patients treated with BCT+RT and 13 of them treated with axillary dissection, of 13 patients none showed axillary metastasis(Recht et al., 1985). NSABP-B06 also reported 78 cases of DCIS randomized into BCT or mastectomy with or without RT. All patients had level I-II axillary dissections and none of them had axillary metastasis(E. R. Fisher et al., 1999, p. 6). Silverstein et. al investigated the need of ALND in 100 patients treated with either mastectomy (n=49) or BCT+RT (n=51) with ALND in all patients. Of 100 patients none of them had axillary lymph node metastasis (Silverstein et al., 1987). With this findings, Silverstein. et al. reported that routine axillary dissection is not mandatory for DCIS patients. In NSABP-B17 and NSABP-B24 trials the axillary recurrence rate was found 0.83 per 1000 patient-years for NSABP-B17 trial and 0.36 per 1000 patient-years for NSABP-B24 trial (Morrow, 2008).

Guideline from NCCN does not recommend ALND in the absence of evidence of invasive cancer or proven axillary metastatic disease in patients with apparent pure DCIS. However, NCCN recommends that SLNB should be "strongly considered" with mastectomy or "excision in an anatomic location compromising the performance of a future sentinel lymph node (National Comprehensive Cancer Network.Breast Cancer (version 4.2022) Retrieved from https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf).American Society of Clinical Oncology (ASCO) also suggest that there is no need for SLNB for patients planned to have BCT for DCIS unless localization of tumor may preclude future node sampling. ASCO also declared that clinicians should offer SLNB for patients planned to have mastectomy for DCIS (Lyman et al., 2017).

RADIOTHERAPHY

First trial to compare Halsted mastectomy and BCT+RT on stage 1 invasive breast cancer patients, showed no difference between two groups in DFS and OS(Veronesi et al., 1981). This study allowed widebroad use of BCT+RT and lead to new studies.

Most patients who underwent BCT for DCIS should have adjuvant RT. RT is so effective for DFS that a shift towards BCT from mastectomy happened in 1980s. First trial to compareBCT+RT vs BCT alonein DCIS patients was NSABP- B17 and was a big success. A total cohort of 818 patients randomized for BCT+RT vs BCT alone,84.4% and 73.8% (p=0.001) patients had 5 year DFS in BCT+RT vs BCT groups respectively(B. Fisher et al., 1993). Eight year results of NSABP- B17 study showed %43 risk reduction on recurrece with RT(6.40 for the group treated by lumpectomy alone vs 3.68 for the group treated by lumpectomy plus breast irradiation; p= .00004.)(B. Fisher et al., 1989). After 15 years of follow-up RT reduced recurrence by 52%(Hazard ratio of risk of invasive ipsilateral breast tumor recurrence = 0.48, 95% confidence interval = 0.33 to 0.69, P < .001)(Wapnir et al., 2011).

Results from NSABP-B17 trials are comparable to subsequent EORTC, sweDCIS and UK/ANZ DCIS studies(Cuzick et al., 2010; Julien et al., 2000; Wärnberg et al., 2014). In UK/ANZ study(Cuzick et al., 2010) 1694 women with DCIS randomized into four groups (RT+tamoxifen, RT alone, tamoxifen alone, no adjuvant treatment). In 12-year follow-upradiotherapy reduced the incidence of all new breast events (hazard ratio0·41, 95% CI 0·30–0·56; p<0·0001).

EORTC trial reported 48% risk reduction of DCIS recurrence and 42% risk reduction of invasive recurrence in 10-year follow-up (log-rank P < .0001; hazard ratio = 0.53)(Bijker et al., 2006).

In light of these studies adjuvant RT after BCT for DCIS patients associated with almost %50 of risk reduction for breast cancer and considered as a standart care for DCIS patients treated with BCT.

There are several radiotheraphy options available for treatment of DCIS. As discussed before, *whole breast radiation theraphy(WBRT)* demostrate approximately 50% reduction in risk for ipsilateral tumor recurrence, thus most patients recieve WBRT after BCT.

Most local recurrence after BCT develops in the same quadrant as the index lesion and in the tumor bed. This knowledge is the rationale for the application of boost RT, a common practice used after WBRT, to tumor bed after WBRT. A multicentric study compared standart WBRT with WBRT+boost RT and the use of the RT boost was associated with reduced recurrence compared with the no-boost group (hazard ratio= 0.73; 95% CI, 0.57-0.94; P = .01)(Moran et al., 2017).

As discussed before, most recurrences develops perimeter to the resection cavity. thus some have suggested that equivelent local control is achievable with *partial breast irradiation(PBI),* which focuses on the tissues around the excision cavity.American Society of Radiation Oncology(ASTRO) released a concensus statement for PBI and stated which patients may be considered for BPI(Castaneda & Strasser, 2017). A prospective study compared PBI and WBRT found WBRT superior. At a 10-years follow-up period 3.9% of WBRT patients and 4.6% of PBI group developed recurrence and results favored WBRT over PBI(Vicini et al., 2019).

Hormonal Theraphy

DCIS subtypes discussed briefly before under histopathology sub-title. DCIS lesions can be classified according to ER, PR and HER-2 status.

Tamoxifen is a selective estrogen receptor modulator used to treat and prevent breast cancer. The use of tamoxifen in DCIS patients has been the subject of many studies. NSABP-B27 trial randomized 1804 patients into two groups: BCT+RT+placebo vs. BCT+RT+tamoxifen. Patients in the tamoxifen group had fewer cumulative breast cancer events (regional recurrence, contralateral breast cancer or distant metastasis) at 5 years (8.2 versus 13.4%, P = 0.0009). In tamoxifen group the risk of local recurrence was lower even when surgical margins were positive in some patients and when DCIS was associated with comedo-necrosis. Use of tamoxifen also lowered risk of contralateral breast cancer risk compared to placebo (Shoker, 1999).

In UK/ANZ DCIS trial patients who underwent BCT were randomized into 3 groups: RT, tamoxifen or both. After 12.7 years of follow up RT reduced the ipsilateral invasive cancer risk as well as ipsilateral DCIS risk, but had no effect on contralateral breast cancer. Tamoxifen also reduced recurrent ipsilateral DCIS but had no effect on ipsilateral invasive disease but reduced contralateral breast cancer risk (Cuzick et al., 2010) . Reduction in ipsilateral new breast events for RT (because it is a local theraphy) and reduction in all breast events for tamoxifen(becouse it is a systemic theraphy) are the primary endpoints of these trials.

In the International Breast Cancer Intervention Study-I (IBIS)-I, 7154 patients deemed to be at high risk of developing breast cancer enrolled into two groups: 20mg tamoxifen or equivalent placebo for 5 years. After median 16 years follow-up study revealed significant reduction in the occurence of all breast cancer in tamoxifen group (Hazard Ratio 0.71 [95% Confidence Interval 0.60–0.83], $p<0.0001$). Tamoxifen reduced risk of ER(+) invasive breast cancer as well as DCIS but did not decrease the risk for ER(-) invasive breast cancer (Cuzick et al., 2015) .

However, aside from the chemopreventive effects of tamoxifen also has several documented side effects. Use of tamoxifen increases risk for endometrium cancer, thromboembolic diseases and cataracts (Condorelli & Vaz-Luis, 2018). Raloxifene is another selective estrogen reseptor modulator with less side effects than tamoxifen and can be used in post-menopausal women. First approved by FDA for treatment and prevention of osteoporosis. Raloxifene also has effects to reduce the risk for breast cancer (Wickerham et al., 2009).

The NSABP Study of Tamoxifen and Raloxifene (STAR) trial randomized 19747 post-menopausal women with increased risk for breast cancer to 20mg/daily tamoxifen and 60mg/daily raloxifene groups. There was no difference between two groups in terms of invasive breast cancer but there were fewer non-invasive cancers in the tamoxifen group(57 and 80 respectively for tamoxifen and raloxifene groups. 36% lobular carcinoma in-situ and 54% DCIS). (Note that, at the time this study published lobular carcinoma in-situ considered as a non-invasive cancer, but as of today it is considered as a independent risk factor for invasive breast cancer.) There was also reduced incidence of utherine cancer in raloxifene group(36 vs 22 in tamoxifen and raloxifene groups respectively.) As a conclusion raloxifene represents an acceptable alternative to tamoxifen for the risk reduction purpose in post-menopausal women bu raloxifene appears to be less effective in reducing the risk of DCIS(Vogel, 2009).

Lastly there are aromatase inhibitors. Aromatase inhibitors block estrogen synthesis by inhibitinng aromatase enzyme which catalyses conversion of adrenal androgens to estrogen. In 2017 NSABP-B35 trial published first results (Margolese et al., 2016, p. 35). Total of 3077 post-menopausal women with ER(+) DCIS treated with BCT+WBRT were randomized into 20mg/day tamoxifen or 1mg/day anostrazole groups. After 9 years of follow-up there were 122 events(local, contralateral or distant) in tamoxifen group and 90 in the anastrazole group. Anostrazole was only found superior to tamoxifen in women younger then 60. There were 17 and 8 uterine cancers in tamoxifen and anastrazole groups but the finding was not statistically significant (Risk Ratio, 0.47; 95% Confidence Interval, 0.18–1.15).

FACTORS ASSOCIATED WITH INCREASED RISK OF RECURRENCE AFTER BREAST CONSERVING THERAPHY

Most important factor effecting prognosis and recurrence after BCT is negative margins. It has been cleared that less then 2mm negative margin status is associated with increased risk for recurrence. In recent update of MD Anderson Cancer Center outcomes, recurrence risk for patients who had WBRT after BCT did not differ when whether patients had more then 2mm negative margins or less (10-year local recurrence rate 4.8% vs 3.3%, p=0.72); but risk was increased for patients with less then 2mm margins who did not underwent WBRT after BCT (10-year LRR 30.9% vs. 5.4%; p=0.003)(Kuerer et al., 2017). Memorial Sloan Kettering Cancer Center study with 2996 cases with median follow-up of 6 years (0-30 years) also reports that among those not recieving RT wider negative margins and lower recurrence was significantly associated (p=0.0003), whereas the association was not significant among those who recieved RT (p=0.99). Wider margins associated with lower risk of recurrence with lower hazard ratios associated with wider margins (0.78, 0.70, and 0.44for negative margin widths of $\leq$2, >2–10, and >10 mm, respectively) compared to positive margins.(Van Zee et al., 2015).

Another study from Memorial Sloan Kettering Cancer Center investigated risk factors in patients treated with mastectomy. More then 3000 patients treated with mastectomy and axillary staging performed in %89 of patients. Age less than 50 years at the time of surgery (hazard ratio=15.0, 95% Confidence Interval 3.58–62.4; p<0.001); presence of microinvasion (hazard ratio 3.35, 95% confidence interval 1.66–6.77; p<0.001) and high nuclear grade (hazard ratio=3.56, 95% confidence interval 1.60–7.88; p=0.001) were significantly associated with local recurrence(Mamtani et al., 2019).

REFERENCES

1. Bane, A. (2013). Ductal Carcinoma In Situ: What the Pathologist Needs to Know and Why. *International Journal of Breast Cancer*, *2013*, 914053. https://doi.org/10.1155/2013/914053
2. Benediktsson, K. P., & Perbeck, L. (2008). Survival in breast cancer after nipple-sparing subcutaneous mastectomy and immediate reconstruction with implants: A prospective trial with 13 years median follow-up in 216 patients. *European Journal of Surgical Oncology (EJSO)*, *34*(2), 143–148. https://doi.org/10.1016/j.ejso.2007.06.010
3. Bent, C. K., Bassett, L. W., D'Orsi, C. J., & Sayre, J. W. (2010). The Positive Predictive Value of BI-RADS Microcalcification Descriptors and Final Assessment Categories. *American Journal of Roentgenology*, *194*(5), 1378–1383. https://doi.org/10.2214/AJR.09.3423
4. Bijker, N., Meijnen, P., Peterse, J. L., Bogaerts, J., Van Hoorebeeck, I., Julien, J.-P., Gennaro, M., Rouanet, P., Avril, A., Fentiman, I. S., Bartelink, H., & Rutgers, E. J. Th. (2006). Breast-Conserving Treatment With or Without Radiotherapy in Ductal Carcinoma-In-Situ: Ten-Year Results of European Organisation for Research and Treatment of Cancer Randomized Phase III Trial 10853—A Study by the EORTC Breast Cancer Cooperative Group and EORTC Radiotherapy Group. *Journal of Clinical Oncology*, *24*(21), 3381–3387. https://doi.org/10.1200/JCO.2006.06.1366
5. Boyages, J., Delaney, G., & Taylor, R. (1999). Predictors of local recurrence after treatment of ductal carcinoma in situ: A meta-analysis. *Cancer*, *85*(3), 616–628.
6. Bragg, A., Candelaria, R., Adrada, B., Huang, M., Rauch, G., Santiago, L., Scoggins, M., & Whitman, G. (2021). Imaging of Noncalcified Ductal Carcinoma In Situ. *Journal of Clinical Imaging Science*, *5*, 1145. https://doi.org/10.25259/JCIS_48_2021
7. Castaneda, S. A., & Strasser, J. (2017). Updates in the Treatment of Breast Cancer with Radiotherapy. *Surgical Oncology Clinics of North America*, *26*(3), 371–382. https://doi.org/10.1016/j.soc.2017.01.013
8. Condorelli, R., & Vaz-Luis, I. (2018). Managing side effects in adjuvant endocrine therapy for breast cancer. *Expert Review of Anticancer Therapy*, *18*(11), 1101–1112. https://doi.org/10.1080/14737140.2018.1520096
9. Cowell, C. F., Weigelt, B., Sakr, R. A., Ng, C. K. Y., Hicks, J., King, T. A., & Reis-Filho, J. S. (2013). Progression from ductal carcinoma in situ

to invasive breast cancer: Revisited. *Molecular Oncology*, *7*(5), 859–869. https://doi.org/10.1016/j.molonc.2013.07.005

10. Cuzick, J., Sestak, I., Cawthorn, S., Hamed, H., Holli, K., Howell, A., & Forbes, J. F. (2015). Tamoxifen for prevention of breast cancer: Extended long-term follow-up of the IBIS-I breast cancer prevention trial. *The Lancet Oncology*, *16*(1), 67–75. https://doi.org/10.1016/S1470-2045(14)71171-4
11. Cuzick, J., Sestak, I., Pinder, S. E., Ellis, I. O., Forsyth, S., Bundred, N. J., Forbes, J. F., Bishop, H., Fentiman, I. S., & George, W. D. (2010). Effect of tamoxifen and radiotherapy in women with locally excised ductal carcinoma in situ: Long-term results from the UK/ANZ DCIS trial. *The Lancet. Oncology*, *12*(1), 21–29. https://doi.org/10.1016/S1470-2045(10)70266-7
12. De La Cruz, L., Moody, A. M., Tappy, E. E., Blankenship, S. A., & Hecht, E. M. (2015). Overall Survival, Disease-Free Survival, Local Recurrence, and Nipple–Areolar Recurrence in the Setting of Nipple-Sparing Mastectomy: A Meta-Analysis and Systematic Review. *Annals of Surgical Oncology*, *22*(10), 3241–3249. https://doi.org/10.1245/s10434-015-4739-1
13. Devolli-Disha, E., Manxhuka-Kërliu, S., Ymeri, H., & Kutllovci, A. (2009). COMPARATIVE ACCURACY OF MAMMOGRAPHY AND ULTRASOUND IN WOMEN WITH BREAST SYMPTOMS ACCORDING TO AGE AND BREAST DENSITY. *Bosnian Journal of Basic Medical Sciences*, *9*(2), 131–136.
14. Dinkel, H. P., Gassel, A. M., & Tschammler, A. (2000). Is the appearance of microcalcifications on mammography useful in predicting histological grade of malignancy in ductal cancer in situ? *The British Journal of Radiology*, *73*(873), 938–944. https://doi.org/10.1259/bjr.73.873.11064645
15. Doke, K., Butler, S., & Mitchell, M. P. (2018). Current Therapeutic Approaches to DCIS. *Journal of Mammary Gland Biology and Neoplasia*, *23*(4), 279–291. https://doi.org/10.1007/s10911-018-9415-1
16. EORTC Breast Cancer Cooperative Group, EORTC Radiotherapy Group, Bijker, N., Meijnen, P., Peterse, J. L., Bogaerts, J., Van Hoorebeeck, I., Julien, J.-P., Gennaro, M., Rouanet, P., Avril, A., Fentiman, I. S., Bartelink, H., & Rutgers, E. J. T. (2006). Breast-conserving treatment with or without radiotherapy in ductal carcinoma-in-situ: Ten-year results of European Organisation for Research and Treatment of Cancer randomized phase III trial 10853--a study by the EORTC Breast Cancer

Cooperative Group and EORTC Radiotherapy Group. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *24*(21), 3381–3387. https://doi.org/10.1200/JCO.2006.06.1366

17. Feig, B. W., Ching, C. D., & M. D. Anderson Cancer Center (Eds.). (2019). *The MD Anderson surgical oncology handbook* (Sixth edition). Wolters Kluwer.
18. Feinberg, J., Wetstone, R., Greenstein, D., & Borgen, P. (2018). Is DCIS Overrated? In W. J. Gradishar (Ed.), *Optimizing Breast Cancer Management* (Vol. 173, pp. 53–72). Springer International Publishing. https://doi.org/10.1007/978-3-319-70197-4_5
19. Fisher, B., Costantino, J., Redmond, C., Fisher, E., Margolese, R., Dimitrov, N., Wolmark, N., Wickerham, D. L., Deutsch, M., Ore, L., Mamounas, E., Poller, W., & Kavanah, M. (1993). Lumpectomy Compared with Lumpectomy and Radiation Therapy for the Treatment of Intraductal Breast Cancer. *New England Journal of Medicine*, *328*(22), 1581–1586. https://doi.org/10.1056/NEJM199306033282201
20. Fisher, B., Redmond, C., Poisson, R., Margolese, R., Wolmark, N., Wickerham, L., Fisher, E., Deutsch, M., Caplan, R., Pilch, Y., Glass, A., Shibata, H., Lerner, H., Terz, J., & Sidorovich, L. (1989). Eight-Year Results of a Randomized Clinical Trial Comparing Total Mastectomy and Lumpectomy with or without Irradiation in the Treatment of Breast Cancer. *New England Journal of Medicine*, *320*(13), 822–828. https://doi.org/10.1056/NEJM198903303201302
21. Fisher, E. R., Dignam, J., Tan-Chiu, E., Costantino, J., Fisher, B., Paik, S., Wolmark, N., & for the National Surgical Adjuvant Breast Bowel Project (NSABP) Collaborating Investigators. (1999). Pathologic findings from the National Surgical Adjuvant Breast Project (NSABP) eight-year update of Protocol B-17: Intraductal carcinoma. *Cancer*, *86*(3), 429–438. https://doi.org/10.1002/(SICI)1097-0142(19990801)86:3<429::AID-CNCR11>3.0.CO;2-Y
22. Fletcher, C. D. M. (Ed.). (2021). *Diagnostic histopathology of tumors* (Fifth edition). Elsevier.
23. Galimberti, V., Morigi, C., Bagnardi, V., Corso, G., Vicini, E., Fontana, S. K. R., Naninato, P., Ratini, S., Magnoni, F., Toesca, A., Kouloura, A., Rietjens, M., De Lorenzi, F., Vingiani, A., & Veronesi, P. (2018). Oncological Outcomes of Nipple-Sparing Mastectomy: A Single-Center Experience of 1989 Patients. *Annals of Surgical Oncology*, *25*(13), 3849–3857. https://doi.org/10.1245/s10434-018-6759-0

24. Gradishar, W. J., Moran, M. S., Abraham, J., Aft, R., Agnese, D., Allison, K. H., Anderson, B., Burstein, H. J., Chew, H., Dang, C., Elias, A. D., Giordano, S. H., Goetz, M. P., Goldstein, L. J., Hurvitz, S. A., Isakoff, S. J., Jankowitz, R. C., Javid, S. H., Krishnamurthy, J., … Kumar, R. (2022). Breast Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network: JNCCN*, *20*(6), 691–722. https://doi.org/10.6004/jnccn.2022.0030
25. Greenway, R. M., Schlossberg, L., & Dooley, W. C. (2005). Fifteen-year series of skin-sparing mastectomy for stage 0 to 2 breast cancer. *The American Journal of Surgery*, *190*(6), 933–938. https://doi.org/10.1016/j.amjsurg.2005.08.035
26. Harris, A. T. (2001). Case 41: Ductal Carcinoma in Situ. *Radiology*, *221*(3), 770–773. https://doi.org/10.1148/radiol.2213000890
27. Heywang-Köbrunner, S. H., Schauml**ffel, U., Viehweg, P., H**fer, H., Buchmann, J., & Lampe, D. (1998). Minimally invasive stereotaxic vacuum core breast biopsy. *European Radiology*, *8*(3), 377–385. https://doi.org/10.1007/s003300050398
28. Hoda, S. A., Chiu, A., Prasad, M. L., Giri, D., & Hoda, R. S. (2000). Are microinvasion and micrometastasis in breast cancer mountains or molehills? *American Journal of Surgery*, *180*(4), 305–308. https://doi.org/10.1016/s0002-9610(00)00464-5
29. Holland, R., Peterse, J. L., Millis, R. R., Eusebi, V., Faverly, D., van de Vijver, M. J., & Zafrani, B. (1994). Ductal carcinoma in situ: A proposal for a new classification. *Seminars in Diagnostic Pathology*, *11*(3), 167–180.
30. Iatrakis, G., & Zervoudis, S. (2021). *Epidemiology of Ductal Carcinoma In Situ*. 7.
31. Izumori, A., Takebe, K., & Sato, A. (2010). Ultrasound findings and histological features of ductal carcinoma in situ detected by ultrasound examination alone. *Breast Cancer*, *17*(2), 136–141. https://doi.org/10.1007/s12282-009-0134-8
32. Jaffer, S., & Bleiweiss, I. J. (2002). Histologic classification of ductal carcinoma in situ. *Microscopy Research and Technique*, *59*(2), 92–101. https://doi.org/10.1002/jemt.10180
33. Julien, J.-P., Bijker, N., Fentiman, I. S., Peterse, J. L., Delledonne, V., Rouanet, P., Avril, A., Sylvester, R., Mignolet, F., Bartelink, H., & Van Dongen, J. A. (2000). Radiotherapy in breast-conserving treatment for ductal carcinoma in situ: First results of the EORTC randomised phase III

trial 10853. *The Lancet*, *355*(9203), 528–533. https://doi.org/10.1016/S0140-6736(99)06341-2

34. Kalli, S., Semine, A., Cohen, S., Naber, S. P., Makim, S. S., & Bahl, M. (2018). American Joint Committee on Cancer's Staging System for Breast Cancer, Eighth Edition: What the Radiologist Needs to Know. *RadioGraphics*, *38*(7), 1921–1933. https://doi.org/10.1148/rg.2018180056
35. Kuerer, H. M., Smith, B. D., Chavez-MacGregor, M., Albarracin, C., Barcenas, C. H., Santiago, L., Edgerton, M. E., Rauch, G. M., Giordano, S. H., Sahin, A., Krishnamurthy, S., Woodward, W., Tripathy, D., Yang, W. T., & Hunt, K. K. (2017). DCIS Margins and Breast Conservation: MD Anderson Cancer Center Multidisciplinary Practice Guidelines and Outcomes. *Journal of Cancer*, *8*(14), 2653–2662. https://doi.org/10.7150/jca.20871
36. Kuhl, C. K., Schrading, S., Bieling, H. B., Wardelmann, E., Leutner, C. C., Koenig, R., Kuhn, W., & Schild, H. H. (2007). MRI for diagnosis of pure ductal carcinoma in situ: A prospective observational study. *Lancet (London, England)*, *370*(9586), 485–492. https://doi.org/10.1016/S0140-6736(07)61232-X
37. Lagios, M. D., Margolin, F. R., Westdahl, P. R., & Rose, M. R. (1989). Mammographically detected duct carcinoma in situ. Frequency of local recurrence following tylectomy and prognostic effect of nuclear grade on local recurrence. *Cancer*, *63*(4), 618–624. https://doi.org/10.1002/1097-0142(19890215)63:4<618::AID-CNCR2820630403>3.0.CO;2-J
38. Lagios, M. D., Westdahl, P. R., Margolin, F. R., & Rose, M. R. (1982). Duct carcinoma in situ. Relationship of extent of noninvasive disease to the frequency of occult invasion, multicentricity, lymph node metastases, and short-term treatment failures. *Cancer*, *50*(7), 1309–1314. https://doi.org/10.1002/1097-0142(19821001)50:7<1309::aid-cncr2820500716>3.0.co;2-#
39. Liberman, L., Gougoutas, C. A., Zakowski, M. F., LaTrenta, L. R., Abramson, A. F., Morris, E. A., & Dershaw, D. D. (2001). Calcifications Highly Suggestive of Malignancy: Comparison of Breast Biopsy Methods. *American Journal of Roentgenology*, *177*(1), 165–172. https://doi.org/10.2214/ajr.177.1.1770165
40. Lyman, G. H., Somerfield, M. R., Bosserman, L. D., Perkins, C. L., Weaver, D. L., & Giuliano, A. E. (2017). Sentinel Lymph Node Biopsy for Patients With Early-Stage Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. *Journal of*

Clinical Oncology, *35*(5), 561–564. https://doi.org/10.1200/JCO.2016.71.0947

41. Mamtani, A., Nakhlis, F., Downs-Canner, S., Zabor, E. C., Morrow, M., King, T. A., & Van Zee, K. J. (2019). Impact of Age on Locoregional and Distant Recurrence After Mastectomy for Ductal Carcinoma In Situ With or Without Microinvasion. *Annals of Surgical Oncology*, *26*(13), 4264–4271. https://doi.org/10.1245/s10434-019-07693-1
42. Margolese, R., Cecchini, R. S., Julian, T. B., Ganz, P. A., Costantino, J. P., Vallow, L. A., Albain, K. S., Whitworth, P. W., Cianfrocca, M. E., Brufsky, A. M., Gross, H. M., Soori, G. S., Hopkins, J. O., Fehrenbacher, L., Sturtz, K., Wozniak, T. F., Seay, T. E., Mamounas, E. P., & Wolmark, N. (2016). Primary results, NSABP B-35/NRG Oncology: A clinical trial of anastrozole vs tamoxifen in postmenopausal patients with DCIS undergoing lumpectomy plus radiotherapy A randomized clinical trial. *Lancet (London, England)*, *387*(10021), 849–856. https://doi.org/10.1016/S0140-6736(15)01168-X
43. Marinovich, M. L., Azizi, L., Macaskill, P., Irwig, L., Morrow, M., Solin, L. J., & Houssami, N. (2016). The Association of Surgical Margins and Local Recurrence in Women with Ductal Carcinoma In Situ Treated with Breast-Conserving Therapy: A Meta-Analysis. *Annals of Surgical Oncology*, *23*(12), 3811–3821. https://doi.org/10.1245/s10434-016-5446-2
44. Moran, M. S., Zhao, Y., Ma, S., Kirova, Y., Fourquet, A., Chen, P., Hoffman, K., Hunt, K., Wong, J., Halasz, L. M., Freedman, G., Prosnitz, R., Yassa, M., Nguyen, D. H. A., Hijal, T., Haffty, B. G., Wai, E. S., & Truong, P. T. (2017). Association of Radiotherapy Boost for Ductal Carcinoma In Situ With Local Control After Whole-Breast Radiotherapy. *JAMA Oncology*, *3*(8), 1060. https://doi.org/10.1001/jamaoncol.2016.6948
45. Morrow, M. (2008). Axillary Surgery in DCIS: Is Less More? *Annals of Surgical Oncology*, *15*(10), 2641–2642. https://doi.org/10.1245/s10434-008-0083-z
46. Morrow, M., Van Zee, K. J., Solin, L. J., Houssami, N., Chavez-MacGregor, M., Harris, J. R., Horton, J., Hwang, S., Johnson, P. L., Marinovich, M. L., Schnitt, S. J., Wapnir, I., & Moran, M. S. (2016). Society of Surgical Oncology-American Society for Radiation Oncology-American Society of Clinical Oncology Consensus Guideline on Margins for Breast-Conserving Surgery With Whole-Breast Irradiation in Ductal

Carcinoma in Situ. *Practical Radiation Oncology*, *6*(5), 287–295. https://doi.org/10.1016/j.prro.2016.06.011

47. Peila, R., Arthur, R., & Rohan, T. E. (2020). Risk factors for ductal carcinoma in situ of the breast in the UK Biobank cohort study. *Cancer Epidemiology*, *64*, 101648. https://doi.org/10.1016/j.canep.2019.101648
48. Pettit, K., Swatske, M. E., Gao, F., Salavaggione, L., Gillanders, W. E., Aft, R. L., Monsees, B. S., Eberlein, T. J., & Margenthaler, J. A. (2009). THE IMPACT OF BREAST MRI ON SURGICAL DECISION-MAKING: ARE PATIENTS AT RISK FOR MASTECTOMY? *Journal of Surgical Oncology*, *100*(7), 553–558. https://doi.org/10.1002/jso.21406
49. Poller, D. N., Barth, A., Slamon, D. J., Silverstein, M. J., Gierson, E. D., Coburn, W. J., Waisman, J. R., Gamagami, P., & Lewinsky, B. S. (1995). Prognostic classification of breast ductal carcinoma-in-situ. *The Lancet*, *345*(8958), 1154–1157. https://doi.org/10.1016/S0140-6736(95)90982-6
50. Rakovitch, E., Pignol, J.-P., Hanna, W., Narod, S., Spayne, J., Nofech-Mozes, S., Chartier, C., & Paszat, L. (2007). Significance of Multifocality in Ductal Carcinoma In Situ: Outcomes of Women Treated With Breast-Conserving Therapy. *Journal of Clinical Oncology*, *25*(35), 5591–5596. https://doi.org/10.1200/JCO.2007.11.4686
51. Recht, A., Danoff, B. S., Solin, L. J., Schnitt, S., Connolly, J., Botnick, L., Goldberg, I., Goodman, R. L., & Harris, J. R. (1985). Intraductal carcinoma of the breast: Results of treatment with excisional biopsy and irradiation. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *3*(10), 1339–1343. https://doi.org/10.1200/JCO.1985.3.10.1339
52. Rojas, K., & Stuckey, A. (2016). Breast Cancer Epidemiology and Risk Factors. *Clinical Obstetrics & Gynecology*, *59*(4), 651–672. https://doi.org/10.1097/GRF.0000000000000239
53. Sakurai, T., Zhang, N., Suzuma, T., Umemura, T., Yoshimura, G., Sakurai, T., & Yang, Q. (2013). Long-term follow-up of nipple-sparing mastectomy without radiotherapy: A single center study at a Japanese institution. *Medical Oncology*, *30*(1), 481. https://doi.org/10.1007/s12032-013-0481-3
54. Sanders, M. E., Schuyler, P. A., Simpson, J. F., Page, D. L., & Dupont, W. D. (2015). Continued observation of the natural history of low-grade ductal carcinoma in situ reaffirms proclivity for local recurrence even after more than 30 years of follow-up. *Modern Pathology*, *28*(5), Article 5. https://doi.org/10.1038/modpathol.2014.141

55. Shoker, B. (1999). Tamoxifen treatment for DCIS - NSABP B-24 trial. *Breast Cancer Research*, *1*(1), 66585. https://doi.org/10.1186/bcr-1999-66585
56. Sikand, K. (2005). Sections of the nipple and quadrants in mastectomy specimens for carcinoma are of limited value. *Journal of Clinical Pathology*, *58*(5), 543–545. https://doi.org/10.1136/jcp.2004.022665
57. Silverstein, M. J. (2003). The University of Southern California/Van Nuys prognostic index for ductal carcinoma in situ of the breast. *The American Journal of Surgery*, *186*(4), 337–343. https://doi.org/10.1016/S0002-9610(03)00265-4
58. Silverstein, M. J., Cohlan, B. F., Gierson, E. D., Furmanski, M., Gamagami, P., Colburn, W. J., Lewinsky, B. S., & Waisman, J. R. (1992). Duct carcinoma in situ: 227 cases without microinvasion. *European Journal of Cancer (Oxford, England: 1990)*, *28*(2–3), 630–634. https://doi.org/10.1016/s0959-8049(05)80114-5
59. Silverstein, M. J., Lagios, M. D., Groshen, S., Waisman, J. R., Lewinsky, B. S., Martino, S., Gamagami, P., & Colburn, W. J. (1999). The Influence of Margin Width on Local Control of Ductal Carcinoma in Situ of the Breast. *New England Journal of Medicine*, *340*(19), 1455–1461. https://doi.org/10.1056/NEJM199905133401902
60. Silverstein, M. J., Recht, A., Lagios, M. D., Bleiweiss, I. J., Blumencranz, P. W., Gizienski, T., Harms, S. E., Harness, J., Jackman, R. J., Klimberg, S. V., Kuske, R., Levine, G. M., Linver, M. N., Rafferty, E. A., Rugo, H., Schilling, K., Tripathy, D., Whitworth, P. W., & Willey, S. C. (2009). Image-Detected Breast Cancer: State-of-the-Art Diagnosis and Treatment. *Journal of the American College of Surgeons*, *209*(4), 504–520. https://doi.org/10.1016/j.jamcollsurg.2009.07.006
61. Silverstein, M. J., Rosser, R. J., Gierson, E. D., Waisman, J. R., Gamagami, P., Hoffman, R. S., Fingerhut, A. G., Lewinsky, B. S., Colburn, W., & Handel, N. (1987). Axillary lymph node dissection for intraductal breast carcinoma—Is it indicated? *Cancer*, *59*(10), 1819–1824. https://doi.org/10.1002/1097-0142(19870515)59:10<1819::aid-cncr2820591023>3.0.co;2-v
62. Spak, D. A., Plaxco, J. S., Santiago, L., Dryden, M. J., & Dogan, B. E. (2017). BI-RADS® fifth edition: A summary of changes. *Diagnostic and Interventional Imaging*, *98*(3), 179–190. https://doi.org/10.1016/j.diii.2017.01.001
63. Sundara Rajan, S., Verma, R., Shaaban, A. M., Sharma, N., Dall, B., & Lansdown, M. (2013). Palpable ductal carcinoma in situ: Analysis of

radiological and histological features of a large series with 5-year follow-up. *Clinical Breast Cancer*, *13*(6), 486–491. https://doi.org/10.1016/j.clbc.2013.08.002

64. Tamimi, R. M., Baer, H. J., Marotti, J., Galan, M., Galaburda, L., Fu, Y., Deitz, A. C., Connolly, J. L., Schnitt, S. J., Colditz, G. A., & Collins, L. C. (2008). Comparison of molecular phenotypes of ductal carcinoma in situand invasive breast cancer. *Breast Cancer Research*, *10*(4), R67. https://doi.org/10.1186/bcr2128
65. The Consensus Conference Committee. (1997). Consensus conference on the classification of ductal carcinoma in Situ. *Cancer*, *80*(9), 1798–1802. https://doi.org/10.1002/(SICI)1097-0142(19971101)80:9<1798::AID-CNCR15>3.0.CO;2-0
66. Van Zee, K. J., Subhedar, P., Olcese, C., Patil, S., & Morrow, M. (2015). Relationship Between Margin Width and Recurrence of Ductal Carcinoma In Situ: Analysis of 2996 Women Treated With Breast-conserving Surgery for 30 Years. *Annals of Surgery*, *262*(4), 623–631. https://doi.org/10.1097/SLA.0000000000001454
67. Veronesi, U., Saccozzi, R., Del Vecchio, M., Banfi, A., Clemente, C., De Lena, M., Gallus, G., Greco, M., Luini, A., Marubini, E., Muscolino, G., Rilke, F., Salvadori, B., Zecchini, A., & Zucali, R. (1981). Comparing Radical Mastectomy with Quadrantectomy, Axillary Dissection, and Radiotherapy in Patients with Small Cancers of the Breast. *New England Journal of Medicine*, *305*(1), 6–11. https://doi.org/10.1056/NEJM198107023050102
68. Vicini, F. A., Cecchini, R. S., White, J. R., Arthur, D. W., Julian, T. B., Rabinovitch, R. A., Kuske, R. R., Ganz, P. A., Parda, D. S., Scheier, M. F., Winter, K. A., Paik, S., Kuerer, H. M., Vallow, L. A., Pierce, L. J., Mamounas, E. P., McCormick, B., Costantino, J. P., Bear, H. D., … Wolmark, N. (2019). Long-term primary results of accelerated partial breast irradiation after breast-conserving surgery for early-stage breast cancer: A randomised, phase 3, equivalence trial. *The Lancet*, *394*(10215), 2155–2164. https://doi.org/10.1016/S0140-6736(19)32514-0
69. Virnig, B. A., Tuttle, T. M., Shamliyan, T., & Kane, R. L. (2010). Ductal Carcinoma In Situ of the Breast: A Systematic Review of Incidence, Treatment, and Outcomes. *JNCI Journal of the National Cancer Institute*, *102*(3), 170–178. https://doi.org/10.1093/jnci/djp482
70. Virnig, B. A., Wang, S.-Y., Shamilyan, T., Kane, R. L., & Tuttle, T. M. (2010). Ductal Carcinoma In Situ: Risk Factors and Impact of Screening.

JNCI Monographs, *2010*(41), 113–116. https://doi.org/10.1093/jncimonographs/lgq024

71. Vogel, V. G. (2009). The NSABP Study of Tamoxifen and Raloxifene (STAR) trial. *Expert Review of Anticancer Therapy*, *9*(1), 51–60. https://doi.org/10.1586/14737140.9.1.51
72. Wang, S.-Y., Chu, H., Shamliyan, T., Jalal, H., Kuntz, K. M., Kane, R. L., & Virnig, B. A. (2012). Network meta-analysis of margin threshold for women with ductal carcinoma in situ. *Journal of the National Cancer Institute*, *104*(7), 507–516. https://doi.org/10.1093/jnci/djs142
73. Wapnir, I. L., Dignam, J. J., Fisher, B., Mamounas, E. P., Anderson, S. J., Julian, T. B., Land, S. R., Margolese, R. G., Swain, S. M., Costantino, J. P., & Wolmark, N. (2011). Long-Term Outcomes of Invasive Ipsilateral Breast Tumor Recurrences After Lumpectomy in NSABP B-17 and B-24 Randomized Clinical Trials for DCIS. *JNCI Journal of the National Cancer Institute*, *103*(6), 478–488. https://doi.org/10.1093/jnci/djr027
74. Ward, E. P., Weiss, A., & Blair, S. L. (2017). Incidence and treatments of DCIS in octogenarians: Grade matters. *Breast Cancer Research and Treatment*, *165*(2), 403–409. https://doi.org/10.1007/s10549-017-4330-5
75. Wärnberg, F., Garmo, H., Emdin, S., Hedberg, V., Adwall, L., Sandelin, K., Ringberg, A., Karlsson, P., Arnesson, L.-G., Anderson, H., Jirström, K., & Holmberg, L. (2014). Effect of Radiotherapy After Breast-Conserving Surgery for Ductal Carcinoma in Situ: 20 Years Follow-Up in the Randomized SweDCIS Trial. *Journal of Clinical Oncology*, *32*(32), 3613–3618. https://doi.org/10.1200/JCO.2014.56.2595
76. Watanabe, T., Yamaguchi, T., Tsunoda, H., Kaoku, S., Tohno, E., Yasuda, H., Ban, K., Hirokaga, K., Tanaka, K., Umemoto, T., Okuno, T., Fujimoto, Y., Nakatani, S., Ito, J., & Ueno, E. (2017). Ultrasound Image Classification of Ductal Carcinoma In Situ (DCIS) of the Breast: Analysis of 705 DCIS Lesions 1. *Ultrasound in Medicine & Biology*, *43*(5), 918–925. https://doi.org/10.1016/j.ultrasmedbio.2017.01.008
77. Weber, W. P., Haug, M., Kurzeder, C., Bjelic-Radisic, V., Koller, R., Reitsamer, R., Fitzal, F., Biazus, J., Brenelli, F., Urban, C., Paulinelli, R. R., Blohmer, J.-U., Heil, J., Hoffmann, J., Matrai, Z., Catanuto, G., Galimberti, V., Gentilini, O., Barry, M., … Gnant, M. (2018). Oncoplastic Breast Consortium consensus conference on nipple-sparing mastectomy. *Breast Cancer Research and Treatment*, *172*(3), 523–537. https://doi.org/10.1007/s10549-018-4937-1
78. Wickerham, D. L., Costantino, J. P., Vogel, V. G., Cronin, W. M., Cecchini, R. S., Ford, L. G., & Wolmark, N. (2009). The Use of

Tamoxifen and Raloxifene for the Prevention of Breast Cancer. In H.-J. Senn, U. Kapp, & F. Otto (Eds.), *Cancer Prevention II* (Vol. 181, pp. 113–119). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-69297-3_12

79. Williams, K. E., Barnes, N. L. P., Cramer, A., Johnson, R., Cheema, K., Morris, J., Howe, M., & Bundred, N. J. (2015). Molecular phenotypes of DCIS predict overall and invasive recurrence. *Annals of Oncology*, *26*(5), 1019–1025. https://doi.org/10.1093/annonc/mdv062

The Role Of Circadian Rhythm In Neurodegenerative Diseases

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Circadian Rhythm

The word "circadian" means about a day and covers nearly 24 hours. The circadian rhythm is a regulatory mechanism found in all living systems, from the simplest to the most complex (Tabibzadeh, 2021). This internal control mechanism regulates biochemical and physiological processes and in maintaining homeostasis in mammals. In the central nervous system, this clock-modulated learning affects behavioral situations, including memory and mood (Hoyt & Obrietan, 2022). The circadian clock network is necessary for the sustainability of physiological function, mental and physical health. Evidence has shown that rhythm disturbance increases the severity of many diseases and many diseases can also disrupt rhythm. Recent data indicates the link between circadian rhythm disorders with psychiatric, neurodegenerative, and neurodevelopmental disorders (Fishbein, Knutson, & Zee, 2021; McCarthy et al., 2022). Beyond its association with disease severity, circadian disruption increases the lifetime risk of neurological disorders (Fishbein et al., 2021).

Brain Molecular Circadian Clock

The circadian clock is a system that regulates and manages its own rhythm independently of external stimuli (Peng, Li, Xiao, Zhao, & Sun, 2022). The center of the rhythm is the suprachiasmatic nucleus (SCN) located above the optic chiasm in the anterior hypothalamus, which is stimulated mainly by light stimulus and stimulates clock-controlled genes by neuroendocrine and neuronal pathways (Rosenwasser & Turek, 2022). In humans, this center consists of approximately 50,000 neurons and the light stimulus is transmitted to the SCN nucleus using the glutamate signal via the retinohypothalamic pathway (RHT). The rhythmic impulsesare transmitted to other parts of the hypothalamus, the brain, and peripheral organs from SCN. The efferent network of the SCN extends to the sub-paraventricular region, dorsomedial hypothalamus, thalamus, lateral septum, stria-terminalis, and intergeniculate nuclei. Also, SCN is directly connected several brain regions (Sharma, Sethi, et al., 2021). Substantia nigra and ventral tegmental area function are under circadian rhythm control. The circadian rhythm is an important regulatory mechanism that affects physical and mental health. Disruption of rhythm is associated with mental and physical illness (Meyer, Harvey, Lockley, & Dijk, 2022; Oh, Lim, Park, Moon, & Park, 2022; Peng et al., 2022). The circadian rhythm has positive and negative molecular feedback loops to maintain rhytmicity (Reddy, Reddy, & Sharma, 2022). Transcription factors BMAL1; (Brain and Muscle ARNT-Like 1) and CLOCK (Circadian Locomotor Output Kaput) form heterodimers and bind to E-box elements of target genes and activate their transcription. The PER

(Period) and CRY(Cryptochrome) proteins form heterodimers, interact with BMAL1/CLOCK and counteract BMAL1/CLOCK-mediated transcription activation, while the nuclear receptor REV-ERB represses BMAL1 transcription by binding to ROR elements in the promoter/enhancer regions of BMAL1. Some key clock genes,particularly BMAL1, PER2 and REVERB-α, exhibit robust circadian oscillations (Kim et al., 2017). Negative feedback regulation works as a stopping mechanism after the levels of transcripts and proteins reach a certain threshold (Peng et al., 2022). The activity of these genes drives circadian rhythms, and external signals called zeitgeber, meaning "time-giver" in German, enable the organism to adapt to the environment (Fowler, Hoedt, Talley, Keely, & Burns, 2022). E3 ubiquitin ligase complexes are important for resetting the system by mediating the degradation of CRY and PER proteins, and as a result, CLOCK:BMAL1 can once again facilitate transcription of CRY and PER (Hunt et al., 2022).

Neurodegeneration and the Circadian Rhythm

Neurodegenerative disease is a general definition used to describe a group of diseases characterized by progressive loss of neuron function and structural deterioration. In these diseases, there is an accumulation of misfolded proteins that impair the structure and function of neuron cells. (Colwell, 2021). Recent studies suggest a relationship between circadian disorders and neurodegenarative disorders such as Alzheimer's Disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). Circadian rhythm clock genes play a critical role in neurodegenerative disorders and dementia. In addition, exposure to shorter light-dark cycles is known to cause metabolic and neuropsychiatric changes (Mathew, Luo, & Bhatwadekar, 2022). It is clear that circadian clock has an impact on the function of neurons, astrocytes, microglia.In the light of these results, it can be said that clock disturbances are an early symptom of this type of neurodegenerative diseases(Wang & Li, 2021). In this section, we focus on AD, PD and HD, age-related neurodegenerative diseases.

Alzheimer's Disease

Alzheimer's disease is characterized by impaired cognitive abilities and often initially manifested by a deterioration in short-term memory. AD progresses β-amyloid (Aβ) deposition and the formation of neurofibrillary tangles of the tau protein in the central nervous system(Li, Shao, Mou, Zhang, & Ping, 2021). Recent studies showed that circadian timing is disrupted in AD, which accelerates the pathogenesis of AD. Most of AD patients show circadian rhythm disorders including sleep-wake disorders (Dong, Cheng, & Zhao, 2022). A

disruption in the sleep/wake cycle (interrupted sleep or altered sleep time) is a circadian rhythm disorder that has been reported as a component of mid- or late-stage AD in preclinical studies. In addition, changes in core body temperature rhythms, activity rhythms, and melatonin rhythm are also common features of AD (Hoyt & Obrietan, 2022). However,orexin regulates the sleep-wake cycle, are inversely proportional to tau and Aβ levels. Orexin inhibition leads to decreased alertness and lower Aβ levels (Niu et al., 2022; Sharma, Moon, Kim, & Kang, 2021).

In Tg4510 mice used as the AD model, Per2 and Bmal1 expression in the hippocampus was clearly impaired (Huang et al., 2021). In addition, rhythmic methylation of BMAL1 has been shown to be altered in AD, suggesting that changes in DNA methylation of circadian genes may increase cognitive loss and behavioral changes (Maiese, 2018). It suggests that this change in the circadian expression of clock genes and especially RNAs of BMAL1 may have a role in dementia (Maiese, 2021). In addition, although SNPs (single nucleotide polymorphisms) in clock genes are associated with many diseases, there is very limited data explaining the relationship of these polymorphisms with AD. It has been shown that BMAL-1 and CLOCK gene polymorphisms increase the risk of AD, however, especially in CLOCK mutations, they affect biochemical and neurophysiological parameters together with sleep-related parameters (Bacalini et al., 2022). In particular, it has been shown that the oscillation in CLOCK gene expression is impaired in patients with AD (Thome, Coogan, Woods, Darie, & Hassler, 2011). Moreover, in an experimental model with tau pathology and marked neurodegeneration, taupathy was observed in the SCN and the rhythm of PER2, one of the main clock genes, was decreased, resulting in impaired SCN rhythm (Hoyt & Obrietan, 2022).

Animal experiments and postmortem studies indicate that there are pathological changes in the SCN in patients with AD. Along with a decrease in cell number and size in the SCN, there is a decrease in cells that express vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) and neurotensin neurons (Colwell, 2021). VIP has significanteffects in SCN function, including photic phase reset, and output signaling. In AD, the circadian rhythm is disrupted in the synthesis and release of pineal melatonin. While the epiphyses of healthy subjects express strong daily clock genes rhythms, these rhythms are lost in preclinical and clinical AD (Duncan, 2020).

Circadian rhythm disorders such as sleep deprivation can lead to disruption of the metabolism of tau protein. Studies have shown that merely tau pathology may cause sleep disorderswhich lead to neuronal degenaration. As a matter of fact, tau causes neurodegenerative damage in sleep-related regions of the brain.

The glamphatic system, managed by glial cells, works like a metabolic cleansing system and is an important component to illuminate the healing effect of sleep. Recent data exhibited that sleep increases the interstitial fluid area in the brain by 60%, allowing clearance of metabolites, and plays a role in maintaining metabolic homeostasis. Glamphatic flow decreases by about 40% in old age, which is an important risk factor for AD in old age (Homolak, Mudrovcic, Vukic, & Toljan, 2018; Xiong et al., 2022).

Parkinson's Disease

Parkinson's diseaseis the second prevalent neurodegenerative disease, and there is a progressive loss of dopaminergic neurons in the substantia nigra pars compacta (Gros & Videnovic, 2020; Shkodina et al., 2022). In last decade, the impact of circadian rhythms in Parkinson's disease has become more evident, and even circadian rhythm disorder is shown among the non-motor dysfunctions of Parkinson's (Liu et al., 2020). Although sleep disorders are quite common in patients; deviations in circadian oscillations have also been reported in experimental models of the disease. Disorders such as insomnia, intermittent sleep, daytime sleepiness, and waking up at very early hours seriously affect the circadian rhythm and mood of the patient. It is thought that the disruption in sleep-wake rhythm occurs as a result of neurodegeneration caused by the disease in PD, and damage to the sleep regulation center and/or circadian center in the hypothalamus (Fishbein et al., 2021; Gros & Videnovic, 2020). In addition, histological changes in SCN and photosensitivity retinal ganglion cells have been reported in these patients (Zuzuarregui & During, 2020). Recent data showed that sleep disturbance may occur before the onset of motor symptoms in PD patients (Maiese, 2021). Therefore, rhythm disturbance can be used as an early marker in neurodegenerative diseases.

In experimental PD models, SCN neurons had lower amplitude and/or misalignment of circadian rhythms. In addition, Parkinson's patients with circadian disrupted changed in the phase and amplitude of rest/activity and melatonin rhythms. As a matter of fact, melatonin rhythm is lower in patients with PD compared to their controls (Obayashi et al., 2021). Circadian disruption may occur even before the onset of clinical motor symptoms (Fishbein et al., 2021).

Mood disorder, a non-motor disorder in Parkinson's and Alzheimer's, is quite common and is often accompanied by Sundown syndrome, which causes tantrums during the day. Local injection of REV-ERB α antagonists or inhibitors, an important clock component, creates a hyperdopaminergic environment in the ventral midbrain. The regulation of dopamine levels, which are known to show circadian oscillation in patients with PD, plays a promising

role in controlling patients' emotional fluctuations and depression (Kim et al., 2017).

Different studies have shown that circadian gene expressions are altered in Parkinson's disease. Loss of Daily transcriptional rhythm of BMAL1, CRY1,CRY2 in the SCN in Parkinson's model has been reported, but the expression of the PER2 gene has been shown to remain unchanged. Additionally, the expression clock genessignificantly reduced. It has been reported that Bmal1 regulates microglia-mediated neuroinflammation in the survival of dopaminergic neurons and also in the regulation of the dopaminergic pathway (Liu et al., 2020). By examining clock gene expression in peripheral blood mononuclear cells taken from early stage PD patients; loss of rhythm in core clock genes have been identified (Colwell, 2021). In experimental animal Parkinson models, BMAL1 and RORα expressions were decreased with levodopa treatment. This shows that long-term treatment of levadopa used in the treatment of Parkinson's may disrupt the circadian rhythm function (Maiese, 2018). In addition, there are data that polymorphisms in clock genes are associated with PD (Shkodina et al., 2022).

Huntington's disease

Huntington's disease is a rare, inherited disease.In this diesease there is an anormal expansion of a glutamine-encoding CAG repeat in the first exon of the HTT gene encoding the huntingtin protein (Farago, Zsindely, & Bodai, 2019). It is possible that the transcriptional defect in HD also affects the circadian clock because ryhthm is regulated by transcriptional feedback-feedforward loops. Patients with HD often present with psychiatric symptoms and cognitive decline, as well as clock disorders. Therefore, the clinical presentation of HD often precedes progressive motor dysfunction with non-motor features (Diago et al., 2018). Circadian abnormalities are also common in HD. Transgenic animal models of HD have shown progressively diminished circadian rhythmicity, with events such as decreased daily activity patterns, heart rate, and body temperature variability. Clinical studies have provided data to support this (Voysey, Fazal, Lazar, & Barker, 2021). Studies in mice have shown that the SCN is greatly affected in this disease, however, VIP levels are reduced (Colwell, 2021; Prakash, Pradhan, & Sheeba, 2022). R6/2 model of HD found that mutant mice showed an increase in duration ofpassive period and a decrease in active period, which eventually led to a complete disruption of the circadian oscillation of locomotor activity. In another experimental animal study, it was shown that subjects with HD showed deterioration in locomotor activity rhythms due to age and genotype compared to wild-type (WT) controls

(Griffis et al., 2022). In the same model, it has been shown that PER2 and BMAL1 rhythms are disrupted in the SCN (Colwell, 2021).

Several studies reported dysregulation of circadian rhythm markers and sleep-wake cycles in these patients. In particular, increased morning cortisol production has been reported in patients in the early phase of HD. In addition, it was reported that the sleep-wake cycle was disrupted and the sleep phase was delayed in patients with a phase shift in melatonin release (Bartlett et al., 2019).

Chronotherapy in Neurodegenerative Diseases

The purpose of chronotherapy is circadian rhythm-based medical treatment. Chronotherapy aims both to provide sleep patterns to heal pathologies and to develop the most appropriate treatment timing according to the rhythm of the individual (Cardinali, Brown, & Pandi-Perumal, 2021). Chronopharmacological treatment increases the efficacy of treatment by considering individual differences in patients' hours, changes in organism sensitivity to therapeutic and side effects of drugs (Sion & Begou, 2021). In addition, considering the timing of drug administration is of great importance in treatment because circadian rhythms have an effect on drug distribution, absorption, metabolism, elimination and toxicity (Chang & Kim, 2020).

In neurodegenerative diseases such as AD and PD, deformities that occur in both the SCN and the pathways that receive and transmit light impulses disrupt the circadian rhythm. Disruption of sleep patterns increases the severity and progression of such diseases (Ruben, Hogenesch, & Smith, 2019). Early morning light therapy, gamma frequency light treatment and pharmacotherapeutic applications are methods for eliminating circadian rhythm disorders in NDD diseases in order to both alleviate the symptoms and prevent the progression of the disease (Fishbein et al., 2021; Hoyt & Obrietan, 2022). This type of circadian-based therapy to ensure regular sleep rhythm is increasingly becoming a part of standard care and helps to preserve cognitive functions (Fishbein et al., 2021). Evaluation of dim light melatonin onset (DLMO), a biomarker for the circadian clock phase in PD patients, showed that the phase drift angle was greater in PD patients on medication (Fifel & Videnovic, 2019).

The options applied in this type of disease are bright light therapy (BLT) or melatonin administration, or a combination of both treatments. Other clinical studies with AD patients exposed to BLT or melatonin administration also showed improvements in sleep problems, cognitive function, sunset or dementia (Chang & Kim, 2020). For example; melatonin supplementation and a combined natural light-like light therapy have been shown to improve sleep

onset, shorten sleep latency, and reinforce nocturnal sleep episodes (Coogan et al., 2013). Small molecule therapies are also a treatment modality that can be applied with other therapies that affect protein functions. Basically, these molecules are the main components of the circadian clocks or their main regulators are agonist modulators that target rhythmic processes and regulate the period lengths, phases or amplitudes of rhythmic processes.

Conclusion

Circadian rhythm disruption is a common feature of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. In such diseases, treatments targeting the circadian rhythm may create a neuroprotective effect and improve cognitive functions.

The explanation of the clock components and modulatory mechanisms may affect the success of chronotherapy and reduce the progression of these neurodegenerative diseases.

References

1. Bacalini, M. G., Palombo, F., Garagnani, P., Giuliani, C., Fiorini, C., Caporali, L., . . . Santoro, A. (2022). Association of rs3027178 polymorphism in the circadian clock gene PER1 with susceptibility to Alzheimer's disease and longevity in an Italian population. *Geroscience, 44*(2), 881-896. doi:10.1007/s11357-021-00477-0
2. Bartlett, D. M., Dominguez, D. J., Reyes, A., Zaenker, P., Feindel, K. W., Newton, R. U., . . . Cruickshank, T. (2019). Investigating the relationships between hypothalamic volume and measures of circadian rhythm and habitual sleep in premanifest Huntington's disease. *Neurobiol Sleep Circadian Rhythms, 6*, 1-8. doi:10.1016/j.nbscr.2018.07.001
3. Cardinali, D. P., Brown, G. M., & Pandi-Perumal, S. R. (2021). Chronotherapy. *Handb Clin Neurol, 179*, 357-370. doi:10.1016/B978-0-12-819975-6.00023-6
4. Chang, Y. C., & Kim, J. Y. (2020). Therapeutic implications of circadian clocks in neurodegenerative diseases. *J Neurosci Res, 98*(6), 1095-1113. doi:10.1002/jnr.24572
5. Colwell, C. S. (2021). Defining circadian disruption in neurodegenerative disorders. *J Clin Invest, 131*(19). doi:10.1172/JCI148288
6. Coogan, A. N., Schutova, B., Husung, S., Furczyk, K., Baune, B. T., Kropp, P., . . . Thome, J. (2013). The circadian system in Alzheimer's disease: disturbances, mechanisms, and opportunities. *Biol Psychiatry, 74*(5), 333-339. doi:10.1016/j.biopsych.2012.11.021
7. Diago, E. B., Martinez-Horta, S., Lasaosa, S. S., Alebesque, A. V., Perez-Perez, J., Kulisevsky, J., & Del Val, J. L. (2018). Circadian Rhythm, Cognition, and Mood Disorders in Huntington's Disease. *J Huntingtons Dis, 7*(2), 193-198. doi:10.3233/JHD-180291
8. Dong, Y., Cheng, L., & Zhao, Y. (2022). Resetting the circadian clock of Alzheimer's mice via GLP-1 injection combined with time-restricted feeding. *Front Physiol, 13*, 911437. doi:10.3389/fphys.2022.911437
9. Duncan, M. J. (2020). Interacting influences of aging and Alzheimer's disease on circadian rhythms. *Eur J Neurosci, 51*(1), 310-325. doi:10.1111/ejn.14358
10. Farago, A., Zsindely, N., & Bodai, L. (2019). Mutant huntingtin disturbs circadian clock gene expression and sleep patterns in Drosophila. *Sci Rep, 9*(1), 7174. doi:10.1038/s41598-019-43612-w
11. Fifel, K., & Videnovic, A. (2019). Chronotherapies for Parkinson's disease. *Prog Neurobiol, 174*, 16-27. doi:10.1016/j.pneurobio.2019.01.002

12. Fishbein, A. B., Knutson, K. L., & Zee, P. C. (2021). Circadian disruption and human health. *J Clin Invest, 131*(19). doi:10.1172/JCI148286
13. Fowler, S., Hoedt, E. C., Talley, N. J., Keely, S., & Burns, G. L. (2022). Circadian Rhythms and Melatonin Metabolism in Patients With Disorders of Gut-Brain Interactions. *Front Neurosci, 16*, 825246. doi:10.3389/fnins.2022.825246
14. Griffis, C. G., Mistry, J., Islam, K., Cutler, T., Colwell, C. S., & Garfinkel, A. (2022). Circadian and ultradian rhythms in normal mice and in a mouse model of Huntington's disease. *Chronobiol Int, 39*(4), 513-524. doi:10.1080/07420528.2021.2014516
15. Gros, P., & Videnovic, A. (2020). Overview of Sleep and Circadian Rhythm Disorders in Parkinson Disease. *Clin Geriatr Med, 36*(1), 119-130. doi:10.1016/j.cger.2019.09.005
16. Homolak, J., Mudrovcic, M., Vukic, B., & Toljan, K. (2018). Circadian Rhythm and Alzheimer's Disease. *Med Sci (Basel), 6*(3). doi:10.3390/medsci6030052
17. Hoyt, K. R., & Obrietan, K. (2022). Circadian clocks, cognition, and Alzheimer's disease: synaptic mechanisms, signaling effectors, and chronotherapeutics. *Mol Neurodegener, 17*(1), 35. doi:10.1186/s13024-022-00537-9
18. Huang, J., Peng, X., Fan, R., Dong, K., Shi, X., Zhang, S., . . . Yang, Y. (2021). Disruption of Circadian Clocks Promotes Progression of Alzheimer's Disease in Diabetic Mice. *Mol Neurobiol, 58*(9), 4404-4412. doi:10.1007/s12035-021-02425-7
19. Hunt, J., Coulson, E. J., Rajnarayanan, R., Oster, H., Videnovic, A., & Rawashdeh, O. (2022). Sleep and circadian rhythms in Parkinson's disease and preclinical models. *Mol Neurodegener, 17*(1), 2. doi:10.1186/s13024-021-00504-w
20. Kim, J., Jang, S., Choe, H. K., Chung, S., Son, G. H., & Kim, K. (2017). Implications of Circadian Rhythm in Dopamine and Mood Regulation. *Mol Cells, 40*(7), 450-456. doi:10.14348/molcells.2017.0065
21. Li, Y., Shao, L., Mou, Y., Zhang, Y., & Ping, Y. (2021). Sleep, circadian rhythm and gut microbiota: alterations in Alzheimer's disease and their potential links in the pathogenesis. *Gut Microbes, 13*(1), 1957407. doi:10.1080/19490976.2021.1957407
22. Liu, W. W., Wei, S. Z., Huang, G. D., Liu, L. B., Gu, C., Shen, Y., . . . Liu, C. F. (2020). BMAL1 regulation of microglia-mediated

neuroinflammation in MPTP-induced Parkinson's disease mouse model. *FASEB J, 34*(5), 6570-6581. doi:10.1096/fj.201901565RR

23. Maiese, K. (2018). Novel Treatment Strategies for the Nervous System: Circadian Clock Genes, Non-coding RNAs, and Forkhead Transcription Factors. *Curr Neurovasc Res, 15*(1), 81-91. doi:10.2174/1567202615666180319151244
24. Maiese, K. (2021). Cognitive Impairment and Dementia: Gaining Insight through Circadian Clock Gene Pathways. *Biomolecules, 11*(7). doi:10.3390/biom11071002
25. Mathew, D., Luo, Q., & Bhatwadekar, A. D. (2022). Circadian rhythm disruption results in visual dysfunction. *FASEB Bioadv, 4*(6), 364-378. doi:10.1096/fba.2021-00125
26. McCarthy, M. J., Gottlieb, J. F., Gonzalez, R., McClung, C. A., Alloy, L. B., Cain, S., . . . Murray, G. (2022). Neurobiological and behavioral mechanisms of circadian rhythm disruption in bipolar disorder: A critical multi-disciplinary literature review and agenda for future research from the ISBD task force on chronobiology. *Bipolar Disord, 24*(3), 232-263. doi:10.1111/bdi.13165
27. Meyer, N., Harvey, A. G., Lockley, S. W., & Dijk, D. J. (2022). Circadian rhythms and disorders of the timing of sleep. *Lancet, 400*(10357), 1061-1078. doi:10.1016/S0140-6736(22)00877-7
28. Niu, L., Zhang, F., Xu, X., Yang, Y., Li, S., Liu, H., & Le, W. (2022). Chronic sleep deprivation altered the expression of circadian clock genes and aggravated Alzheimer's disease neuropathology. *Brain Pathol, 32*(3), e13028. doi:10.1111/bpa.13028
29. Obayashi, K., Saeki, K., Yamagami, Y., Kurumatani, N., Sugie, K., & Kataoka, H. (2021). Circadian activity rhythm in Parkinson's disease: findings from the PHASE study. *Sleep Med, 85*, 8-14. doi:10.1016/j.sleep.2021.06.023
30. Oh, C. E., Lim, H. J., Park, J., Moon, E., & Park, J. K. (2022). Relationship of Circadian Rhythm in Behavioral Characteristics and Lipid Peroxidation of Brain Tissues in Mice. *Clin Psychopharmacol Neurosci, 20*(4), 649-661. doi:10.9758/cpn.2022.20.4.649
31. Peng, F., Li, X., Xiao, F., Zhao, R., & Sun, Z. (2022). Circadian clock, diurnal glucose metabolic rhythm, and dawn phenomenon. *Trends Neurosci, 45*(6), 471-482. doi:10.1016/j.tins.2022.03.010
32. Prakash, P., Pradhan, A. K., & Sheeba, V. (2022). Hsp40 overexpression in pacemaker neurons delays circadian dysfunction in a Drosophila

model of Huntington's disease. *Dis Model Mech, 15*(6). doi:10.1242/dmm.049447
33. Reddy, S., Reddy, V., & Sharma, S. (2022). Physiology, Circadian Rhythm. In *StatPearls*. Treasure Island (FL).
34. Rosenwasser, A. M., & Turek, F. W. (2022). Neurobiology of Circadian Rhythm Regulation. *Sleep Med Clin, 17*(2), 141-150. doi:10.1016/j.jsmc.2022.02.006
35. Ruben, M. D., Hogenesch, J. B., & Smith, D. F. (2019). Sleep and Circadian Medicine: Time of Day in the Neurologic Clinic. *Neurol Clin, 37*(3), 615-629. doi:10.1016/j.ncl.2019.03.004
36. Sharma, A., Moon, E., Kim, G., & Kang, S. U. (2021). Perspectives of Circadian-Based Music Therapy for the Pathogenesis and Symptomatic Treatment of Neurodegenerative Disorders. *Front Integr Neurosci, 15*, 769142. doi:10.3389/fnint.2021.769142
37. Sharma, A., Sethi, G., Tambuwala, M. M., Aljabali, A. A. A., Chellappan, D. K., Dua, K., & Goyal, R. (2021). Circadian Rhythm Disruption and Alzheimer's Disease: The Dynamics of a Vicious Cycle. *Curr Neuropharmacol, 19*(2), 248-264. doi:10.2174/1570159X18666200429013041
38. Shkodina, A. D., Tan, S. C., Hasan, M. M., Abdelgawad, M., Chopra, H., Bilal, M., . . . Alexiou, A. (2022). Roles of clock genes in the pathogenesis of Parkinson's disease. *Ageing Res Rev, 74*, 101554. doi:10.1016/j.arr.2021.101554
39. Sion, B., & Begou, M. (2021). Can chronopharmacology improve the therapeutic management of neurological diseases? *Fundam Clin Pharmacol, 35*(3), 564-581. doi:10.1111/fcp.12659
40. Tabibzadeh, S. (2021). CircadiOmic medicine and aging. *Ageing Res Rev, 71*, 101424. doi:10.1016/j.arr.2021.101424
41. Thome, J., Coogan, A. N., Woods, A. G., Darie, C. C., & Hassler, F. (2011). CLOCK Genes and Circadian Rhythmicity in Alzheimer Disease. *J Aging Res, 2011*, 383091. doi:10.4061/2011/383091
42. Voysey, Z., Fazal, S. V., Lazar, A. S., & Barker, R. A. (2021). The sleep and circadian problems of Huntington's disease: when, why and their importance. *J Neurol, 268*(6), 2275-2283. doi:10.1007/s00415-020-10334-3
43. Wang, X. L., & Li, L. (2021). Circadian Clock Regulates Inflammation and the Development of Neurodegeneration. *Front Cell Infect Microbiol, 11*, 696554. doi:10.3389/fcimb.2021.696554

44. Xiong, X., Hu, T., Yin, Z., Zhang, Y., Chen, F., & Lei, P. (2022). Research advances in the study of sleep disorders, circadian rhythm disturbances and Alzheimer's disease. *Front Aging Neurosci, 14*, 944283. doi:10.3389/fnagi.2022.944283
45. Zuzuarregui, J. R. P., & During, E. H. (2020). Sleep Issues in Parkinson's Disease and Their Management. *Neurotherapeutics, 17*(4), 1480-1494. doi:10.1007/s13311-020-00938-y

Investigation of the Effect of Mobile Phone Sourced 2100 MHz Radiofrequency Radiation on Rat Kidney Tissue

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1. Introduction

Technological devices facilitate human life every day and provide advantages in terms of time and energy, and both the development and the ownership rate of the society are increasing. People are constantly or partially interacting with high voltage lines, base stations, electrical home and office appliances and mobile phones for a long time. All these devices emit electromagnetic radiation to their environment and the society interacts with these areas(Yavaş et al., 2018; Atakır et al., 2022). In addition, there are data that very low frequency electromagnetic fields have negative effects on human serum biochemistry, hormones and whole blood(Yavaş, 2020).With technological developments, we are exposed to wired and wireless electromagnetic radiations in our daily lives, and discussions and concerns continue about their effects on human health(Kurnaz and Mutlu, 2021).In the light of experimental and epidemiological studies with radiofrequency electromagnetic field exposures, they have been included in the group 2B probable carcinogen class by the International Agency for Research on Cancer (IARC) (IARC, 2013).Although radiofrequency electromagnetic radiations work in different frequency bands (900-2100 MHz, 2600 MHz, 3.5 GHz etc.), it is stated that as a result of exposure to these areas, they increase oxidative stress in living tissues and disrupt the antioxidant balance. In some studies, it is stated that these areas have a partial effect and that more detailed long-term studies are needed(Yavaş et al., 2021). It has been reported that radiation (900 MHz) produced by a mobile phone causes oxidative stress and peroxidation in rat kidney tissue(Devrim et al., 2008). The biological effects of exposure to electromagnetic fields originating from radiofrequency vary depending on the duration of application, the condition of the affected organ and the distance. Researchers report that radiofrequency radiation applied in the prenatal period may cause significant histopathological changes in the tissues of rat juveniles (Türedi, et al., 2022).

The area exposures emitted by these devices, which affect daily life, almost everyone has a smartphone and are used for a long time, arouse curiosity and the effects on human health are reported by researchers. In this study, we will investigate the effect of 2100 MHz radiofrequency radiation on rat kidney tissue in the light of available data.

2. Exposure, Animal model and Findings

We used Sprague Dawley male rats in our study. 7 rats in the control group did not receive exposure, while 7 rats in the experimental group were exposed

to 2100 MHz for 5 hours a day for 14 days. Exposure was performed with a signal generator (Rohde & Schwartz, Germany), and area measurement was performed with Narda EMR 300. Area measurement SAR values are presented in Table 1.

Table1. SAR analysis values

Body part-SAR	10g	1g
Whole body	305.5 mW/kg	720 mW/kg
Kidney	203 mW/kg	645 mW/kg

Histopathological evaluation of kidney tissue

The kidney tissues taken after the application were fixed. After the tissues were embedded in paraffin blocks, they were stained with hematoxylin and eosin (H&E). In addition, Masson trichrome staining was performed histochemically. Histopathological findings were evaluated under the light microscope by the pathologist who did not know the experimental groups. A minimum of 10 fields for each kidney slide were analyzed and evaluated for severity of changes.

Histopathological scoring was made according to the highest area. By semi-quantitative semi-quantitative analysis; Four categories were determined (0: None, 1: Minimal, 2: Mild, 3: Moderate, and 4: Severe) and parameters were scored accordingly. To determine the degree of tubular damage, glomerular damage and interstitial damage, “tubular dilatation, proteinous material accumulation in the tubule, tubular epithelial cell change, glomerular damage (fibrosis/atrophy/thrombosis), interstitial fibrosis, interstitial congestion/hemorrhage, interstitial mononuclear cell infiltration We used the " parameters. Analysis results are shown in Figure 1-4, histopathological evaluation, and in Table 2, histopathological scoring.

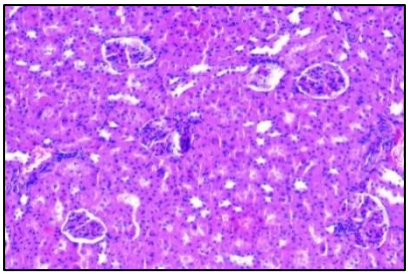

Figure 1: Usual histomorphological appearance of kidney tissue from the control group (H&E, x100).

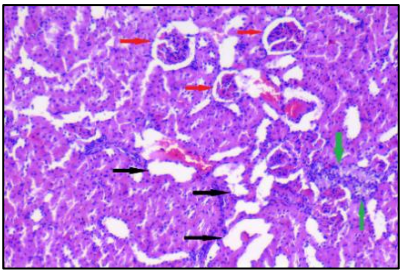

Figure 2: There are mild dilatation (black arrows) in some tubules, atrophy in glomeruli (red arrows) and reactive changes (green arrows) in tubular epithelial cells in the kidney of the rat from the experimental group (H&E, x100).

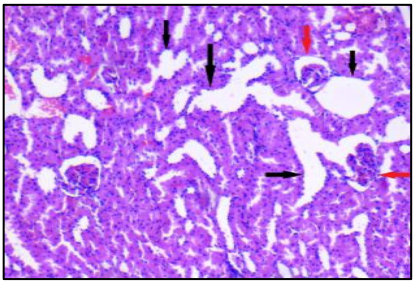

Figure 3: There are glomeruli (red arrows) showing slight atrophy in places and dilatation (black arrows) in some tubules in the rat belonging to the experimental group (H&E, x100).

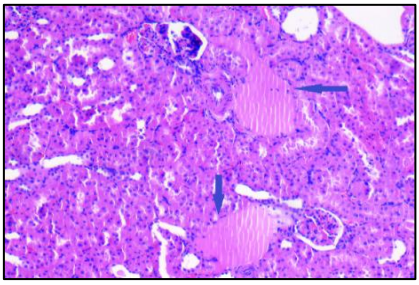

Figure 4: A minimal dilatation and content of eosinophilic proteinous material is seen in some tubules in the kidney of the rat from the experimental group (blue arrows) (H&E, x100).

Table 2. Histopathological scoring results of kidney tissues

Kidney histopathology		Sham-control group Median (Min-Max)	Exposure group Median (Min-Max)	*P*
Tubular Damage Scoring	Dilation	1,00 (1-3)	1,00 (1-3)	0.620
	Deposition of proteinous material	0,00 (0-1)	0,00 (0-1)	1000
	Epithelial cell changes	0,00 (0-1)	1,00 (0-1)	0.209
Glomerular Damage Scoring	Fibrosis, atrophy, thrombosis	1,00 (0-1)	1,00 (1-2)	0.128
Interstitial Damage Scoring	Vascular congestion/hemorrhage	1,00 (1-2)	1,00 (1-2)	0.710
	Fibrosis	0,00 (0-0)	0,00 (0-0)	1.000

3. Conclusion

In the study conducted by Öktem et al., it was determined that 900 MHz radiofrequency exposure increased the MDA level in the kidney tissue of rats(Öktem et al., 2005).Researchers exposed kidney tissue to 4G cell phone radiation on mice. As a result of the study, they showed that it can affect blood hemostasis and inflammation in the kidney tissue of mice. It was also stated that it is important to inform the public about the potential negative effects of exposure to electromagnetic radiation from mobile phones (Hasan et al., 2021).In the 2100 MHz radiofrequency study model, some pathological changes were reported in the kidney tissue of postnatal male rats(Bedir et al., 2018). In a different modeled study, the effect of 2100 and 1800 MHz mobile phone-induced radiations on chick embryos was investigated. As a result of the study, it was concluded that there were histopathological changes in the chick kidney kidneys(Dsilva et al., 2022).

In our study, we found similar findings with the literature. In the exposure group of the study (experiment), mild dilatation in some tubules, atrophy in glomeruli and reactive changes in tubular epithelial cells, mild dilatation in some tubules, atrophy in glomeruli and reactive changes in tubular epithelial cells were detected. In our histopathological scoring (Tubular Damage, Glomerular Damage and Interstitial Damage), we could not detect a statistically significant difference between the control and experimental groups.

It is seen that scientific studies on radiofrequency electromagnetic field exposures on kidney tissue are limited. Therefore, it is clear that more work is

needed in this area. In the light of our studies, it is seen that there are possible biological effects of radiofrequency radiations originating from mobile phones and it is essential for the society to raise awareness and take protective measures in this regard.

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References

1. K. Atakır, G. Özevci, B. Ceyhan (2022). Elektromanyetik Radyasyon ve İnsan Sağlığına etkisi. *Environmental Toxicology and Ecology*, 2(1), ss. 9-21.
2. Yavaş M.C., (2020). Changes in the serum of individuals exposed to extremely low frequency electromagnetic fields for a long time. *Adıyaman Üniversitesi Sağlık Bilimleri Dergisi*, 6(1),13-18.https://doi.org/10.30569/adiyamansaglik.558935
3. Yavaş, M.C., Akpolat, V., Deveci, E., et al,. (2018). Determining the effect of an electromagnetic field generated by a high voltage power line on rat spermatogonia cells. *Dicle Tıp Dergisi,* 45 (4), 447-461. DOI: 10.5798/dicletip.497923
4. Kurnaz, Ç., & Mutlu, M. (2021). Uzun süreli radyofrekansı elektromanyetik alan seviyesinin izlenmesi ve modellenmesi. *Gazi Üniversitesi Mühendislik Mimarlık Fakültesi Dergisi*, *36*(2), 669-684.https://doi.org/10.17341/gazimmfd.641287
5. IARC, Monographs on the evaluation of carcinogenic risks to humans non-ionizing radiation, Part 2: Radiofrequency Electromagnetic Fields, 102. IARC, Lyon, 2013. https://publications.iarc.fr/126 accessed:08.012.2022.
6. Yavaş M.C., Yegin Y., Oruç S., et al., (2021). Analysis of thiol/disulphide homeostasis and oxidant-antioxidant status as a result of exposure to radio-frequency electromagnetic fields, Electromagnetic Biology and Medicine, 40:1, 84-91, DOI: 10.1080/15368378.2021.1874970
7. Türedi S. , Hancı H. , Odaci E. , Çelik H. (2022).Prenatal dönemleri boyunca günde bir saat kesintisiz 900 MHz elektromanyetik alan etkisine maruz kalan 75 günlük Sprague Dawley dişi sıçanların böbrek ve mesane dokularının histopatolojik olarak incelenmesi. *ADYÜ Sağlık Bilimleri Derg.* 8(2): 142-150.https://doi.org/10.30569/adiyamansaglik.1023728
8. Devrim et al., (2008). Effects of electromagnetic radiation use on oxidant/antioxidant status and DNA turn-over enzyme activities in erythrocytes and heart, kidney, liver, and ovary tissues from rats: possible protective role of vitamin C.*Toxicol. Mech.*Methods, 18.pp. 679-683 https://doi.org/10.1080/15376510701380182
9. Oktem, F., Ozguner, F., Mollaoglu, H., Koyu, A., & Uz, E. (2005). Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone: protection by melatonin. *Archives of Medical Research*, *36*(4), 350-355. https://doi.org/10.1016/j.arcmed.2005.03.021

10. Hasan, I., Amin, T., Alam, M. R., & Islam, M. R. (2021). Hematobiochemical and histopathological alterations of kidney and testis due to exposure of 4G cell phone radiation in mice. *Saudi Journal of Biological Sciences*, *28*(5), 2933-2942.https://doi.org/10.1016/j.sjbs.2021.02.028
11. Bedir, R., Tumkaya, L., Mercantepe, T., & Yilmaz, A. (2018). Pathological findings observed in the kidneys of postnatal male rats exposed to the 2100 MHz electromagnetic field. *Archives of Medical Research*, *49*(7), 432-440. https://doi.org/10.1016/j.arcmed.2018.12.010
12. Dsilva, M. H., Swer, R. T., & Anbalagan, J. (2022). Histomorphometric Analysis of Chick Embryo Kidneys on Exposure to 1800 MHz and 2100 MHz Radiofrequency Radiation Emitted from Cell Phone. *Journal of Clinical & Diagnostic Research*, *16*(10). DOI: 10.7860/JCDR/2022/58391.16862

Homalothecium Sericeum (Hedw.) Schimp. As A New Potential Medical Pharmaceutical

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Introduction

Nowadays, there has been a noticeable increase in diseases caused by external factors and sinful lifestyles. Antioxidants and the by-products of their mechanisms have attracted attention for their role in preventing diseases. Antioxidants have protective, therapeutic, and prognostic roles against ageing, degenerative disease, autoimmune diseases, cancer, and many other diseases[1]. Studies have shown that antioxidant molecules play an essential role against intracellular stress and oxidative damage caused by free radicals/reactive oxygen species (ROS), triggering diseases [2].

Oxidative stress can occur due to a disruption of the oxidative imbalance due to a lack of antioxidant molecules in the cell, which can detoxify ROS when they increase during cellular metabolism[3].

Free radicals (ROS) are molecules that contain at least one unpaired electron in their structure and attack biomolecules in the cell to complete the last electron level. For example, ROS attacks structural groups in cells containing double bonds. It initiates chain oxidation reactions by cleaving a hydrogen atom from the double bonds of DNA bases, lipids, and proteins leading to cell damage or death [2].

ROS molecules formed due to increased oxidative stress in the cell attack lipid and protein groups containing double bonds and double bonds of bases on DNA and initiate oxidation chain reactions by cleaving hydrogen atoms. As a result, double-bonded macromolecules in the cell are damaged, and cell damage can result in apoptosis. Based on this, the search for naturally occurring antioxidant molecules in plants and biological organisms has accelerated.

Unstable free radicals have one or more unpaired electrons in the cell, are short-lived, have low molecular weight and have active molecules produced in cells. In aerobic organisms, ROS can produce free radicals in the cell through metabolic pathways, such as electron transport chains in mitochondria or active phagocytosis. During this production, hydroxyl radical (HO-), superoxide anion (O_2-), hydrogen peroxide (H_2O_2), alkyl radical (RO^-), peroxynitrite ($ONOO^-$), hydrochloric acid(HCl) can be formed [2, 4].

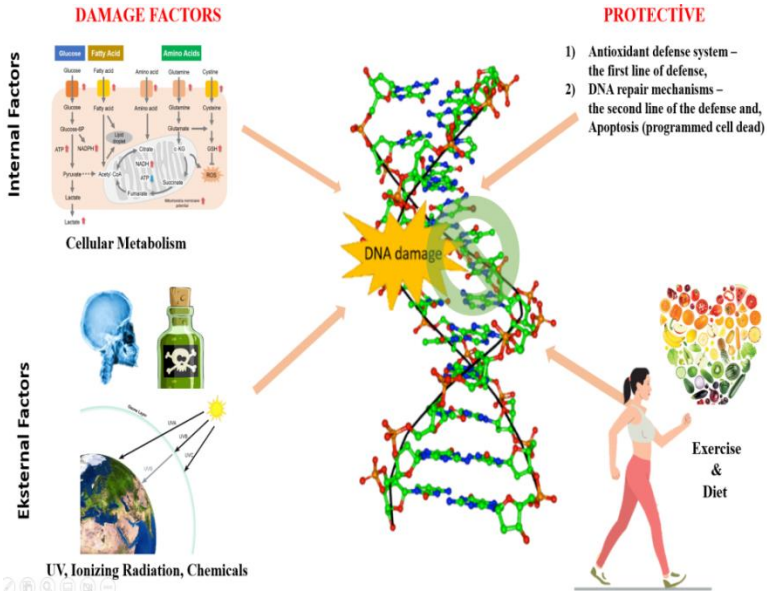

Figure 1.External and internal factors that cause ROS and protective mechanisms to fight against ROS stress

Molecules called antioxidants help to eliminate oxidative stress triggered by free radicals in our bodies. Antioxidants are substances easily oxidized by oxygen, which may occur in circumstances where they may impact changes even at tiny concentrations. Antioxidants can inhibit or be promoted by O_2 and peroxide; different inhibitors or retarders of oxidative reactions are substances with a chemical structure.

Antioxidant substances stabilize oxidation-sensitive nutrients such as vitamins A, K, beta carotene, xanthophyll, and tocopherol, increasing the durability of biological molecules in danger of oxidation. Today, individuals take synthetic antioxidant drugs as part of preventive medicine against conditions associated with oxidative damage, such as ageing and health problem (degenerative disease etc.). Plants have high antioxidant properties as they are rich in secondary metabolites such as isoflavones, flavonoids, anthocyanins, lignans, coumarin, catechins, flavones and is catechins. [5]. Plant antioxidants are now being used to escape synthetic molecules' adverse effects and obtain antioxidant activities more naturally.

Vascular plants are known to be natural sources of antioxidants, but little is known about other plant groups, especially mosses. Mosses, historically the oldest land plants, can produce a variety of secondary metabolites to combat many stresses (temperature, salt, UV etc.), radiation-related damage and microbial degradation [6]. Many species of these plants have different biological

activities besides antioxidant properties, such as antimicrobial, antifungal, and antineoplastic, due to their excellent sources of secondary compounds. The COVID-19pandemic highlighted the importance of natural products to improve the immune system against microorganisms; thus, antioxidant molecules and antimicrobial peptides have become popular topics.

The antibacterial and antifungal properties of bryophytes are thought to result from various antimicrobial peptides (AMPs) that form structurally or in response to mechanical injury from pathogen infection, abiotic stress, and various pest damages [7]. Plant AMPs are part of the innate immune system to protect plants against microbes. Thesedefencessucceed with natural chemicals like cyclotides, calprotectin, defensins, hepcidin, hevein-like proteins, lactoferrin, lipid transfer proteins, snakes, thionins and vicilin-like proteins. They are classified according to their structure and the presence of disulfide bonds [8]. For example, defensins induce apoptosis [9-10], attract microbial cell walls, and inhibit pathogen metabolism by inhibiting activity against proteinase and amylase[11].

Evolutionarily more advanced than algae, vascular plants are at a more primitive level than ferns, horsetails, matchsticks, and seed plants. Bryophytes are among the first green plants to set foot on land. They broke away from their aquatic ancestors and successfully spread over large areas in terrestrial habitats 470 million years ago. Two main evolutionary hypotheses have been put forward as a result of recent phylogenetic analyzes. First, liverworts and mosses form a monophyletic clade within the bryophytes, while hornworts are an independent lineage. Latter: all bryophyte groups are in a single clade as sister taxa to vascular plants [12]

Mosses, which are thought to be between 20,000 and 25,000 in the world [13,14] and are called the most primitive living plants, are represented by around 1000 taxa in our country (leafy mosses: ~ 800; liverworts: ~180; hornworts: 4). In the period from 1829, when the first moss record was given in our country, to the present day, most of them were systematic studies, including sociology, ecology, and antimicrobial etc. of moss. Numerous articles have been published on its effects.

H. sericeum, a bryophyte species, shows high levels of antifungal, antibacterial and antioxidant properties; There is research that it can be used in disease prevention and treatment phases as a natural antioxidant of plant origin. *H. sericeum* is a plant species in temperate and tropical climates, generally on the northern and southern slopes[15]. In the forest ecosystem, it occurs in hills and hollows in a carpet of brown or red colours, with bright green colours on the

ground. However, they are found as semi-saprophytes on stones, rocks, water, trunks, and branches of dead/alive trees and decaying organic matter in moist dry places.

In this respect, it can be said that bryophyteshave rich biological activity. It is believed to have potent antioxidant and antifungal properties, and based on this information, its use should be increased today.

Antioxidant, Antibacterial and Antifungal Compounds Contained in Mosses

Many plants have a great reservoir of secondary metabolites [16-17]. Plant phenols (stilbenes, curcumin, catechins, flavonoids, etc.) are effective antioxidants. There is growing evidence that they can protect cells from oxidative damage and thus limit oxidative-related degenerative disease risk [18]. Extensive clinical research has been conducted on the phenolic compound potentials on cardiovascular, inflammatory, and degenerative diseases and neoplasm [19].

1stGroupAntioxidants(Enzymes)	Catalase, Superoxidedismutase, Peroxidase, Glutathionereductase
2nd GroupAntioxidants (BiomoleculesasVitaminsand Aminoacids)	Glutathione, VitaminA, Cand E, Lipoicacid, Albumin, Phenolicsflavonoids.
3rd Group(ComplexEnzymes)	Repair enzymes for biomolecules such as DNA, lipids and proteins

Table 1: Antioxidants molecules

Ertürk et al. (2015) have identified the total phenolic compound and potential antioxidant effects of moss species of Turkey. In their study, *H. sericeum* and *Eurhynchium striatum (Plasteurhynchium striatum)*(Pico) had the highest activity[3].

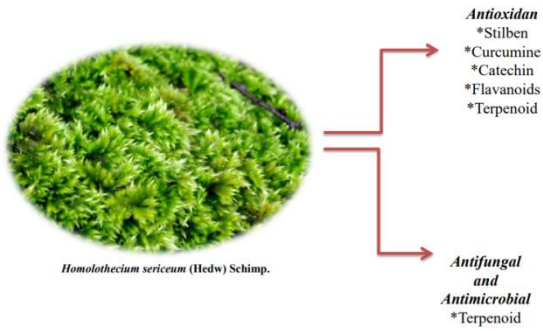

Figure 2. Secondary metabolites of *Homalothecium sericeum* (Hedw) Schimp.

The primary elements causing antioxidant action are the polyphenols in bryophyte extracts, with flavonoids, bioflavonoids, and isoflavonoids in each species [20]. Flavonoids, a group of polyphenols abundant in fruits, vegetables, cereals, and green plants, are involved in many biological processes, including responses to external environmental factors. Flavonoids are widely used in the human diet and have antibacterial, anti-inflammatory, antimicrobial, antimicrobial and high-antioxidant properties that reduce the risk and help treat diseases[21].

Terpenoids, also called isoprenoids, are important to gain pathogen resistance and are found in almost every organism [22-23]. Together with theirimmune regulatory effect (anti-inflammatory, anti-viral), terpenoids are essential to support the human body against ageing and neoplasm through oxidative stress [23]. One study observed that the antifungal activities of *H.sericeum* could extract be attributed to active terpenoids[24].

Studies have shown that the biological activity of bryophytes is due to flavonoids and terpenoids. Based on these studies, it is seen that secondary metabolites are used in the pharmaceutical sector as antifungal, anticancer and antibacterial, and studies are being carried out to expand their use.

Antioxidant Properties of *Homalothecium sericeum* (Hedw.) Schimp.

Scientists use different methods and materials to add new ones to known antioxidants and obtain stronger antioxidants. One of the valuable and hopeful materials used in antioxidant activity research is mosses. Unlike seed plants, which constitute a crucial part of plants, bryophytes stand out with their ability to remove free radicals.

	TPC (mg GAE/100 g sample)	CUPRAC (µmol Trolox /100 g sample)	FRAP (µmol Trolox /100 g sample)	DPHH EC50
H.cupressiforme	4.10±0.003	58.42±0.13	12.92±0.10	079±0.05
H.sericeum	8.21±0.04	142.91±0.23	36.91±0.22	052±0.06
T.delicatulum	5.71±0.03	95.43±0.24	24.91±0.13	0.87±0.06
H.lutescens	3.33±0.02	52.65±0.18	16.66±0.10	2.83±0.08
H.nitens	3.49±0.03	64.90±0.22	17.18±0.16	4.40±0.09
L.sciurodies	5.32±0.02	76.01±0.31	28.58±0.23	0.49±0.04
C.molluscum	4.42±0.01	60.31±0.20	14.37±0.16	1.96±0.07
E.striatulum	7.12±0.05	118.12±0.42	33.59±0.20	0.22±0.01

Table 2: Antioxidant activities of moss samples adapted from [22]

In the study by Ertürk et al. (2015), *Hypnum cupressiforme* (Hedw.) EC_{50} 0.79±0.05 µg/mL, *Homalothecium sericeum* (Hedw.) EC_{50} 0.52±0, respectively, from the samples used in the study of determining the antioxidant capacity of mosses. 06 µg/mL, *Thuidium delicatulum* (Hedw.) EC_{50} 0.87±0.06 µg/mL, *Homalothecium lutescens* (Hedw.) EC_{50} 2.83±0.08 µg/mL, *Homalothecium nitens* (Hedw.) EC_{50} 4, 40±0.09 µg/mL, *Leucodon sciuroides* (Hedw.) EC_{50} 0.49±0.04 µg/mL, *Ctenidium molluscum* (Hedw.) EC_{50} 1.96±0.07 µg/mL, *Eurhynchium striatulum* (Spruce) EC_{50} 0.22±0.01 µg/mL results were obtained. obtained from the samples used in this study [25].

Sahilli et al. have been shown that radical scavenging activity and due to its reducing power determination, *H. sericeum* plant was defined as the natural antioxidant source. This circumstance supports the notion that bryophyte species can provide natural antioxidants in place of manufactured antioxidants[26]. Antioxidants are important because they strengthen the immune system and regulate the physiological activity of the cell by preventing DNA, protein, and lipid damage. Prevention of possible DNA damage supports the regulatory mechanisms that ensure uncontrolled cell division. Oztopçu et al. showed antineoplastic activity in a glioma cell line with *H. sericeum* extracts. The demonstration of dose-dependent cytotoxicity of *H. sericeum* in the study is evidence that the contents of bryophytes can be investigated in more detail, and research can be carried out for drugs such as vascular plants.

Antifungal, Antibacterial and Antimicrobial Properties of *Homalothecium sericeum* (Hedw.) Schimp.

Studies carried out for many years on the use of mosses in treating some diseases, antimicrobial activity analysis of species, determination of related substances and purification determinations make it essential to use it in drug designs.[27].

According to studies, *H. cupressiforme*'s polycyclic aromatic hydrocarbons, hypnogenols, bioflavonoids, and hydroxyflavonoids have antifungal action.[28]. The extract of *H. sericeum*showed both antibacterial and antifungal effects*on Y. enterocolitica, S. typhimurium and S. cerevisiae*[25].

This study also suggests that extracts of black algae may be used as natural antimicrobials. According to the literature, the antimicrobial and antifungal activity of the mosses originated from flavonoids and terpenoids.

Savaraoglu et al. have shown an antiproliferative effect of *H. sericeum* for the first time, which means further purification of these extracts may be developed as a chemotherapeutic agent against cancer treatment [29].

Conclusion

Today, the inadequacy of synthetic drugs against diseases and the detection of their side effects have increased the necessity of using natural products. For this purpose, many plants have been investigated in microbiological pharmacological aspects and plant defence mechanisms in recent years when biological warfare is on the agenda.

If we analyze the context of the investigations, we can see that the bryophyte species *H. sericeum* has a wide range of biological functions. Antifungal, antimicrobial, antibacterial, and antioxidant characteristics stand out among these biological activities. However, when scanned, little information about *H. sericeum* can be gleaned from the studies.

By examining the extracts of various plant species with several antioxidant activities that can be utilized in place of synthetic antioxidants today, it should be considered that antioxidant activity can be employed as a natural antioxidant source in light of the information collected. In cosmetics, food, and functional medicine fields, natural antioxidant sources derived from plant extracts represent a promising alternative.

As natural plant extracts like *H.sericeum*have fewer negative effects than commercially available synthetic antioxidants, more study is still required.

The literature has shown that secondary metabolites of bryophyteextracts, such as flavonoids and isoprenoids, exhibit antifungal properties. These sub-

stances are thought to play a role in plants' defence mechanisms against environmental influences. When the mechanisms of action in the human body are examined, it is discovered that these compounds, which are present in many organisms, exhibit a wide range of biological activities, including antiviral, antibacterial, anti-inflammatory, antiproliferative, and antioxidant ones, which lower the risk of disease and aid in treatment.

This research has shown that isoprenoids play a role in antifungal bioactivity. *H. sericeum*is thought to be explored for drug manufacturing, as in vascular plants, by considering its antineoplastic action in the future, based on its cytotoxicity.

Despite the deficiency of material in the literature, all investigations indicate that further research is necessary to fully understand the potent biological activities of the bryophyte species *Homalothecium sericeum* (Hedw.).

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References

1. Ratnam, D.V., Ankoola D.D., Bhardwaj, V., Sahana, D.K., Kumar N.V.K, (2006), "Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective,"*J. Control. Relese,* 113(3), 189-207
2. Mittler R., (2017*)*"ROS Are Good," *Trends Plant Sci.,* 22(1), 11-19
3. Türker H.and Türkyılmaz Ünal B. (2020)"Bryophytes as The Potentıal Source ofAntioxidant," *Anatol. Bryol*
4. HerbM., Shramm M., (2021)"Functions of ROS in Macrophages and Antimicrobial Immunity",*Antioxidants ,*10(2), 313
5. Agbar H. M.,Shakya Z A, Khalaf A , (2008) "Comparative Antioxidant Activity of Some Edible Plants," *Turk J Biol*, 32, 193-196
6. Xie C.-F. and LouH.-X., (2009) "Secondary Metabolites in Bryophytes: An Ecological Aspect,"*Chem. Biodivers*. 6(3),303-312
7. Sathoff A. E. SamacD. A., (2019)"Antibacterial Activity of Plant Defensins,"*Mol. Plant- Microbe Interact.,*32(5),507-514
8. Goyal R. K., MattooA. K., (2014)"Multitasking antimicrobial peptides in plant development and host defense against biotic/abiotic stress," *Plant Sci.*,228,135-149

9. StotzH. U., ThomsonJ., and WangY., (2009)"Plant defensins,"*Plant Signal. Behav.,* 4(11), 1010-1012
10. CoolsT. L., StruyfsC., CammieB. P., and ThevissenK., (2017)"Antifungal plant defensins: increased insight in their mode of action as a basis for their use to combat fungal infections," *Future Microbiol.* ,12(5), 441-454
11. De ConinckB., CammieB. P. A., and ThevissenK., (2013) "Modes of antifungal action and in planta functions of plant defensins and defensin-like peptides,"*Fungal Biol. Rev.,* 26(4), 109-120
12. Budke Jessica M., Bernard Ernest C., Gray Dennis J., Sanna Huttunen, Birgit Piechulla & Robert N. Trigiano (2018) 'Introduction to the Special Issue on Bryophytes',*Critical Reviews inPlant Sciences,*37:2-3, 102-112
13. Gradstein, S. & Churchill, Steven & Salazar Allen, Noris. (2001). *Guide to the bryophytes of tropical America*
14. Crum, H. A. (2001). *'Structural diversity of bryophytes.'University of Michigan Press.*
15. Smith A. J. E., SmithR., (2004) The Moss Flora of Britain and Ireland. *Cambridge University Press*
16. Bhattarai, H. D., Paudel, B., Lee, H. K., Oh, H., & Yim, J. H. (2009)" In vitro Antioxidant Capacities of Two Benzonaphthoxanthenones: Ohioensins F and G, Isolated from the Antarctic Moss Polytrichastrum alpinum "*Zeitschrift Für Naturforschung* C, 64(3-4), 197–200
17. Pandey K. B. and RizviS. I., (2009) "Plant Polyphenols as Dietary Antioxidants in Human Health and Disease,"*Oxid. Med. Cell. Longev.,*2(5), 270-278
18. Pandey K. B. and Rizvi S. I.*,* (2010)"Protective effect of resveratrol on markers of oxidative stress in human erythrocytes subjected to in vitro oxidative insult,"*Phytother. Res.,* 24, S1, S11-S14
19. ZenkovN. K., ChechushkovA. V., KozhinP. M., KandalintsevaN. V., MartinovichG. G., and MenshchikovaE. B., (2016)"Plant phenols and autophagy," *Biochem.,* 81 (4), 297-314
20. Basile A., Giordano S., López-Sáez J. A., and Cobianchi R. C., (1999)"Antibacterial activity of pure flavonoids isolated from mosses," *Phytochemistry,* 52(8),1479-1482
21. ShenN., WangT., GanQ., LiuS., WangL., and Jin,B. (2022)"Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity,"*Food Chem.,* 383, 132531

22. Colak Emel, K. Recep et al. (2011) "Investigation of antimicrobial activity of some Turkish pleurocarpic mosses,"*African J. Biotechnol,*10(60), 12905-12908
23. ChenY., HuB., XingJ., and LiC., (2021) "Endophytes: the novel sources for plant terpenoid biosynthesis,"*Appl. Microbiol. Biotechnol.,*105(11),4501-4513
24. DaviesF. K., JinkersonR. E., and PosewitzM. C., (2015)"Toward a photosynthetic microbial platform for terpenoid engineering,"*Photosynth. Res.,*123(3),265-284
25. Ertürk Ö., Sahin H., Ertürk E. Y., Hotaman H. E., Koz B., and Özdemir Ö., (2015*)*"The antimicrobial and antioxidant activities of extracts obtained from some moss species in Turkey,"*Herba Pol.,*61(4), 52-65
26. Çakır Salihli A. M., Korkmaz Y.V, (2020)"Antıoxıdant Propertıes of Homalothecıum Serıceum (Hedw.) Schımp. Taxon," *İn 1st Internatıonal Iğdır Congress on Multıdıscıplınary Studıes,* 1-6
27. CanliK., AlterE. M., and AkataI., (2015*)*"Antimicrobial screening of Mnium stellare," *Bangladesh J. Pharmacol*., vol. 10(2), 321
28. VeljićM., TarbuckM., MarinP. D., ĆirićA., SokovićM., and MarinM., (2008*)*"Antimicrobial Activity of Methanol Extracts of Mosses from Serbia,"*Pharm. Biol*.,46(12), 871- 875
29. Oztopcu-Vatan U. R., Savaroglu P.,Filik İşçen F., Kabadere C., İlhanS., (2011)"Antımıcrobıal and Antıprolıferatıve Actıvıtıes of Homalotheciumsericeum (Hedw.) Schimp. Extracts," *Fresenius Environ. Bull.*, 20, 461-466

Mysterious Molecules That Might Provide Light On The Complexity Nature Of Leukemia: Circular RNAs

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INTRODUCTION

The term "leukemia" is derived from the Greek terms "leukos" (white) and "haima" (blood). Leukemiarefers to a large number of white blood cells in the blood of pantients and itis currently used to describe a variety of hematological cancers classified into subtypes based on morphological structure, cytogenetic and molecular abnormalities, immunophenotypic features, and clinical conditions (Arber et al., 2016). Leukemia is a life-threatening disease and most commonly diagnosed in adults and it is a kind of blood cancer distinguished by the oligoclonal growth of hematopoietic cells that have penetrated the bone marrow and can enter the blood as well as other extramedullary regions.It is often classified as either myeloid or lymphoid in origin, as well as acute or chronic, which is crucial in choosing the therapeutic strategy. Clinical onset and illness progression rate are considered in the second step of categorisation. Although there are many other types of leukemia, the most prevalent and well-studied include acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia(CLL), and chronic myeloid leukemia (CML).Leukemia subtypes are distinguished by the presence of cancerous white blood cells in the bloodstream. It should be noted, however, that blood-borne tumor cells are frequently found in epithelial malignancies and other hematological illnesses, such as lymphomas and multiple myeloma, and frequently in connection with more severe diseases(Gharbaran, Park, Kim, Goy, & Suh, 2014). Unfortunately with no obvious preventive or control measures in today, leukemia remains a deadly illness and a hazard to public health.In solid tumors, metastasis occurs as a result of complicated reprogramming processes influenced by multiple genetic and epigenetic variables. The situation is different in leukemia because leukemic cancer cells have a high intrinsic predisposition for migration and invasion. In the undifferentiated condition, malignant leukocyte cells are more likely to divide rapidly and uncontrollably, whereas benign leukocyte cells are more mobile and have a better chance of surviving in the circulation. For these reasons, while leukemias are not commonly thought of as metastatic tumors, they can be viewed as examples of extensive metastatic dissemination(Whiteley, Price, Cantelli, & Sipkins, 2021).

Circular RNAs

Circular RNAs (circRNAs), which were discovered in a plant virus using electron microscopy in 1976, are non-coding RNAs having a circular structure and no 3′-poly(A) tails or 5′-heads. With the advancement of sequencing technology investigations, it has been shown that 70 to 90 percent of the entire genome is expressed into RNAs, but only 2 percent of RNAs encode proteins.

This means that non-coding RNAs (ncRNAs) may undertake important biological activities, and that a comprehensive investigation of ncRNAs is required. CircRNAs have been linked to illness initiation and progression through acting in a range of biological processes in the last few years. Studies demonstrate that circRNAs may be important molecules in leukemia diagnosis, potential therapeutic approaches and new drug screening strategies in the future. CircRNAs having covalently closed circular forms are created from precursor mRNAs as 'backsplicing' (Z. Wu, Sun, Li, & Jin, 2019).CircRNAs may also exhibit bioactivity through binding with proteins. For instance Circ-Foxo3 was shown to be involved in cell cycle progression and proliferation through the formation of ternary complexes involving p21 and CDK2(Du et al., 2016). Although there were other significant advances in the following years, such as the first identification of circRNAs in humans and the finding of four circRNAs transcribed from the DCC (deleted in colorectal cancer) gene the significance of circRNAs was not well recognized until 2013. Before 2013 circRNAs were formerly thought to be the consequence of faulty pre-mRNA splicing. The advancement of RNA sequencing technologies (RNA-seq) and bioinformatics have revealed that circRNAs are very numerous and dynamically expressed in a wide range of eukaryotic cells. CircRNAs consist of three basic parts according to their structure: circRNA 'ecircRNA' originating from exons, 'ciRNA' formed by 3′,5′- or 2′,5′- phosphodiester bonds, 'EIciRNA' consisting of junction of exon-intron. EcircRNAs are the most numerous subclass of circRNAs, representing roughly 85 percent of all circRNAs. EcircRNAs are formed of solely exons, which might be one or more, although the majority of ecircRNAs have less than five exons(Q. Wu, Li, Wu, & Liu, 2019).Despite the fact that much of the research in this field has concentrated on the discovery of novel prognostic biomarkers, data shows that circRNAs have the capacity to distinguish between cancer subtypes(Kristensen, Jakobsen, Hager, & Kjems, 2022).In NSCLC, for instance, downregulation of circACVR2A and overexpression of circCCNB1 could be utilized to discriminate among squamous cell carcinoma and adenocarcinoma(C. Wang et al., 2019).Different circRNA expression patterns in breast cancer have been found to have the capacity to distinguish between ER+, HER2+, and triple negative subtypes of breast cancers(Nair et al., 2016).CircRNAs can be identified non-invasively and used as possible diagnostic candidates. Tan et al., for example, discovered F-circEA, a novel circRNA produced from the NSCLC-associated EML4-ALK fusion variant 3b. This circRNA's oncogenic role has been shown by researchers. They found this circRNA in plasma and emphasized that F-circEA has the potential to guide targeted therapy with approved ALK inhibitors for the

diagnosis of EML4-ALK-positive NSCLC(S. Tan et al., 2018).Numerous studies have shown that ciRS-7 exhibits oncogenic properties. It has been reported that miR-7 is the most common sponge miRNA of ciRS-7, which is understood to act as a sponge for many miRNAs. CiRS-7 has been found to be a poor prognostic marker for many types of cancer(Zou et al., 2020).CircRNAs may potentially be valuable in predicting therapy response, assisting clinicians in achieving optimal patient care. For example, it has been underlined that the response to endocrine treatment in breast and prostate malignancies may be expected by evaluating the expression levels of certain circRNAs. Overexpression of circCNOT2 has been linked to earlier progression with aromatase inhibitors in breast cancer. It has been reported that this circRNA can be identified in plasma and might be a promising option for monitoring therapeutic response(Smid et al., 2019).Given the difficulty of treating many forms of cancer after metastasis, it is evident that early identification of cancer is critical in reducing both morbidity and death. There have been tremendous improvements in the development of diagnostics based on different circulating proteins and extracellular tumor DNA.CircRNAs have the potential to be less invasive biomarkers for early cancer diagnosis due to their more stable tissue-specific expression. It has been demonstrated, for example, that plasma-derived circRNA biomarkers for early detection of HCC outperform -fetoprotein in identifying people with HBV-associated HCC, including both healthy and probable patients, from persons without this illness(Kristensen et al., 2022).CircRNAs are also common in serum exosomes and have been demonstrated to have promise for early diagnosis of colorectal cancer. Another study found that circHIPK3 and circSMARCA5circRNAs produced from serum extracellular vesicles, had a high potential for early detection of glioblastoma(Y. Li et al., 2015; Stella et al., 2021).

Despite strong evidence that circRNAs are essential in leukemia biosynthesis,maintenance, and development, a comprehensive analysis of circRNAs in leukemia is absent. In this chapter,we evaluated key leukemia research in order to better understand the activities and regulation mechanisms of circRNAs in leukemia.

The Relationship Between Circular RNAs and Acute Myeloid Leukemia

Acute Myeloid Leukemia**(**AML) is the most frequent kind of acute leukemia. Prevalence of AML rises with age, and individuals exhibit substantial biochemical and clinical variation. CircRNAs have recently been demonstrated to be valuable in AML studies. While the level of expression of circRNAs may be abnormal in leukemia cells, changed circRNAs may also be implicated in

leukemogenesis (Guarnerio et al., 2016). Cytogenetic and molecular genetic features are important prognostic variables for patients with AML. CircRNAs assist in understanding the pathogenic process of AML in particular circumstances. Due to the fact that certain genomic amplicons are related to a poor outcome, the structural features of amplicon development and their probable causes in AML remain unknown. Several research have looked into the possible utility of circRNAs in the diagnosis and prognosis of AML. Varying expression of particular circRNAs have been discovered to be informative in AML in alternative model types. Yi et al. found that circ-VIM was upregulated in AML individuals' bone marrow specimens using RT-qPCR compared to healthy individuals. High circ-VIM expression was foun to be associated with shorter leukemia-free and overall survival in multivariate analyses (Singh, Uddin, Zonder, Azmi, & Balasubramanian, 2021; Y. Y. Yi et al., 2019). circ-ANAPC7, circRNA-DLEU2, hsa_circ_100290, which has been demonstrated to be frequently upregulated and circ-Foxo3 which has been shown to be downregulated in AML sufferers, has been suggested as a potential indicator for the diagnosis of AML (Z. Wu et al., 2019; J. Zhou et al., 2019). In a microarray study, 147 overxpressed and 317 downregulated circRNAs were detected in six cytogenetically normal newly diagnosed AML individuals compared to four healthy controls. One of the essential and interesting results of this study was that Hsa_circ_000427 was down-regulated in AML patients at different treatment stages, and was down-regulated again in disease relapse, where it showed normal expression during remission (W. Li et al., 2017). Overexpressed circ-PTK2 was found in AML bone marrow samples as compared to the controls. Circ-PTK2 knockdown inhibited AML cell proliferation by diminishing the expression of cyclin D1 and BCL-2 and increasing the expression of the pro-apoptotic protein Bax. In-vivo studies have shown that circ-PTK2 sponges miR-330-5p, resulting in increased FOXM1 expression. Overexpression of circ-PTK2 was linked to a shorter survival rate in AML patients, probably due to its influence on cell proliferation and apoptosis (L. Yi, Zhou, Luo, & Yang, 2021).Gene fusions are formed by chromosomal rearrangements as a result of breaking both strands of the DNA helix. Fusion genes constitute the central class of somatic mutations in hematological cancers. AML1/ETO,MLL/AF9 and PML/RARa fusion proteins, which are considered high risk factors for AML and specific biomarkers for prognosis, are among the important and well-known fusion proteins(Chen & Zhou, 2012).Translocations have been proven in studies to produce fusion mRNAs as well as fusion circRNAs(Wang, Han, Sun, Chen, & Chen, 2019). Although their precise processes are unknown, fusion circRNAs have been found to be oncogenic in in

vitro and in vivo studies (Guarnerio et al., 2016).MLL genes are involved as partners in many fusion gene formations such as AF9 (MLLT3). Examples of fusion circRNAs consisting of the MLL gene are MLL/ENL, MLL/AF9 and MLL/AF4 (Dal Molin et al., 2019). The MLL/AF9 fusion has been reported to be highly expressed in AML. Presence of f-circM9_2 and f-circM9_1 was detected in THP1 cells by Sanger sequencing method. It has been shown that overexpression of F-circM9 in K562 cells suppressed apoptosis induced by cytarabine and arsenic trioxide(Guarnerio et al., 2016).

Circular RNA Studies on Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disease that is malignant, myeloproliferative, and myeloproliferative. The main genetic abnormality in the development of CML is the Philadelphia chromosome (Ph), which is formed as a result of reciprocal translocation between chromosomes 9 and 22. The BCR and ABL genes combine as a consequence of translocation to generate the BCR-ABL chimeric gene.The ABL gene normally encodes tyrosine kinase under strict regulation. BCR-ABL-encoded oncoprotein exhibits enhanced tyrosine kinase activity independent of biological regulation. Tyrosine kinase inhibitors (TKIs) that target the BCR-ABL oncoprotein are effective and first-line treatment for the majority of CML patients(Comert, Baran, & Saydam, 2013).Imatinib was the first BCR-ABL tyrosine kinase inhibitor to be utilized in clinical trials. Imatinib and the subsequent new generation TKIs (dasatinib, ponatinib, nilotinib, bosutinib) have dramatically improved overall survival and prognosis in the therapy of CML by managing the illness.Second and third generation TKIs are more efficient and specific than imatinib; they have a distinct pharmacological profile and sensitivity depending on patient and illness parameters such as comorbidities, stage of cancer, and BCR-ABL1 mutation type.In around 15% of individuals who acquire resistance, the underlying cause is still unclear, and much study is being conducted on this issue. TKIs are physically resistant to CML leukemic stem cells, according to recent research(Corbin et al., 2011).The function of circRNAs, particularly in the group with treatment resistance, may be essential in explaining the disease's molecular mechanism.

Hsa_circ_0058493 has been shown to be overexpressed in PBMCs from CML patients, and increased circ 0058493 level has been linked to poor clinical effect of imatinib. The Silencing of circ_0058493was shown to drastically decrease the development of imatinib-resistant CML cells. It has been revealed that miR-548b-3p, which has been identified as a potential target of circ_0058493 in bioinformatics databases, is overexpressed in CML cells where

circ_0058493is silenced. Furthermore, hsa circ 0058493 was shown to be significantly enriched in exosomes which derived from imatinib resistant CML cells (Zhong et al., 2021).

circHIPK3 was found to be considerably elevated in peripheral blood mononuclear cells from CML patients' serum samples when compared to the control group. A high elevation in circHIPK3 expression has been linked to a poor prognosis in CML patients. Following functional tests, it was revealed that circHIPK3 may have an oncogenic role in CML. According to the findings of the study it was emphasized that circHIPK3 may have a prominent participation in the therapy of CML (Feng et al., 2020).

Circ_0080145 Improves Imatinib Resistance in CML by Modulating the miR-326/PPFIA1 axis, according to Hong Che et al (Che, Ding, & Jia, 2020). F-circBA1, a new fusion circular RNA generated from the BCR-ABL fusion gene, was found to be oncogenic in CML cells (Y. Tan et al., 2021). Yao-Hua Lu et al. demonstrated that dysregulation of circ_0080145 and circ_0051886 is responsible for the establishment of IM chemoresistance in CML via controlling ABL1 expression via modulation of miR-203 and miR-637 expression.

In another investigation to detect differently expressed circRNAs in CML cells, circRNA sequencing was performed. Circ_100053 was found to be considerably up-regulated in peripheral blood mononuclear cells in CML serum samples compared to the healthy control group. This significant rise in circ_100053 expression has been linked to clinical stage and BCR/ABL mutant status. High_circ_100053 expression has been linked to a poor prognosis in CML patients as well as imatinib resistance. Circ_100053 was highlighted as a potential biomarker for CML patients (Ping, Jian-Jun, Chu-Shu, Guang-Hua, & Ming, 2019).

The Relationship Between Chronic Lymphocytic Leukemia and Circular RNAs

Chronic lymphocytic leukemia (CLL), the most prevalent kind of adult leukemia, is a kind of hematological malignancy that is distinguished by the monoclonal expansion of B lymphocytes in peripheral blood, lymphoid tissue, and bone marrow. CLL cell viability can be extended by both autocrine and survival cues from the microenvironment. Despite the fact that CLL patients have a good response rate to therapy, knowledge concerning the development and progression of CLL is still restricted(Xia et al., 2018). It is estimated that roughly 20% of CLL patients do not have chromosomal abnormalities. Epigenetic alterations and changes in ncRNAs have been proposed as potential causes of cancer severity(Lingua, Carrà, Maffeo, & Morotti, 2021).When the

literature is reviewed, it is observed that the number of research on circRNAs that have a role in the pathogenesis of CLL is remarkably low compared to other kinds of leukemia.Transcriptomic profiling of 21 de novo CLL cases indicated that 859 circRNAs expressed differently in CLL cells than in normal B cells(Raz, Granot, Pasmanik-Chor, Raanani, & Rozovski, 2020).The role of circ-CBFB in CLL was explored in one research, and it was shown that the expression of circ-CBFB was statistically substantially higher in CLL patients compared to the normal control group. The elimination of Circ-CBFB decreased CLL cell growth, interrupted cell cycle progression, and triggered cellular apoptosis, according to the findings of the study. Circ-CBFB has been shown to contribute to tumorigenesis via the miR-607/FZD3/Wnt/b-catenin axis. It has been emphasized that circ-CBFB may be a diagnostic and prognostic biomarker for CLL patients (Xia et al., 2018). In another study the downregulation of circ-0132266 has been demonstrated to increase cell viability in CLL via the miR-337-3p/PML axis (W. Wu et al., 2019).

Circular RNAs in Acute Lymphocytic Leukemia

Acute lymphocytic leukemia (ALL) is a kind of leukemia characterized by malignant transformation and proliferation of lymphoid progenitor cells found in the bone marrow, blood, and extramedullary areas. Approximately 80% of ALL cases are observed in children, and the condition has a devastating effect on adults.While dose-intensification approaches have resulted in dramatic improvements in pediatric treatment outcomes, the prognosis for the elderly continues quite poor. Despite the excellent return rate to induction chemotherapy, approximately 30-40% of adult ALL individuals will achieve long-term remission. The incidence of ALL in the United States is around 1.6 per 100,000 individuals(Terwilliger & Abdul-Hay, 2017).In an one year, it is predicted that over 6500 people will be diagnosed with ALL, and over 1400 will die from the disease(Paul, Kantarjian, & Jabbour, 2016).In the etiology of ALL, abnormal proliferation and differentiation of the clonal lymphoid cell population is notable. In a limited population of children, research have revealed that several genetic disorders such as Fanconi anemia, Down syndrome, and Bloom syndrome may play a role in vulnerability to ALL(German, 1997; Shah, John, & Sondhi, 2013).Ionizing radiation, some pesticides, the Epstein-Barr virus, and immunodeficiency have all been linked to an increased risk of ALL(Sehgal, Mujtaba, Gupta, Aggarwal, & Marwaha, 2010).However, the great majority of cases of the illness occur in previously healthy individuals. Although many chromosomal defects characterise ALL, they are insufficient to induce leukemia.In adults, Acute B-lymphocytic

leukemia (B-ALL) represents for 75% of cases, with Acute T-lymphocytic leukemia (T-ALL) accounting for the remaining instances(Terwilliger & Abdul-Hay, 2017).

B-ALL is a malignancy with a higher death rate in the absence of an appropriate treatment strategy(B. Zhou et al., 2022).The discovery of novel B-ALL diagnostic and prognostic indicators can facilitate the creation of innovative treatment approaches and medications that can enhance the survival results of B-ALL patients.T-ALL is distinguished by the uncontrollable development of T lymphoid tumor precursor cells in thebone marrow and thymus.During the development of T-ALL, leukocytosis grows and infiltrates lymph nodes and also other tissues such as the nervous system, and a mediastinal mass originating from the thymus appears.Recurrence is a key issue in T-ALL, which is less lethal than B-ALL(Zheng et al., 2021). In the work by Zhou et al., circRNAs were investigated in tissue samples from 6 patients with B-ALL and 6 individuals without ALL. They identified 263 circRNAs, 76 of which were up-regulated and 187 of which were down-regulated. According to enrichment analysis, both up-regulated and down-regulated circRNAs may be related with processes such as'macromolecule modification' and 'cellular protein modification.' Two of the down-regulated circRNAs (hsa circ 0000745 and chr15:8794959487966067) were revealed to be downregulated in both B-ALL samples and cell lines after confirmation using RTqPCR. One significant limitation of the study was that it did not analyze which miRNA-gene axis the identified circRNAs were related with(B. Zhou et al., 2022). In the study of Zheng et al., it was determined that miR-20a-5p expression decreased, while circPRKCI and SOX4 gene expression increased in clinical T-ALL samples. Silencing of circPRKCI was observed to suppress T-ALL cell viability. In the study, it was determined that there was a significant diminish in the luciferase activity of circPRKCI in T-ALL cells after mimic miR-20a-5p treatment. Silencing of circPRKCI was found to increase the expression level of miR-20a-5p in T-ALL cells. These findings support that circPRKCI may act as a sponge for miR-20a-5p. According to the findings of the study, it has been shown that circPRKCI may contribute to the malignant progression of T- ALL via the miR-20a-5p/SOX4 axis(Zheng et al., 2021).CircPVT1 located on chromosome 8q24, which is the cancer-related region where the Myc gene is also located, is one of the important circRNAs whose dysregulation has been reported in many cancers such as breast cancer, gastric cancer and colorectal cancer. CircPVT1 has been shown to be a prognostic marker for AML and gastric cancer (Hu et al., 2018; Kong et al., 2015; Takahashi et al., 2014; Zeng et al., 2015). Hu et al. reported that circPVT1 was overexpressed in ALL relative to normal bone marrow

samples in their investigation. It has been demonstrated that circPVT1 knockdown causes apoptosis in ALL cell lines by suppressing c-Myc and increasing the production of antiapoptotic Bcl-2 protein, resulting in cell growth inhibition(Hu et al., 2018).

CONCLUSION

According to research, circRNAs provide a variety of biological functions as sponges for miRNAs and proteins. These unique and intriguing molecules are common in cells, and these non-coding RNAs are evolutionarily conserved across species and have a stable structure. CircRNAs were considered to arise as byproducts in the early years, but these molecules have been discovered in a wide range of clinical processes in the recent years. After discovering the secret traits and abilities of circRNAs, investigations demonstrate that these mystery molecules can serve as perfect biomarkers in several pathologies.Because of their great stability and particular expression pattern, circRNAs have important prognostic or diagnostic significance. Understanding the molecular mechanisms of circRNAs in different transcriptional and translation pathways is critical for illuminating many human diseases, including cancer.Aside from the well-known role of circRNAs as miRNA sponges, more research is needed to uncover additional processes shown by circRNAs. Despite substantial research into circRNA expression, their role in normal physiological systems and human health remains unknown. Despite the fact that circRNAs have been found in a vast number of cancers including leukemia, the roles of circRNAs have yet to be completely identified. The exact mechanism of circRNA regulation in leukemia is unknown. It is unclear whether dysregulated circRNAs represent a main event or an epiphenomenon in pathogenesis of leukemia. Furthermore, the majority of research included bone marrow samples, with only a minority using peripheral blood samples. There has also been a deficiency of association studies between bone marrow specimens and peripheral blood (Z. Wu et al., 2019).

In this regard, it still has a long way to go before circRNAs can be employed as an effective and helpful therapeutic or prognostic biomarker in leukemia.

REFERENCES

1. Arber, D. A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M. J., Le Beau, M. M., . . . Vardiman, J. W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood, 127*(20), 2391-2405. doi:10.1182/blood-2016-03-643544
2. Che, H., Ding, H., & Jia, X. (2020). circ_0080145 Enhances Imatinib Resistance of Chronic Myeloid Leukemia by Regulating miR-326/PPFIA1 Axis. *Cancer Biother Radiopharm*. doi:10.1089/cbr.2020.3600
3. Chen, S. J., & Zhou, G. B. (2012). Targeted therapy: The new lease on life for acute promyelocytic leukemia, and beyond. *IUBMB Life, 64*(8), 671-675. doi:10.1002/iub.1055
4. Comert, M., Baran, Y., & Saydam, G. (2013). Changes in molecular biology of chronic myeloid leukemia in tyrosine kinase inhibitor era. *Am J Blood Res, 3*(3), 191-200.
5. Corbin, A. S., Agarwal, A., Loriaux, M., Cortes, J., Deininger, M. W., & Druker, B. J. (2011). Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. *J Clin Invest, 121*(1), 396-409. doi:10.1172/jci35721
6. Dal Molin, A., Bresolin, S., Gaffo, E., Tretti, C., Boldrin, E., Meyer, L. H., . . . Bortoluzzi, S. (2019). CircRNAs Are Here to Stay: A Perspective on the MLL Recombinome. *Front Genet, 10*, 88. doi:10.3389/fgene.2019.00088
7. Du, W. W., Yang, W., Liu, E., Yang, Z., Dhaliwal, P., & Yang, B. B. (2016). Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res, 44*(6), 2846-2858. doi:10.1093/nar/gkw027
8. Feng, X. Q., Nie, S. M., Huang, J. X., Li, T. L., Zhou, J. J., Wang, W., . . . Meng, F. J. (2020). Circular RNA circHIPK3 serves as a prognostic marker to promote chronic myeloid leukemia progression. *Neoplasma, 67*(1), 171-177. doi:10.4149/neo_2018_181129N908
9. German, J. (1997). Bloom's syndrome. XX. The first 100 cancers. *Cancer Genet Cytogenet, 93*(1), 100-106. doi:10.1016/s0165-4608(96)00336-6
10. Gharbaran, R., Park, J., Kim, C., Goy, A., & Suh, K. S. (2014). Circulating tumor cells in Hodgkin's lymphoma - a review of the spread of HL tumor cells or their putative precursors by lymphatic and hematogenous means, and their prognostic significance. *Crit Rev Oncol Hematol, 89*(3), 404-417. doi:10.1016/j.critrevonc.2013.09.004

11. Guarnerio, J., Bezzi, M., Jeong, J. C., Paffenholz, S. V., Berry, K., Naldini, M. M., . . . Pandolfi, P. P. (2016). Oncogenic Role of Fusion-circRNAs Derived from Cancer-Associated Chromosomal Translocations. *Cell, 165*(2), 289-302. doi:10.1016/j.cell.2016.03.020
12. Hu, J., Han, Q., Gu, Y., Ma, J., McGrath, M., Qiao, F., . . . Ge, Z. (2018). Circular RNA PVT1 expression and its roles in acute lymphoblastic leukemia. *Epigenomics, 10*(6), 723-732. doi:10.2217/epi-2017-0142
13. Kong, R., Zhang, E. B., Yin, D. D., You, L. H., Xu, T. P., Chen, W. M., . . . Zhang, Z. H. (2015). Long noncoding RNA PVT1 indicates a poor prognosis of gastric cancer and promotes cell proliferation through epigenetically regulating p15 and p16. *Mol Cancer, 14*, 82. doi:10.1186/s12943-015-0355-8
14. Kristensen, L. S., Jakobsen, T., Hager, H., & Kjems, J. (2022). The emerging roles of circRNAs in cancer and oncology. *Nat Rev Clin Oncol, 19*(3), 188-206. doi:10.1038/s41571-021-00585-y
15. Li, W., Zhong, C., Jiao, J., Li, P., Cui, B., Ji, C., & Ma, D. (2017). Characterization of hsa_circ_0004277 as a New Biomarker for Acute Myeloid Leukemia via Circular RNA Profile and Bioinformatics Analysis. *Int J Mol Sci, 18*(3). doi:10.3390/ijms18030597
16. Li, Y., Zheng, Q., Bao, C., Li, S., Guo, W., Zhao, J., . . . Huang, S. (2015). Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. *Cell Res, 25*(8), 981-984. doi:10.1038/cr.2015.82
17. Lingua, M. F., Carrà, G., Maffeo, B., & Morotti, A. (2021). Non-Coding RNAs: The "Dark Side Matter" of the CLL Universe. *Pharmaceuticals (Basel), 14*(2). doi:10.3390/ph14020168
18. Nair, A. A., Niu, N., Tang, X., Thompson, K. J., Wang, L., Kocher, J. P., . . . Kalari, K. R. (2016). Circular RNAs and their associations with breast cancer subtypes. *Oncotarget, 7*(49), 80967-80979. doi:10.18632/oncotarget.13134
19. Paul, S., Kantarjian, H., & Jabbour, E. J. (2016). Adult Acute Lymphoblastic Leukemia. *Mayo Clin Proc, 91*(11), 1645-1666. doi:10.1016/j.mayocp.2016.09.010
20. Ping, L., Jian-Jun, C., Chu-Shu, L., Guang-Hua, L., & Ming, Z. (2019). High circ_100053 predicts a poor outcome for chronic myeloid leukemia and is involved in imatinib resistance. *Oncol Res*. doi:10.3727/096504018x15412701483326
21. Raz, O., Granot, G., Pasmanik-Chor, M., Raanani, P., & Rozovski, U. (2020). Profiling and bioinformatics analyses reveal chronic lymphocytic

leukemia cells share a unique circular RNA expression pattern. *Exp Hematol, 85*, 8-12. doi:10.1016/j.exphem.2020.04.001

22. Sehgal, S., Mujtaba, S., Gupta, D., Aggarwal, R., & Marwaha, R. K. (2010). High incidence of Epstein Barr virus infection in childhood acute lymphocytic leukemia: a preliminary study. *Indian J Pathol Microbiol, 53*(1), 63-67. doi:10.4103/0377-4929.59186
23. Shah, A., John, B. M., & Sondhi, V. (2013). Acute lymphoblastic leukemia with treatment--naive Fanconi anemia. *Indian Pediatr, 50*(5), 508-510.
24. Singh, V., Uddin, M. H., Zonder, J. A., Azmi, A. S., & Balasubramanian, S. K. (2021). Circular RNAs in acute myeloid leukemia. *Mol Cancer, 20*(1), 149. doi:10.1186/s12943-021-01446-z
25. Smid, M., Wilting, S. M., Uhr, K., Rodríguez-González, F. G., de Weerd, V., Prager-Van der Smissen, W. J. C., . . . Martens, J. W. M. (2019). The circular RNome of primary breast cancer. *Genome Res, 29*(3), 356-366. doi:10.1101/gr.238121.118
26. Stella, M., Falzone, L., Caponnetto, A., Gattuso, G., Barbagallo, C., Battaglia, R., . . . Barbagallo, D. (2021). Serum Extracellular Vesicle-Derived circHIPK3 and circSMARCA5 Are Two Novel Diagnostic Biomarkers for Glioblastoma Multiforme. *Pharmaceuticals (Basel), 14*(7). doi:10.3390/ph14070618
27. Takahashi, Y., Sawada, G., Kurashige, J., Uchi, R., Matsumura, T., Ueo, H., . . . Mimori, K. (2014). Amplification of PVT-1 is involved in poor prognosis via apoptosis inhibition in colorectal cancers. *Br J Cancer, 110*(1), 164-171. doi:10.1038/bjc.2013.698
28. Tan, S., Gou, Q., Pu, W., Guo, C., Yang, Y., Wu, K., . . . Peng, Y. (2018). Circular RNA F-circEA produced from EML4-ALK fusion gene as a novel liquid biopsy biomarker for non-small cell lung cancer. *Cell Res, 28*(6), 693-695. doi:10.1038/s41422-018-0033-7
29. Tan, Y., Huang, Z., Wang, X., Dai, H., Jiang, G., & Feng, W. (2021). A novel fusion circular RNA F-circBA1 derived from the BCR-ABL fusion gene displayed an oncogenic role in chronic myeloid leukemia cells. *Bioengineered, 12*(1), 4816-4827. doi:10.1080/21655979.2021.1957749
30. Terwilliger, T., & Abdul-Hay, M. (2017). Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J, 7*(6), e577. doi:10.1038/bcj.2017.53
31. Wang, C., Tan, S., Liu, W. R., Lei, Q., Qiao, W., Wu, Y., . . . Li, W. (2019). RNA-Seq profiling of circular RNA in human lung

adenocarcinoma and squamous cell carcinoma. *Mol Cancer, 18*(1), 134. doi:10.1186/s12943-019-1061-8

32. Wang, W. T., Han, C., Sun, Y. M., Chen, T. Q., & Chen, Y. Q. (2019). Noncoding RNAs in cancer therapy resistance and targeted drug development. *J Hematol Oncol, 12*(1), 55. doi:10.1186/s13045-019-0748-z
33. Whiteley, A. E., Price, T. T., Cantelli, G., & Sipkins, D. A. (2021). Leukaemia: a model metastatic disease. *Nat Rev Cancer, 21*(7), 461-475. doi:10.1038/s41568-021-00355-z
34. Wu, Q., Li, P., Wu, M., & Liu, Q. (2019). Deregulation of Circular RNAs in Cancer From the Perspectives of Aberrant Biogenesis, Transport and Removal. *Front Genet, 10*, 16. doi:10.3389/fgene.2019.00016
35. Wu, W., Wu, Z., Xia, Y., Qin, S., Li, Y., Wu, J., . . . Li, J. (2019). Downregulation of circ_0132266 in chronic lymphocytic leukemia promoted cell viability through miR-337-3p/PML axis. *Aging (Albany NY), 11*(11), 3561-3573. doi:10.18632/aging.101997
36. Wu, Z., Sun, H., Li, J., & Jin, H. (2019). Circular RNAs in leukemia. *Aging (Albany NY), 11*(13), 4757-4771. doi:10.18632/aging.102091
37. Xia, L., Wu, L., Bao, J., Li, Q., Chen, X., Xia, H., & Xia, R. (2018). Circular RNA circ-CBFB promotes proliferation and inhibits apoptosis in chronic lymphocytic leukemia through regulating miR-607/FZD3/Wnt/β-catenin pathway. *Biochem Biophys Res Commun, 503*(1), 385-390. doi:10.1016/j.bbrc.2018.06.045
38. Yi, L., Zhou, L., Luo, J., & Yang, Q. (2021). Circ-PTK2 promotes the proliferation and suppressed the apoptosis of acute myeloid leukemia cells through targeting miR-330-5p/FOXM1 axis. *Blood Cells Mol Dis, 86*, 102506. doi:10.1016/j.bcmd.2020.102506
39. Yi, Y. Y., Yi, J., Zhu, X., Zhang, J., Zhou, J., Tang, X., . . . Deng, Z. Q. (2019). Circular RNA of vimentin expression as a valuable predictor for acute myeloid leukemia development and prognosis. *J Cell Physiol, 234*(4), 3711-3719. doi:10.1002/jcp.27145
40. Zeng, C., Yu, X., Lai, J., Yang, L., Chen, S., & Li, Y. (2015). Overexpression of the long non-coding RNA PVT1 is correlated with leukemic cell proliferation in acute promyelocytic leukemia. *J Hematol Oncol, 8*, 126. doi:10.1186/s13045-015-0223-4
41. Zheng, Y., Niu, B., Zhang, W., Ru, X., Gao, Y., Li, C., & Wu, X. (2021). Circular RNA circPRKCI contributes to malignant progression of T-cell acute lymphoblastic leukemia by modulating miR-20a-5p/SOX4 axis. *Aging (Albany NY), 13*(20), 23757-23768. doi:10.18632/aging.203647

42. Zhong, A. N., Yin, Y., Tang, B. J., Chen, L., Shen, H. W., Tan, Z. P., . . . Xu, P. (2021). CircRNA Microarray Profiling Reveals hsa_circ_0058493 as a Novel Biomarker for Imatinib-Resistant CML. *Front Pharmacol, 12*, 728916. doi:10.3389/fphar.2021.728916
43. Zhou, B., Zhong, L., Tian, L., Zhang, Y., Wang, R., He, Q., & Zhao, Y. (2022). Identification of two downregulated circRNAs in patients with acute B-lymphocytic leukemia. *Oncol Lett, 23*(1), 40. doi:10.3892/ol.2021.13158
44. Zhou, J., Zhou, L. Y., Tang, X., Zhang, J., Zhai, L. L., Yi, Y. Y., . . . Deng, Z. Q. (2019). Circ-Foxo3 is positively associated with the Foxo3 gene and leads to better prognosis of acute myeloid leukemia patients. *BMC Cancer, 19*(1), 930. doi:10.1186/s12885-019-5967-8
45. Zou, Y., Zheng, S., Deng, X., Yang, A., Kong, Y., Kohansal, M., . . . Xie, X. (2020). Diagnostic and prognostic value of circular RNA CDR1as/ciRS-7 for solid tumours: A systematic review and meta-analysis. *J Cell Mol Med, 24*(17), 9507-9517. doi:10.1111/jcmm.15619

Regulatory Role Of Irisin in Gestational Diabetes Mellitus

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Introduction

Gestational diabetes (GDM) is a glucose tolerance disorder that starts during pregnancy or is diagnosed for the first time during pregnancy (1, 2). Although its frequency varies from society to society, its incidence is increasing day by day. The most important reason for this is the increasing obesity worldwide. incidence and the decrease in threshold values in diagnostic tests (3).

The American Diabetes Association (ADA) (2), the World Health Organization (WHO) (4), and the International Federation of Gynecology and Obstetrics (5) recognize women with pre-pregnancy diabetes who were first diagnosed during pregnancy and women who experience temporary pregnancy-related insulin resistance. does not separate them. Therefore, these organizations include gestational diabetes; Diagnosed with an increase in glycemic index at 20 weeks or more of pregnancy they define it as intolerance (6).

The observation that diabetes can develop during pregnancy and resolve later dates back to the 19th century. The first published case diagnosed during pregnancy and resolved upon termination of pregnancy was published by Heinrich in 1828. Gottleib Made by Bennewitz . In this study, the patient's symptoms are described as exhibiting elevated hyperglycemia and excessive glucose production. Later, the term " gestational diabetes" was used by ER Carrington in 1957 (7).

obesity rates worldwide lead to increased diabetes rates in pregnancy (8). GDM is seen in 7% of all pregnancies. This rate varies between 1 and 22%, depending on the population and the diagnostic methods used. (9). International Diabetes Federation (International Diabetes According to Federation (IDF) 2017 data; Of women aged 20-79, 204 million have diabetes. It is estimated that this number will increase to 308 million in 2045. Women with gestational hyperglycemia are predominantly low- or middle-income individuals living in countries with limited maternal care. National Institute of Health and Clinical Excellence (The national Institute of Clinical Excellence (NICE) identifies additional risk factors such as maternal ethnicity, advanced maternal age, multiple pregnancy, and a history of GDM or macrosomia in a previous pregnancy. NICE guidelines recommend that women with any of these risk factors diabetes It recommends further diagnostic tests for diabetes mellitus (10).

GDM pathophysiology in pregnancy

In women with a healthy pregnancy, a series of physiological changes begin to occur in order to meet the demands of the growing fetus, such as nutrients and energy, along with some changes in the mother's body during the pregnancy

period. These physiological requirements include the respiratory, hematological, renal, cardiovascular and metabolic systems, as well as adaptation and maturation processes. One of the metabolic processes that occur after these requirements and changes is insulin sensitivity. This insulin sensitivity changes during the pregnancy period according to the requirements of pregnancy. In the early pregnancy period, insulin sensitivity increases. Later, in the later stages of midwifery, glucose is encouraged to be stored in fat stores in response to energy requirements (11).However, as pregnancy progresses, estrogen, progesterone , leptin , cortisol , placental Insulin resistance occurs with the increase in local and placental hormones , including lactogen and placental growth hormone (12). As a result, blood glucose rises slightly and this glucose is transported across the placenta to accelerate the growth of the fetus. The resulting mild insulin resistance increases the endogenous glucose production and the breakdown of fat stores, leading to an increase in glucose and free fatty acid concentrations in the blood (13).

During the fasting period in a normal pregnancy, the blood glucose level is 10-15% lower than in a non-pregnant woman. This is due to increased glycogen storage with pregnancy, peripheral glucose utilization, hepatic It occurs due to glucose production and increased glucose consumption by the fetus . maternal glucose is distributed to both mother and fetus, that is, the area where glucose is distributed is expanding. Due to insulin resistance, keeping glucose levels within normal limits can be achieved by the release of large amounts of insulin. Insulin and insulin-like growth factor-1; energy metabolism regulation , cellular proliferation , tissue development and differentiation are of vital importance (14). A subclinical metabolic disorder in women with normal pre-pregnancy blood glucose levels but who develop gestational diabetes later in pregnancy. considered to be dysfunctional . The 60% decrease in insulin sensitivity during normal pregnancy leads to hyperglycemia or GDM in these women. Maternal coexistence with gestational diabetes obesity ; maternal It is associated with increased inflammation in adipose tissue and placenta . Acute inflammation its effects on platelet indices are also mentioned (15).A response occurs by secreting anti-inflammatory cytokines such as leptin, adipokines, adiponectin, tumor necrosis factor-alpha (TNF-α), interleukin-6 from the adipose tissue.The placenta also shows a similar cytokine gene expression profile, with the exception of adiponectin. It is thought that inflammation caused by secreted cytokines may be associated with increased insulin resistance in pregnant women with gestational diabetes. GDM may develop if maternal pancreatic β-cells cannot secrete enough insulin to meet the increasing insulin requirement. (16).

Irisin

Irisin was first described as an exercise-induced myokine by Bostrom et al. at Harvard University in 2012. The name of the iris comes from the goddess named Iris, who was tasked with carrying messages to the gods in Greek mythology (17). Having a peptide structure of 112 amino acids , irisin is the proteolytic product of the type I membrane protein fibronectin type III domain 5 (FNDC5) . The FNDC5 gene encodes this membrane protein, and FNDC5 is stimulated by exercise. structure of FNDC5; It consists of a 29 - amino -acid signal peptide, a 94-amino acid chain, and the C-terminus, the region where the iris cleaves before it is secreted into the circulation. Although irisin is mainly secreted in skeletal muscle, it is also secreted from adipose tissue, pancreas, sebaceous glands and heart muscle (18). It has been observed that the iris is highly conserved in mammalian species, and it is stated that the structure of the iris is 100% similar in mice and humans. This similarity drops to 90% for glucagon , 85% for insulin and 83% for leptin (17).

First of all, irisin, which is found in skeletal muscle, is synthesized and secreted in tissues with different functions in the body as a result of research. Irisin has been seen mainly in skeletal muscle and adipose tissue, heart tissue, intratanial arteries, kidney, myelin sheath, neural cells, ovaries , Purkinje cells, rectum, salivary glands, sweat glands, stomach, testes, and tongue tissues (Figure 1) (19). The irisin level increases as a result of the proteolysis of FNDC5 from the specified tissues . Although there are conflicting results in studies, irisin has been reported to have many physiological properties such as weight loss, decrease in insulin resistance, association with obesity, glucose regulation and effects on lipid metabolism (20). Irisin is synthesized by the production and cleavage of protein 5 (FNDC5) containing the membrane protein fibronectin type III . Irisin enters the bloodstream and is converted to lipid and glucose in some organs, such as skeletal muscle, liver, and adipose tissue. metabolic participates in its regulation (21).

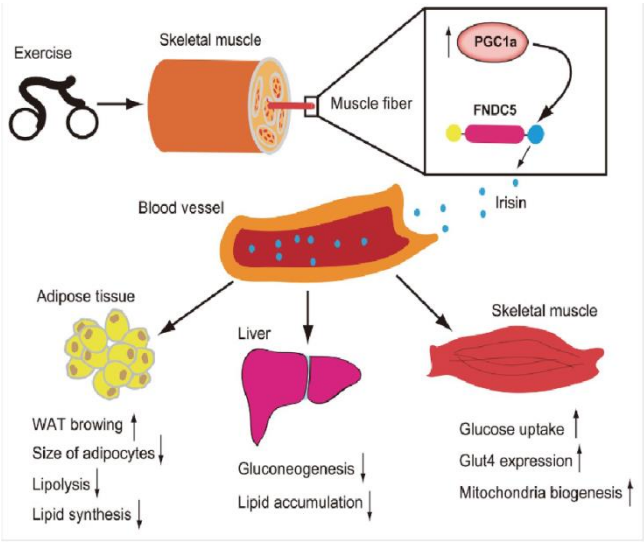

Figure 1. Regulation of the iris (21)

Irisin and Diabetes Mellitus

Irisin has numerous positive effects on glucose homeostasis and insulin sensitivity. Stimulates energy expenditure, glucose uptake and glycogenolysis; It reduces gluconeogenesis, adipogenesis , and lipid accumulation. While irisin levels are high in type 1 diabetes patients; found to be low in type 2 diabetes patients. There was a positive relationship with fasting blood sugar and a negative relationship with insulin sensitivity. Iris supplementation should be considered in the complications of diabetes and in the treatment of type 2 diabetes (22). Irisin acts as a messenger by sending signals to specific cells such as skeletal muscle, liver, pancreas, heart, fat and brain. With the positive regulation of irisin hormone in type 2 diabetes and insulin resistance, insulin receptor sensitivity in the heart and skeletal muscle increases. In addition, irisin, which is known to improve hepatic glucose and lipid metabolism and regulate pancreatic β-cell functions, shows that it does a great job in terms of energy by transforming white adipose tissue into brown adipose tissue (23). Two large-scale meta-analysis studies have shown that individuals with type 2 diabetes have lower irisin levels than healthy individuals (24, 25). A recent meta-analysis of 3667 participants also found that irisin levels were significantly lower in type 2 diabetes patients. Similarly, irisin levels were found to be lower in pregnant women with gestational diabetes compared to healthy pregnant women (26). When the relationship between diabetes and complications was evaluated, irisin levels were found to be significantly lower in patients with macroalbuminuria , and irisin was correlated with kidney function indicators

such as blood urea nitrogen (BUN) and creatinine (27). Physical activity is seen as the most effective component in the treatment of insulin resistance in type 2 diabetes, and irisin stimulated by exercise has a positive effect on metabolism and glucose tolerance (25). Contrary to type 2 diabetes, studies have shown that irisin levels are high in type 1 diabetes patients (28-30). This is attributed to the absence of insulin resistance and high physical activity levels in these patients (29). In many studies, irisin has a negative relationship with Hemoglobin A1c (HbA1c) and fasting blood glucose in type 2 diabetes patients (25, 31). However, in a study conducted in patients with type 1 diabetes, this relationship was positive. The positive relationship between irisin and HbA1c, which is an indicator of hyperglycemia , has been associated with a positive relationship between irisin and blood glucose (28). It has been observed that irisin has a negative correlation with age in type 1 diabetes (29). Faienza et al . conducted the first study investigating irisin levels in children with type 1 diabetes. As a result of this study, high irisin levels were seen in children with type 1 diabetes and a negative relationship between HbA1c and irisin was reached. It has been stated that high irisin levels provide better metabolic control in type 1 diabetes. It is stated that the difference in ELISA kits used in studies may lead to conflicting results (30). Due to the positive effects of irisin on insulin sensitivity, it has been predicted that it may be associated with the formation of gestational diabetes. Since irisin is located in the placenta during pregnancy, serum levels are higher during the entire pregnancy compared to non-pregnant women (32). Many studies have shown that the irisin level of pregnant women with gestational diabetes is lower than healthy pregnant women (33). In a study conducted in 60 healthy pregnant women with gestational diabetes, anthropometric and biochemical parameters and serum irisin levels were evaluated. Serum irisin levels were found to be significantly lower in patients with gestational diabetes compared to healthy pregnant women. However, the relationship of irisin with parameters related to gestational diabetes such as HbA1c, glucose, homeostatic model evaluation-insulin resistance (HOMA-IR) and BMI has not been reached (32).

Irisin in GDM

GDM, which is the most common metabolic disorder in pregnancy, is a disease that can lead to adverse outcomes such as congenital malformation and intrauterine death in infants. In mothers with a history of GDM, it can lead to diabetic complications, leading to nephropathy, neuropathy, and retinopathy. In addition, it leads to serious disorders such as hypoglycemia and diabetic ketoacidosis, resulting in serious consequences that can lead to increases in

morbidity and mortality. During pregnancy, many metabolic and hormonal changes are observed in which hormonal differences are effective. The main purpose of these fluctuations is to provide sufficient energy and food supplement to the fetus with the effect of hormones (33). It is known that gluconeogenesis increases in the first trimester, known as the anabolic period. With this increase, maternal protein, glycogen and fat stores are controlled and balanced. In addition to this balancing, insulin sensitivity increases and hypoglycemia tendency is revealed, followed by ketoacidosis. The second half of pregnancy is called the catabolic period and maternal metabolism is affected by placental hormones, causing some changes. Placental hormones begin to reduce carbohydrate use, while lipolysis begins to increase. Thus, maternal body fat mass decreases and insulin resistance comes into play (35, 36). Glucose and amino acids needed by the fetus begin to be stored, and glycerol, free fatty acids and ketones, which are the main energy content, begin to be used in the mother's metabolism. These metabolic products start to trigger insulin resistance as the gestational week progresses. Human placental lactogen (HPL), also known as anti-insulin, cortisol, growth hormone, progesterone and prolactin hormones are among the most important factors causing insulin resistance (37). As a response to the insulin resistance, hyperplasia begins to develop in pancreatic beta cells and insulin secretion gradually increases. This increase becomes such that it can no longer respond to secretion (38). The 112 amino acid peptide irisin hormone discovered by Bostrom et al. is considered one of the hormones involved in energy metabolism. With exercise, irisin, which is synthesized from muscle tissue, transforms white adipose tissue into brown adipose tissue and releases this energy in the form of heat (17). Irisin, which emerges with the effect of exercise, causes a decrease in insulin resistance and positively affects glucose and lipid metabolism. In studies, irisin, which is said to have a protective role in nutritional obesity and diabetes in experimental animals, has been the subject of research in recent years (39, 40). This study aims to examine in depth irisin, a new hormone involved in energy metabolism in patients with GDM.

Conclusion and Recommendations

Many changes occur in the mother during pregnancy to provide sufficient energy to the fetus. Anabolic processes continue during the first trimester, characterized by increased gluconeogenesis, maternal protein, glycogen and fat stores. In order to meet the needs of the growing fetus in the first trimester, the stored energy is conserved for later use. During pregnancy, fasting blood glucose levels decrease and peripheral glucose use and consumption increase.

The catabolic period, the second half of pregnancy, is a period characterized by increased HPL secreted by syncytiotrophoblasts. Along with HPL, lipolysis increases in adipose tissue and amino acid and glucose biomolecules needed by the fetus are stored. One of the hormones responsible for insulin resistance, HPL disrupts the glucose uptake of cells, as does the effect of other hormones such as progesterone, cortisol and prolactin. However, this deterioration does not lead to a decrease in insulin receptors during pregnancy, unlike type 2 diabetes. However, pregnancies complicated by diabetes are risky pregnancies that require close follow-up from both fetal and maternal aspects. When adequate glycemic control is not achieved, it can cause congenital malformations and intrauterine death in the baby. It is a metabolic disease that can cause morbidity and mortality due to increased maternal hypoglycemia, diabetic ketoacidosis, retinopathy and nephropathy. This study, which draws attention to the close relationship between irisin and gestational diabetes, is needed for larger studies in the future.

The hormone irisin, whose function has been discovered recently, has different effects on metabolism. Irisin, obesity, T2DM, GDM and metabolic A clear analysis of the effects of metabolic disorders such as syndrome will be hopeful for the prevention and treatment of diseases. Therefore, understanding the effect of irisin will certainly help in early diagnosis and appropriate treatment of GDM, reducing adverse maternal and fetal outcomes, and protecting mothers and babies from long-term complications. More human clinical studies should be included for the effect of irisin, the mechanism of which has not yet been fully elucidated in GDM studies.

REFERENCES

1. National Diabetes Data Group. 1979, Classification and diagnosis of diabetes and other categories of glucose intolerance, *Diabetes*, 28:1039-57.
2. American Diabetes Association. 2011, Diagnosis and classification of diabetes mellitus, *Diabetes Care*, 34(Suppl 1):S62- S69.
3. The American College of Obstetricians and Gynocologists. 2013, Gestational diabetes mellitus, *Practice Bullletin*, 122: 406-16.
4. World Health Organization, 2013, Diagnostic Criteria and Classification of Hyperglicaemia First Detected in Pregnancy.
5. (FIGO), International Federation of Gynecology and Obstetrics. 2015, Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. *International Journal of Gynecology & Obstetrics*.
6. Denney, J.M., Quinn, K.H. 2018, Gestational Diabetes: Underpinning Principles, Surveillance, and Management, *Obstetrics and Gynecology Clinics of North America*, 45, 2, s: 299-314.
7. McIntyre, H.D., Colagiuri, S., Roglic, G., Hod, M. 2015, "Diagnosis of GDM: A suggested consensus," *Best Practice & Research Clinical Obstetrics & Gynaecology*, vol. 29, no. 2, s: 194-205.
8. Feig, D.S., Corcoy, R, Jensen, D.M., Kautzky-Willer, A., Nolan C.J., Oats J.J., Sacks D.A., Caimari F, McIntyre H.D. 2015, "Diabetes in pregnancy outcomes: A systematic review and proposed codification of definitions," *Diabetes Metab Res Rev*, vol. 31, s: 680–690.
9. American Diabetes Association. 2012, "Standards of medical care in diabetes," *Diabetes Care*, vol. 35, no. 1.
10. Piper, L.K., Stewart, Z., Murphy, H.R. 2017, Gestational diabetes, Obstetrıcs, *Gynaecology And Reproductıve Medıcıne*,.
11. Di Cianni, G., Miccoli, R., Volpe, L., Lencioni, C. 2003, Intermediate metabolism in normal pregnancy and in gestational diabetes, *Diabetes/Metabolism Research and Review*., no. 19, s: 259-270.
12. Catalano, P., Tyzbir, E., Roman, N., Amini, S., Sims, E. 1991, Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women, *American Journal of Obstetrics & Gynecology*, no. 165, s: 1667–1672.
13. Phelps, R., Metzger, B., Freinkel, N. 1981, Carbohydrate metabolism in pregnancy: XVII. Diurnal profiles of plasma glucose, insulin, free fatty acids, triglycerides, cholesterol, and individual amino acids in late normal

pregnancy., *American Journal of Obstetrics & Gynecology*, no. 140, s: 730-736.
14. Oğuz, A. 2016, Gestasyonel Diyabet, *Kahramanmaraş Sütçü İmam Üniversitesi Tıp Fakültesi Dergisi*, 11, 1.
15. Tahiroğlu, V., Kara, F. 2022, Effects of acute inflammation on platelet indices: Anexperimental study, *Baghdad journal of biochemistry andapplied biological sciences*, vol. 3, 210-219.
16. Öztürk, F.Y., Altuntaş, Y. 2015, Gestasyonel Diabetes Mellitus, *Şişli Etfal Hastanesi Tıp Bülteni*, cilt 49, no. 1, 2015.
17. Boström, P., Wu, J., Jedrychowski, M.P., Korde, A., Ye, L., Lo, J.C, et al. 2012, A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis, *Nature*, 481(7382): 463–468. https://doi.org/10.1038/nature10777.
18. Martinez Munoz, I.Y., Camarillo Romero, E., Garduno Garcia, J.J. 2018, Irisin a Novel Metabolic Biomarker: Present Knowledge and Future Directions, *International Journal of Endocrinology*, 2018; 7816806. https://doi.org/10.1155/2018/7816806.
19. Aydın, S. 2014, Three new players in energy regulation: preptin, adropin and irisin, *Peptides*, 56,94-110.
20. Arhire, L.I., Mihalache, L., Covasa, M. 2019, Irisin: A hope in understanding and managing obesity and metabolic syndrome, *Frontiers in Endocrinology*, 10,524.
21. Ma, C., Ding, H., Deng, Y., Liu, H., Xion, X. 2021, Irisin: a new code uncover the relationship of skeletal muscle and cardiovascular health during exercise, *Frontiers in physiology*, 12:620608. https://doi.org/10.3389/fphys.2021.620608
22. Polyzos, S,A., Anastasilakis, A.D., Efstathiadou, Z.A., Makras, P., Perakakis, N., Kountouras, J., et al. 2018, Irisin in metabolic diseases, *Endocrine,* 59(2): 260–274. https://doi.org/10.1007/s12020-017-1476-1.
23. Gizaw, M., Anandakumar, P., Debela, T. 2017, A Review on the Role of Irisin in Insulin Resistance and Type 2 Diabetes Mellitus, *Journal of pharmacopuncture*, 20(4): 235–242. https://doi.org/10.3831/KPI.2017.20.029 (Retraction published J Pharmacopuncture. 2020 Mar 31; 23(1): 42-43).
24. Zhang, C., Ding, Z., Lv, G., Li, J., Zhou, P., Zhang, J. 2016, Lower irisin level in patients with type 2 diabetes mellitus: a case-control study and meta-analysis, *J. Diabetes*, 8: 56–62. doi: 10.1111/1753-0407.12256.

25. Du, X.L., Jiang, W.X., Lv, Z.T. 2016, Lower circulating irisin level in patients with diabetes mellitus: a systematic review and metaanalysis, *Horm. Metab*, 48: 644–652 doi: 10.1055/s0042-108730.
26. Song, R., Zhao, X., Zhang, D.Q., Wang, R., Feng, Y. 2021, Lower levels of irisin in patients with type 2 diabetes mellitus: A metaanalysis, *Diabetes Research and Clinical Practice*, 175: 108788.
27. Hu, W., Wang, R., Li, J., Zhang, J., Wang, W. 2016, Association of irisin concentrations with the presence of diabetic nephropathy and retinopathy, *Annals of clinical biochemistry*, 53(1): 67–74. https://doi.org/10.1177/0004563215582072.
28. Ates, I., Arikan, M.F., Erdogan, K., Kaplan, M., Yuksel, M., Topcuoglu, C., Yilmaz, N., et al. 2017, Factors associated with increased irisin levels in the type 1 diabetes mellitus, *Endocr. Regul*, 51(1): 1–7. doi: 10.1515/enr2017-0001.
29. Espes, D, Lau, J., Carlsson, P.O. 2015, Increased levels of irisin in people with long-standing type 1 diabetes, *Diabet. Med*., 32: 1172–1176. doi: 10.1111/dme.12731.
30. Faienza, M.F., Brunetti, G., Sanesi, L., Colaianni, G., Celi, M., Piacente, L. et al. 2018, High irisin levels are associated with better glycemic control and bone health in children with Type 1 diabetes, *Diabetes Research and Clinical practice*, 141: 10-17. doi: 10.1016/j.diabres.2018.03.046
31. Choi, Y.K., Kim, M.K.., Bae, K.H., Seo, H.A., Jeong, J.Y., Lee, W.K. et al. 2013, Serum irisin levels in newonset type 2 diabetes, *Diabetes Res Clin Pract*, 2013; 100: 96–101. doi: 10.1016/j.diabres.2013.01.007
32. Al-Ghazali, M.J., Ali, H.A., Al-Rufaie., M.M. 2020, Serum irisin levels as a potential marker for diagnosis of gestational diabetes mellitus, *Acta bio-medica :Atenei Parmensis*, 91(1): 56–63. https://doi.org/10.23750/abm.v91i1.7675.
33. Zhao, L., Li, J., Li, Z.L., Yang, J., Li, M.L., Wang, G.L. 2015, Circulating irisin is lower in gestational diabetes mellitus, *EndocrJ*, 62: 921– 926.
34. Kahraman, G., Kahraman, M., Oterkus, M. 2021, Investigation of the Level of Serum Irisin in Patients With Gestational Diabetes Mellitus, *Kafkas J Med Sci,* 11(3):360–364. doi: 10.5505/kjms.2021.97947
35. Barbour, L.A., McCundy, C.E., Hernandez, T.L. 2007, Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes, *Diabetes Care*, 30:112–119.

36. Butte, N.F. 2000, Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus, *Am J Clin Nutr*, 71:1256–1261.
37. Friedman, J.E., Ishizuka, T., Shao, J., Huston, L., Highman, T., Catalano, P. 1999, Impaired glucose transport and insulin receptor tyrosine phosphorylation in skeletal muscle from obese women with gestational diabetes*,Diabetes*, 48:1807–1814.
38. Yamashita, H., Shao, J., Friedman, J.E. 2000, Physiologic and molecular alterations in carbohydrate metabolism during pregnancy and gestational diabetes mellitus, *Clin Obstet Gynecol*, 43:87– 98.
39. Timmons, J.A., Baar, K., Davidsen, P.K., Atherton, P.J. 2012, Is irisin a human exercise gene? *Nature*, 488:9–10.
40. Aydin, S., Kuloglu, T., et al. 2013, Copeptin, adropin and irisin concentrations inbreast milk and plasma of healthy women and those with gestational diabetes mellitus, *Peptides,* 47:66– 70.

The Relationship Between Idiopathic Pulmonary Fibrosis (Ipf) and Cancer And Perioperative Atifibrotic Treatment

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The relationship between interstitial lung diseases (ILD) and cancer is an issue that needs to be addressed from different perspectives. In particular, when considering the association of fibrotic ILD with cancer, the relationship with both lung and non-lung cancers should be emphasized. There are many reports of the close association between lung cancer and ILD. Although less researched, we know that ILD is also associated with non-pulmonary cancers. Idiopathic pulmonary fibrosis (IPF) is very ımportant because it is one of the most common fıbrotıc ILDs and has sıgnıfıcant relatıonshıp wıth cancer. In a large cohort conducted on this subject, the overall cancer incidence in 25,241 patients with IPF was found to be 29.0 cases/1000 person-years. Cancer was detected significantly more frequently in the IPF group than in the non-IPF group. Lung cancer has the highest incidence among all cancer and IPF patients, followed by lymphoma, skin, uterus, cervical cancers, multiple myeloma, thyroid cancer, leukemia, pancreatic, liver and prostate cancers. The high incidence of cancer is most likely due to the local and systemic consequences of inflammatory and fibrotic processes, as well as the effect of weakening the antineoplastic defense mechanisms of immunomodulatory drugs, which are still frequently used in the treatment of ILD. In patients with fibrotic ILD, the risk of lung cancer was found to be approximately five times higher even in examinationsindependent of common risk factors such as advanced age and smoking history. The most common form is squamous cell carcinoma. Lung cancer is difficult to diagnose and manage in the ILD population. This difficulty contributes to the lower overall survival in patients with ILD with lung cancer than in those with lung cancer with ILD (1, 2).

Patients with IPF have an increased risk of cancer compared to the general population. The most common type of cancer in these patients is lung malignancy. The fact that IPF and cancer have common etiopathogenetic factors is considered to be the most basic reason for their association. Along with the development of cancer, indications for surgical resection emerge in these patients. Although surgical resection under antifibrotic therapy has been discussed in terms of risk-benefit, the importance of an individualized approach has come to the fore considering that surgery can trigger acute exacerbations in IPF patients (1, 2). In the two cases we will present here, we aimed to discuss the relationship between IPF and cancer and the continuation of perioperative atifibrotic treatments.

CASE 1

A 69-year-old male patient was diagnosed with IPF in 2011 and has been followed-up under Pirfenidone treatment for the last 3 years. The clinical,

laboratory and radiological findings of the patient were checked every 3 months. With clinical findings, existing or newly developing symptoms and signs, dyspnea scoring, drug side effects; exercise capacity, pulmonary function tests, oxygen saturation measurement, 6-minute walking test; radiological findings were evaluated with posteroanterior chest radiography. Computed tomography (CT) was performed on the patient after a suspicious nodular lesion was observed in the middle zone of the right lung in the routine examinations performed in the 3rd year of his treatment (Figure 1). Oncological Positron Emission-Computed Tomography (PET-CT) was performed for the suspicion of malignancy and staging of the patient, after a 29x28mm solid nodule, which was not found in previous CT scans, was observed in the medial segment of the right lung middle lobe on CT (Figure 2). In PET-CT, increased FDG uptake (SUV Max: 10.1) was observed in the macrolobule contoured mass. FDG uptake in other body areas was within physiological limits. CT guided transthoracic tru-cut needle aspiration biopsy was performed with the diagnosis of primary lung cancer in the patient (Figure 3). The patient, who was found to have "malignant giant cell tumor" as a result of the pathology, was evaluated in the tumor council with the diagnosis of Stage 1A3 (T1c, N0, M0) lung cancer according to TNM staging, and surgical resection of the tumor was deemed appropriate. The current condition of the patient was evaluated before the surgical treatment, and Pirfenidone treatment was continued. Wedge resection procedure was applied to the patient. Since he could not take oral medication on the day of the operation and on the first postoperative day, the treatment was interrupted. After oral intake was opened in the postoperative period, his general condition and radiological findings were evaluated as stable, and Pirfenidone treatment was continued (Figure 4). In this process, no additional problem was encountered regarding the patient's IPF picture. No complications were encountered in the thoracotomy area and during the surgical wound healing process. No bleeding or delay in wound healing was observed. There was no exacerbation. Pirfenidone treatment of the patient was continued (Figure 1).

CASE 2

A 76-year-old male patient who had been operated on with the diagnosis of colon cancer 3 years ago was consulted in our clinic with the radiological evaluation for tumor metastasis screening. After the usual pattern of interstitial pneumonia was detected on thorax CT, connective tissue and environmental diseases were excluded, and a diagnosis of IPF was made (Figure 5). The patient was evaluated in terms of abdominal surgery and bleeding risk, and

Pirfenidone treatment was started. In the routine follow-up of the patient, a nodular lesion suspicious for malignancy was observed in the paravertebral area in the superior segment of the left lung lower lobe (Figure 6). The patient, who also had a history of rectal cancer, underwent CT-guided transthoracic fine needle aspiration biopsy for the differential diagnosis of second primary/metastasis. As a result of the biopsy, the patient was diagnosed with "squamous cell lung carcinoma". PET-CT, which was taken for the purpose of staging the second primary lung cancer and control of operated colon cancer, revealed pathological FDG uptake in the ascending colon as well as the lesion in the lung (Figure 7). Recurrence of adenocarcinoma was reported in biopsies taken from the colon by colonoscopy. Chemotherapy decision was given to the patient in the tumor council. During the chemotherapy period, pirfenidone treatment was continued. In the follow-up of the patient, an indication for open abdominal surgery occurred due to ileus. Considering the risk of exacerbation of the patient during the surgery, pirfenidone treatment was continued perioperatively. No complications related to wound healing or exacerbation of IPF developed in the postoperative period.

DISCUSSION

IPF patients have a high risk of cancer. It is known to increase the risk of developing lung cancer in IPF by 7% to 20% (3). Among these cancers, it is most frequently associated with squamous cell lung cancer. IPF and cancer share common risk factors such as smoking, chronic tissue damage, viral infection, and environmental exposure. In addition, these two clinical pictures; It also has pathogenic similarities such as genetic and epigenetic changes, altered cell-cell communication, abnormal activation of signal transduction pathways, uncontrolled proliferation, resistance to apoptosis and tissue invasion (4). A population-based cohort study conducted in the Republic of Korea examined the incidence of cancer in 25,241 patients with IPF and 75,723 matched controls. It was reported that the overall incidence of cancer in patients with IPF was found to be significantly higher than the control group (13.89 cases per 1000 person-years), with 29 cases per 1000 person-years compared to the control group. According to this cohort, patients with IPF had the highest incidence of lung cancer, followed by lymphoma, skin cancer, cervical cancer, multiple myeloma, thyroid cancer, leukemia, pancreatic cancer, liver cancer, and prostate cancer. In this study, it was stated that IPF is a cancer risk factor independent of smoking in the comparison adjusted for smoking status in order to prevent the carcinogenic effect of smoking from affecting the results. It is stated that this may be a trigger of chronic inflammation in IPF. In addition;

Myofibroblast activation, endoplasmic reticulum stress, changes in the expression of growth factors, oxidative stress, and dense parenchymal fibrosis in IPF have also been associated with this condition (1).

The development of lung cancer in the follow-up of IPF patients is an issue that should be carefully considered. In addition to those who think that control CT is not necessary in the routine follow-up of the patients, there are also those who argue that it is clinically more appropriate to follow up with low-dose CT at least once a year due to the increased risk of cancer. At this point, the individualized approach seems quite appropriate. There are studies showing that Pirfenidone, which is used in the treatment of IPF, reduces the risk of lung cancer development in these patients (5). Another antifibrotic, Nintedanib, is an agent that also plays a role in the treatment of non-small cell lung cancers (6). In the follow-up of the patient, the necessity of surgical treatment may arise when the diagnosis of newly developing lung cancer is made. At this point, the issue of maintenance of antifibrotic treatments in the pre- and post-surgical period was examined. In the study of Kanayama et al. (7), it was stated that there were no complications related to the use of perioperative pirfenidone and the risk of postoperative acute exacerbation was reduced. Surgical procedures in patients with IPF cause an acute exacerbation risk in approximately 20% of cases. It is known that approximately 50% of these exacerbations result in death. In addition, no adverse effects were found in terms of wound healing or bleeding associated with perioperative use of Pirfenidone in lung surgery, including lung transplantation (8, 9). Considering these points, it is important to continue the treatment of these patients in the perioperative period. Perioperative complications and exacerbations were not observed in the two patients we presented here.

In IPF patients, the risk of cancer outside of the lung also increases. In this regard, indications for extrathoracic surgery may also arise. The number of studies examining the effects of major abdominal surgery and antifibrotics is limited. However, in general, it has been reported that the perioperative use of pirfenidone during abdominal surgery does not cause complications, and reduces the risk of exacerbation and peritoneal adhesions (8, 9). Due to its antiangiogenic effect on vascular endothelial growth factor (VEGF), nintedanib should not be used during abdominal surgery and for 4 weeks postoperatively (10). Although there are no large studies on this subject, cases of increased risk of bleeding and perforation have been reported. On the other hand, larger studies are needed to clarify this issue and to understand the clinical benefit/loss ratio for the patient.

REFERENCES

1. Lee HY, Lee J, Lee C, Han K, Choi SM. Risk of cancer incidence in patients with idiopathic pulmonary fibrosis: A nationwide cohort study. Respirology. July 22 2020.
2. Sebastiano
   Emanuele Torrisi, Mauro Pavone, Ada Vancheri, Carlo Vancheri. When to start and when to stop antifibrotic therapies? European Respiratory Review Sep 2017;26(145):170053.
3. Raghu G, Amatto VC, Behr J, Stowasser S. Comorbidities in idiopathic pulmonary fibrosis patients: a systematic literature review. Eur Respir J 2015;46:1113-30.
4. Vancheri C. Common pathways in idiopathic pulmonary fibrosis and cancer. Eur Respir Rev 2013;22:265-2.
5. Iwata T, Yoshino I, Yoshida S, et al. A phase II trial evaluating the efficacy and safety of perioperative pirfenidone for prevention of acute exacerbation of idiopathic pulmonary fibrosis in lung cancer patients undergoing pulmonary resection: West Japan Oncology Group 6711 L (PEOPLE Study). Respir Res 2016;17:90.
6. Shiratori T, Tanaka H, Tabe C, Tsuchiya J, Ishioka Y, Itoga M, Taima K, Takanashi S, Tasaka S. Effect of nintedanib on non-small cell lung cancer in a patient with idiopathic pulmonary fibrosis: A case report and literature review. Thorac Cancer. 2020;11(6):1720-3.
7. Kanayama M, Mori M, Matsumiya H, Taira A, Shinohara S, Kuwata T, Imanishi N, Yoneda K, Kuroda K, Tanaka F. Perioperative pirfenidone treatment for lung cancer patients with idiopathic pulmonary fibrosis. Surg Today. 2020;50(5):469-74.
8. Mackintosh JA, Munsif M, Ranzenbacher L, Thomson C, Musk M, Snell G, Glanville A, Chambers DC, Hopkins P. Risk of anastomotic dehiscence in patients with pulmonary fibrosis transplanted while receiving anti-fibrotics: Experience of the Australian Lung Transplant Collaborative. J Heart Lung Transplant. 2019;38(5):553-9.
9. Bayhan Z, Zeren S, Kocak FE, Kocak C, Akcılar R, Kargı E, Tiryaki C, Yaylak F, Akcılar A. Antiadhesive and anti-inflammatory effects of pirfenidone in postoperative intra-abdominal adhesion in an experimental rat model. J Surg Res. 2016;201(2):348-55.
10. https://www.ema.europa.eu/en/documents/product-information/ofev-epar-product-information_en.pdf (erişim tarihi: 24.06.2021).

Figure 1. Tomography findings of the patient. A. Thoracic tomography taken during follow-up after diagnosis, B. Detection of a mass in the medial segment of the right lung middle lobe, C. Postoperative tomography.

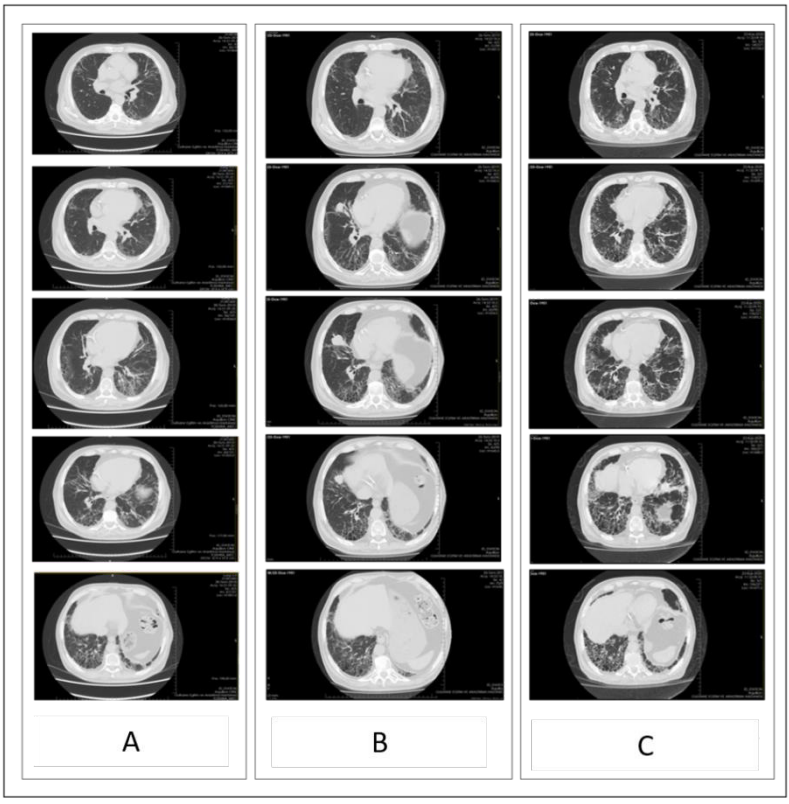


Figure 2. Tomography and Oncological Positron Emission-Computed Tomography (PET-CT) image of the mass in the medial segment of the right lung middle lobe.

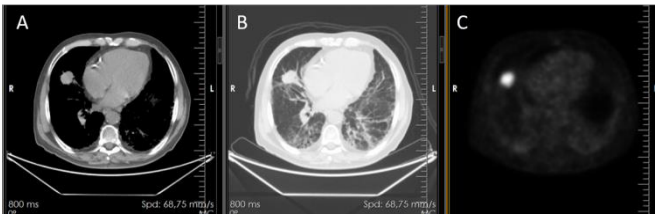


Figure 3. Tomography-guided transthoracic tru-cut needle aspiration biopsy procedure.

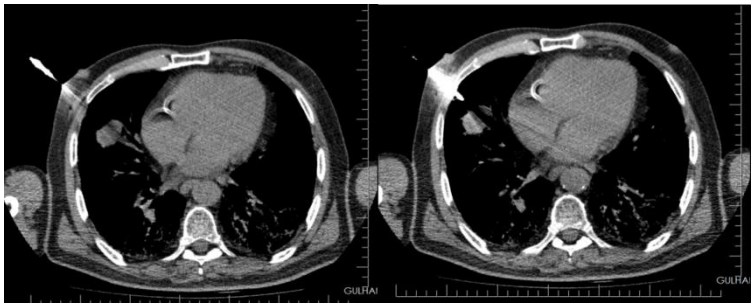


Figure 4. Chest X-ray taken on the 3rd postoperative day.

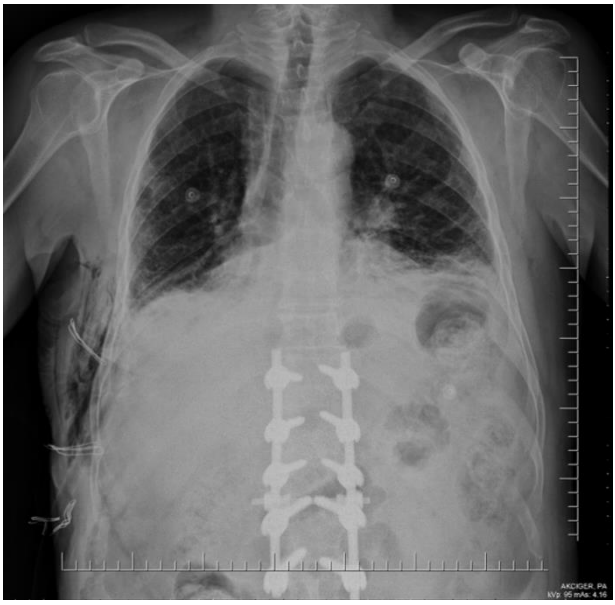


Figure 5. Tomography images taken during the patient's stable follow-up period.

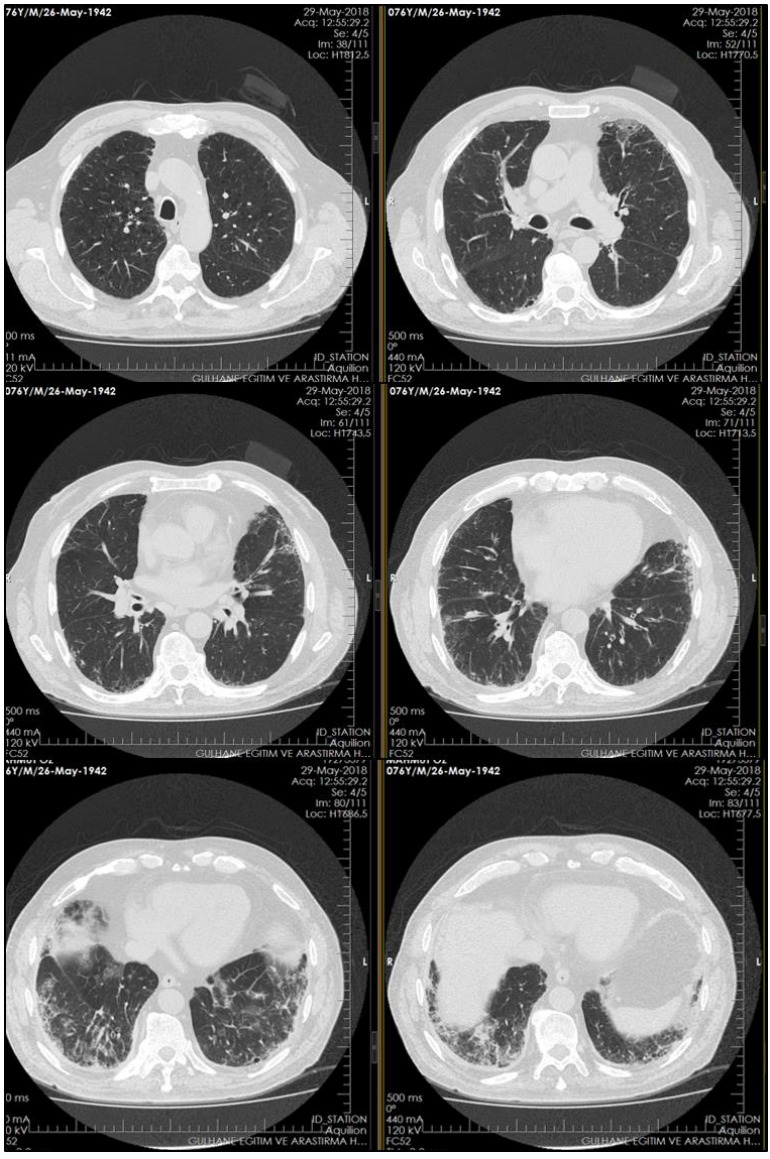


Figure 6. The course of the nodule (squamous cell lung carcinoma) detected during the follow-up of the patient.

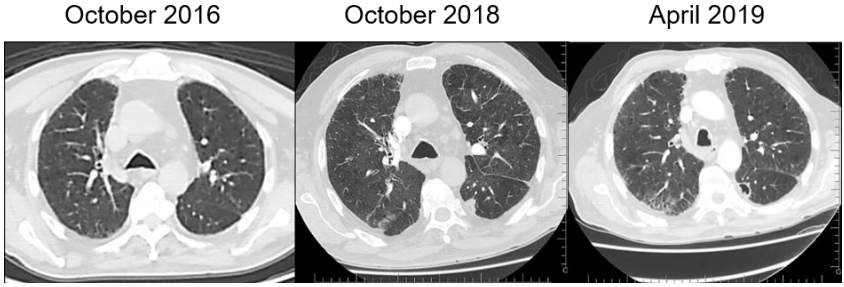
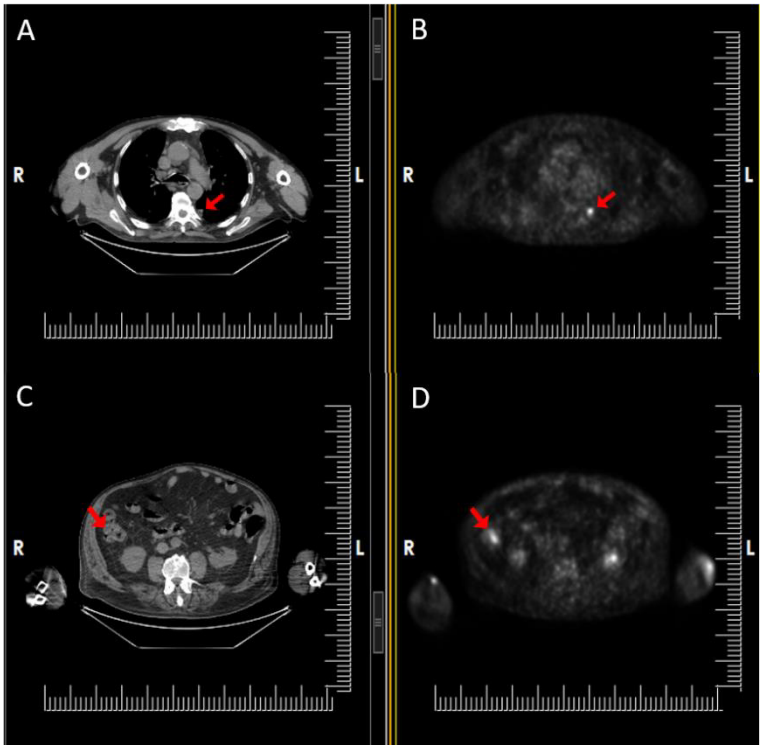


Figure 7. Oncologic Positron Emission-Computed Tomography (PET-CT) images of squamous cell lung carcinoma (A, B) in the superior segment of the left lung lower lobe and adenocarcinoma (C, D) in the ascending colon.



Glass Ionomer Cements in Pediatric Dentistry

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Introduction

Traditional glass ionomer cements, resin modified glass ionomer cements, compomers, and resin composites are among the restorative materials frequently used in pediatric dentistry.[1] Although it was considered the gold standard in restorative dentistry in the past, amalgam has been used today due to the potential toxicity of mercury and the need for excessive tissue loss during cavity preparation. [2,3] For this reason, glass ionomer and resin-based materials are widely used, in accordance with the concept of minimally invasive dentistry, as they provide long-term lifetime and high durability, as well as meeting the aesthetic demands of patients.1 Glass ionomer cements are easy to apply, It is one of the indispensable restorative materials used in pediatric dentistry due to its ability to bond to dental tissues by chemical bonding, advanced aesthetic properties, low toxicity and being a fluorine reservoir.[4,5]

Glass Ionomer Cements

Glass ionomer cements, which can also be called glass polyalkenoate cements and polyalkenoate cements, developed by Wilson and Kent in 1972, have been used in pediatric dentistry in various fields since the late 1970s.[6,7]

Glass ionomer cements, which are the result of combining the advanced properties of silicate cements and polycarboxylate cements; They show various properties such as being a fluorine reservoir and chemically bonding to dental hard tissues.[8,9] It was developed after it was introduced as ASPA (Alumino-Silicate-Polyacrylic-Acid) in 1972 by Wilson and Kent[10] and has taken its place among the restorative materials that are frequently used today.Glass ionomer cements are frequently used because they have caries-preventing effects thanks to fluoride release, have similar color properties with dental tissues, are aesthetically acceptable, and can be chemically bonded to dental tissues. It consists of a powder liquid system whose powder consists of aluminafluoro silicate glass containing high fluorine and its liquid consists of polyacrylic acid. The hardening reaction takes place as an acid-base reaction after mixing the powder and liquid.[11,12]

Powder of Glass Ionomers

The powder part of glass ionomer cements is aluminosilicate glass based on calcium and strontium, to which various polymeric acids containing phosphate (P_2O_5), sodium (Na_2O) and fluoride ions have been added.[13,14,15]The ratio of alumina (Al_2O_3) and silica (SiO_2), which forms the aluminosilicate in its content, is very important in determining the essential properties of glass ionomer cement.[13]

Glass is obtained by melting the mixture containing the essential elements at a temperature between 1200°C and 1500°C and then abruptly cooled on the cold metal and then ground to a coarse frit that will form glass powders.[16]Glass particles of glass ionomer cements consist of 3 main components: aluminum, silicon and calcium.[17]

Liquid of Glass Ionomers

The main component of the liquid part of glass ionomer cements is polyacrylic acid. The activity of polyacrylic acid depends on the content, molecular weight and concentration of the copolymer.The polymer used is highly effective on the mechanical properties of the material.The mechanical and physical properties of the polymer increase as the molecular weight increases, but the viscosity also increases as the molecular weight increases, making it difficult to mix the cement. [18]By adding polyacids with lower viscosity such as itaconic acid, maleic acid, tartaric acid to glass ionomer cements, better physical properties and easy application are ensured during hardening.[6]

Curing Reaction

The curing mechanism of conventional glass ionomer cements occurs with a kind of acid-base reaction that occurs as a result of the neutralization of acid groups by solid glass particle powders after the liquid and powder part are combined.[19,21]The setting reaction mechanism of glass ionomer cements consists of 4 steps. The first stage begins with the dispersion of the glass particles in the polycarboxylic acid solution.As a result of the contact between the powder and the liquid, an acid attack occurs on the surface of the glass particles, the destruction of the glass powders begins and Ca^{+2}, Na^{+1} and Al^{+3} ions are released.[6,22]In the second stage, the liberated metal ions move towards the liquid phase of the glass ionomer cement, and then the acid chains turn into a network structure, with the cations helping the formation of salt bridges between the polyacid chains, and a silica-rich "silica hydrogel layer" is formed on the surfaces of the glass particles. After this layer is formed, while the metal ions outside the glass particles gradually decrease, they become enriched with Ca^{+2} and Al^{+3} ions in the cement structure. At this stage, the cement is extremely sensitive to moisture and if it comes into contact with water in the early period, some of the metal ions in the structure of the material are lost and as a result, a cement that is unstable, has micron-level cracks in its structure and has not reached sufficient hardness is obtained.[17,21,23]The third stage is also known as the setting phase.At this stage, the ion concentrations in the matrix

increase and as a result of the conversion of polyacrylic acid to polyacrylates, the viscosity and pH of the medium increase, calcium and aluminum salts are hydrated, and as a result, metal ions pass into an insoluble phase. This phase is considered to be the phase in which the durability of glass ionomer cement increases and its transparency develops.[23,24] The final stage is maturation, and after the third stage, the setting phase, the reaction continues for several months, resulting in an increase in bond strength.[25]

Connection to Dental Tissues

Glass ionomer cements are bonded to dental hard tissues in two stages as chemical and mechanical bonding. Mechanical bonding occurs first. This bonding occurs by hybridization of collagen fibrils and demineralization by polyacrylic acid. The chemical bonding that takes place in the second stage is; It is formed as a result of the displacement of calcium and phosphorus ions in the hydroxyapatite structure and polyacrylate ions. Due to the less inorganic content of dentin and its more homogeneous structure, the bond of glass ionomer cements to dentin is lower than that of enamel. Glass ionomer cements are the only restorative materials that can be chemically bonded to dental tissues without any prior preparation. The bond of glass ionomer cements to dental tissues is quite low compared to composite resins, but a more reliable and stronger bond is obtained with glass ionomer cements.[26,27,28]

Types and Uses of Glass Ionomer Cements

Glass ionomer cements used in restorative dentistry are examined in 3 parts.

Type I: Lugating glass ionomer cements

Type II: Restorative glass ionomer cements

-Glass ionomer cements used in anterior aesthetic areas

-Reinforced glass ionomer cements used in posterior areas

Type III: Glass ionomer cements used as base material and fissure sealant [29]

Glass Ionomer Cermet Cements

By adding metal powders such as silver, aluminum, tin, titanium dioxide, nickel, chrome, stainless steel, gold into the powder of glass ionomer cement, it is aimed to obtain a more durable and durable material. [30,31]

The mixtures obtained are called cermet cements, which are a mixture of ceramic and metal.[32]The use of these esthetically inadequate cements is limited and they are mostly used as core material.[20,33]

High Viscosity (Condensable) Glass Ionomer Cements

High viscosity glass ionomer cements, also called packable cements, have been obtained by increasing the powder/liquid ratios of conventional (conventional) glass ionomer cements and changing particle sizes, and they have more advanced mechanical properties than conventional glass ionomer cements. [34,35]The curing reactions take place by acid-base reaction like conventional glass ionomer cements. Unlike conventional glass ionomer cements, since these cements harden faster, the disadvantages of the material such as moisture sensitivity in the early period are eliminated. [36]High viscosity glass ionomer cements are generally used in atraumatic restorative treatment in children with cooperation problems, as fissure sealants in children with active caries, in low stress areas or as temporary restorative material. [37,38]These cements have similar fluorine release rates with conventional glass ionomer cements and have several advantages such as non-toxicity, high abrasion resistance, completion of the finishing process without the need for a second polishing appointment, and lack of sensitivity to moisture in the early period.[39,40]

Resin Modified Glass Ionomer Cements

Resin modified glass ionomer cements were produced by synthesizing traditional glass ionomer cements with resin composites, with the aim of removing the negative properties and improving the positive properties of both restorative materials.[41]

Resin modified glass ionomer cements; They consist of 20% composite resin and 80% glass ionomer cement. They are produced to increase the physical properties of conventional glass ionomer cements and to prolong the short working time.[42,20]

Glass Hybrid Restorative Materials

Glass hybrid restorative materials are restorative materials modified with highly reactive small glass particles of different sizes added to conventional glass ionomer cements. These modifications increase reactivity and make the material more durable and longer lasting.[43,44]

In 2007, GC Corporation (Tokyo, Japan) named Equia Elephant; introduced a new restorative material consisting of two components. This material consists of a combination of Fuji IX GP Extra (GC, Tokyo, Japan) high viscosity glass ionomer cement and nano filler coat. Equia Forte Fil glass hybrid restorative material (GC, Tokyo, Japan) was developed from the Equia Fil platform in 2015, and the latest Equia Forte HT Fil material (GC, Tokyo, Japan) was

produced in 2019.[43,44] Glass hybrid restorative materials are also illuminated by light. They contain a cured nano-filled coat Equia Forte Coat (GC, Tokyo, Japan). Equia Forte HT Fil is widely used in pediatric dentistry and is applied in the clinic for long-lasting permanent restorations.[45,46]

Polyacid Modified Composite Resins (Compomers)

Polyacid modified composite resins, also called "compomers", derived from the abbreviation of composite resin and glass ionomer cements, are formed by synthesizing the positive properties of composite resins such as high aesthetics, ease of application and long working time, and fluorine release properties of glass ionomer cements.[47] Polyacid modified composite resins. Although they contain 13% fluorine, they are not fluorine reservoirs.[48] Although they contain different ratios of composite resin and glass ionomer cement, this ratio is generally 80% composite resin and 20% glass ionomer. Although these materials show the properties of both materials, they are more similar to composite resins.[17,49]

Giomeres

Giomers are restorative materials produced by combining the properties of glass ionomer cement and composite resins. They have the fluorine release properties of glass ionomer cements and the light-curing, high aesthetic and good polishability properties of composite resins. Giomers need to be bonded to dental hard tissues such as composite resins with the application of a bonding agent.[50,51]

Giomers are used in pediatric dentistry in class 3, 4 and 5 restorations of permanent teeth, in the restoration of primary teeth in the anterior and posterior regions, as fissure sealant and cavity base material.[52]

Glass Carbomers

Glass carbomers are materials developed from glass ionomer cements containing fluoroapatite and nanoparticles (hydroxyapatite) that support the remineralization of dental tissues thanks to fluoride release.[53,54] They are produced by modifying traditional glass ionomer cements to eliminate the negative properties of glass ionomer cements. It is aimed to create a structure resembling enamel tissue.[56]

Thanks to these particles, their mechanical properties have improved and they have become more resistant to abrasion.[67]

Conclusion

Glass ionomer cements have a wide range of uses, especially in pediatric dentistry. Today, with the development of restorative materials, various modifications have been made to strengthen the structure and eliminate its disadvantages. We think that clinical success rates and indications will be increased with new studies to be done.

Keywords: Pediatric Dentistry, Glass Ionomer Cements

REFERENCES

1. **Dhar V, Hsu KL, Coll JA, Ginsberg E, Ball BM, et al.** Evidence-Based Update of Pediatric Dental Restorative Procedures: Dental Materials. *J Clin Pediatr Dent.* **2015**;39(4):303–10.
2. **Mickenautsch S, Yengopal V, Banerjee A.** Atraumatic Restorative Treatment versus Amalgam Restoration Longevity: A Systematic Review. *Clin Oral Investig.* **2010**;14(3):233–40.
3. **Fuks AB.** The Use of Amalgam in Pediatric Dentistry: New Insights and Reappraising the Tradition. *Pediatr Dent.* **2015**;37(2):125–32.
4. **Fm T, Toras FM.** Faculty of Dentistry; Umm Al-Qura University; Makkah; Arabia., S.; Hamouda, I. M.; Biomaterials, of D.; Faculty of Dentistry; Mansoura University; Mansoura; Egypt. Effect of Nano Filler on Microhardness, Diametral Tensile Strength and Compressive Strength of Nano-Filled Glass Ionomer. *Int J Dent Oral Sci.* **2017**;35(1):413–17.
5. **Preston AJ, Mair LH, Agalamanyi EA, Higham SM.** Fluoride Release from Aesthetic Dental Materials. *J Oral Rehabil.* **1999**;26(2):123–29.
6. **Wilson AD, Kent BE.** A New Translucent Cement for Dentistry. The Glass Ionomer Cement. *Br Dent J.* **1972**; *132*(4):133–35.
7. **Mount GJ.** An Atlas of Glass-Ionomer Cements. A Clinician's Guide 3rd Edition. **2001**,187-96.
8. **Francisconi LF, Scaffa PMC, de Barros VR, dos SP, Coutinho M, Francisconi PAS.** Glass Ionomer Cements and Their Role in the Restoration of Non-Carious Cervical Lesions. *J Appl Oral Sci.* **2009**; 17(5):364-69.
9. **Mickenautsch S, Mount G, Yengopal V.** Therapeutic Effect of Glass-Ionomers: An Overview of Evidence. *Aust Dent J.* **2011**;*56*(1):10–5.
10. **Wilson AD, Kent BE, Clinton D, Miller RP.** The Formation and Microstructure of Dental Silicate Cements. *J Mater Sci*. **1972**;7(1):220–38.
11. **Croll TP, Nicholson JW.** Glass Ionomer Cements in Pediatric Dentistry: Review of the Literature. *Pediatr Dent.* **2002**; *24*(5):423–29.
12. **Burke FM, Ray NJ, McConnell RJ.** Fluoride-Containing Restorative Materials. *Int Dent J.* **2006**;*56* (1):33–43.
13. **Kent BE, Lewis BG, Wilson AD.** Glass Ionomer Cement Formulations: I. The Preparation of Novel Fluoroaluminosilicate Glasses High in Fluorine. *J Dent Res.* **1979**;58(6):1607–19.
14. **Brandt B, Lohbauer U, Göken M, Durst K.** The Influence of Particle Size on the Mechanical Properties of Dental Glass Ionomer Cements. *Advanced Engineering Materials*. **2010**;76(1):684–89.

15. **McLean JW.** Glass-Ionomer Cements. *Br Dent J.* **1988**;164(9):293–300.
16. **Nicholson JW.** Chemistry of Glass-Ionomer Cements: A Review. *Biomaterials* **1998**;*19*(6):485–94.
17. **Kanık Ö, Türkün Ş.** Restoratif Cam İyonomer Simanlarda Güncel Yaklaşımlar. *EÜ Dişhek Fak Derg.* **2016**; 37(2):54-65.
18. **Fareed MA, Stamboulis AN.** Addition to a Conventional Glass Ionomer Cements: Influence on Physical Properties. *Eur J Dent.* **2014**;8(4):456–63.
19. **Al-Angari SS, Hara AT, Chu TM, Platt J, Eckert G et al.** Physicomechanical Properties of a Zinc-Reinforced Glass Ionomer Restorative Material. *J Oral Sci.* **2014**;56(1):11–6.
20. **Kaya DT, Tirali ER.** Cam İyonomer Simanlardaki Gelişmeler. *A Ü Diş Hek Fak Derg.* **2013**;9(7):73-6.
21. **Crisp S, Kent BE, Lewis BG, Ferner AJ, Wilson AD.** Glass-Ionomer Cement Formulations. II. The Synthesis of Novel Polycarobxylic Acids. *J Dent Res.* **1980**;*59*(6):1055–63.
22. **Davidson CL.** Glass Ionomer Cement, an Intelligent Material. *Bull. Group. Int Rech Sci Stomatol. Odontol.* **1998**;*40*(1):38–42.
23. **Hatton PV, Brook IM.** Characterisation of the Ultrastructure of Glass-Ionomer (poly-Alkenoate) Cement. *Br Dent J*. **1992**;173(8):275-77.
24. **Khoroushi M, Mansoori KT, Hadi S.** The Effect of Pre-Warming and Delayed Irradiation on Marginal Integrity of a Resin-Modified Glass-Ionomer. *Gen Dent.* **2012**;*60*(6):383–88.
25. **Khoroushi M, Keshani F.** A Review of Glass-Ionomers: From Conventional Glass-Ionomer to Bioactive Glass-Ionomer. *Dent Res J.* **2013**;*10*(4):411-20.
26. **Davidson CL.** Advances in Glass-Ionomer Cements. *J Appl Oral Sci.* **2006**;8(14):3-9.
27. **Yoshida Y, Van Meerbeek B, Nakayama Y, Snauwaert J, Hellemans L, et al.** Evidence of Chemical Bonding at Biomaterial-Hard Tissue Interfaces. *J Dent Res.* **2000**;*79*(2):709–14.
28. **Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, et al.** Adhesion to Enamel and Dentin: Current Status and Future Challenges. *Oper Dent.* **2003**;*28*(3):215–35.
29. **Sidhu S, Nicholson J.** A Review of Glass-Ionomer Cements for Clinical Dentistry. *J Funct Biomater*. **2016**;7(3):16-9.
30. **Simmons JJ.** The Miracle Mixture. Glass Ionomer and Alloy Powder. *Tex Dent J.* **1983**; 100(10):6–12.

31. **Albers HF.** Tooth-Colored Restoratives: Principles and Techniques; PMPH-USA. **2002,**48-9.
32. **Robertson T, Heymann HO, Swift EJ.** Sturdevant's Art and Science of Operative Dentistry. **2019**,164-8.
33. **Donovan TE, Sulaiman TA, Oliveira GMS, Bayne SC, Thompson JY.** Dental Biomaterials. *Sturdevant's Art and Science of Operative Dentistry*. **2019**,453–510.
34. **Van Duinen RNB, Kleverlaan CJ, de Gee AJ, Werner A, Feilzer AJ.** Early and Long-Term Wear of "Fast-Set" Conventional Glass-Ionomer Cements. *Dent Mater.* **2005**; *21*(8):716–20.
35. **Friedl K, Hiller KA, Friedl KH.** Clinical Performance of a New Glass Ionomer Based Restoration System: A Retrospective Cohort Study. *Dent Mater.* **2011**; 27(10):1031–37.
36. **Ritter A.**Sturdevant's art and science of *Operative Dentistry*. 7th Edition. **2006**,207-10.
37. **Frencken JE, Pilot T, Songpaisan Y, Phantumvanit P.** Atraumatic Restorative Treatment (ART): Rationale, Technique, and Development. *J Public Health Dent.* **1996**; *56* (3):135–40.
38. **Hu JY, Li YQ, Smales RJ, Yip K.** Restoration of Teeth with More-Viscous Glass Ionomer Cements Following Radiation-Induced Caries. *Int Dent J*. **2002**;52(6):445–48.
39. **Anusavice KJ, Shen C, Ralph Rawls H.** *Phillips' Science of Dental Materials*; Elsevier Health Sciences. **2013**, 275-8.
40. **Anusavice, K. J.** Does ART Have a Place in Preservative Dentistry? *Community Dent. Oral Epidemiol.* **1999**; *27*(6):442–48.
41. **Croll TP, Berg JH, Donly KJ.** Dental Repair Material: A Resin-Modified Glass-Ionomer Bioactive Ionic Resin-Based Composite. *Compend Contin Educ Dent.* **2015**; *36*(1):60–5.
42. **Onal B, Pamir T.** The Two-Year Clinical Performance of Esthetic Restorative Materials in Noncarious Cervical Lesions. *J Am Dent Assoc.* **2005**; *136* (11):1547–55.
43. **Najeeb S, Khurshid Z, Zafar M, Khan A, Zohaib S, et al.** Modifications in Glass Ionomer Cements: Nano-Sized Fillers and Bioactive Nanoceramics. *Int J Mol Sci.* **2016**;17(7):1134-7.
44. **Šalinović I, Stunja M, Schauperl Z, Verzak Ž, Malčić AI, Rajić VB.** Mechanical Properties of High Viscosity Glass Ionomer and Glass Hybrid Restorative Materials. *Acta Stomatologica Croatica*. **2019**;53(2):125–131.
45. **Mickenautsch S.** High-Viscosity Glass-Ionomer Cements for Direct

Posterior Tooth Restorations in Permanent Teeth: The Evidence in Brief. *J Dent.* **2016**;55(7):121–23.

46. **Kutuk ZB, Ozturk C, Cakir FY, Gurgan S.** Mechanical Performance of a Newly Developed Glass Hybrid Restorative in the Restoration of Large MO Class 2 Cavities. *Niger J Clin Pract.* **2019**;22(6):833-41.
47. **Cildir SK, Sandalli N.** Fluoride Release/uptake of Glass-Ionomer Cements and Polyacid-Modified Composite Resins. *Dent Mater J.* **2005**; *24*(1):92–7.
48. **Hes KMY, Leung SK, Wei SHY.** Resin-Ionomer Restorative Materials for Children: A Review. *Aust Dent J*. **1999**;44:(1)–11.
49. **Meyer JM, Cattani-Lorente MA, Dupuis V.** Compomers: Between Glass-Ionomer Cements and Composites. *Biomaterials* **1998**; *19*(6):529–39.
50. **Deliperi S, Bardwell DN, Wegley C, Congiu MD.** In Vitro Evaluation of Giomers Microleakage after Exposure to 33% Hydrogen Peroxide: Self-Etch vs Total-Etch Adhesives. *Oper Dent.* **2006**; *31*(2):227–32.
51. **Quader SMA, Shamsul Alam M, Bashar AKM, Gafur A, Al Mansur M.** A. Compressive Strength, Fluoride Release and Recharge of Giomer. *Update Dent Coll J*. **2013**;2(2):28–37.
52. **Noor Saira Wajid Najma Hajira**. Giomer-The intelligent particle. *Int J Dent Oral Health*. **2015**;2(4):1-5.
53. **Menne-Happ, U.; Ilie, N.** Effect of Gloss and Heat on the Mechanical Behaviour of a Glass Carbomer Cement *J Dent.* **2013**; 41(3):223–30.
54. **Chen X, Du MQ, Fan MW, Mulder J, Huysmans MCDNJM, Frencken JE.** Caries-Preventive Effect of Sealants Produced with Altered Glass-Ionomer Materials, after 2 Years. *Dent Mater.* **2012**; *28*(5):554–60.
55. **Cehreli SB, Tirali RE, Yalcinkaya Z, Cehreli ZC.** Microleakage of Newly Developed Glass Carbomer Cement in Primary Teeth. *Eur. J. Dent.* **2013**;7(1):15–21.
56. **Zainuddin N, Karpukhina N, Law RV, Hill RG.** Characterisation of a Remineralising Glass Carbomer® Ionomer Cement by MAS-NMR Spectroscopy. *Dent Mater.* **2012**;28(10):1051–58.

Ozone Therapy

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Introductıon

Ozone (O3) gas is formed by combination of three oxygen (O2) molecules. Ozone means smell in greek as it is colourless and it has own characteristic smell. It was discovered in 1840 by Austrian chemist Christian Schönbein (1,2).Ozone gas takes place in the stratosphere layer in atmosphere. It is formed and destroyed by the effect of ultraviolet radiation naturally. But medical ozone is produced by ozone generators from medical oxygen.Although presence of ozone in the stratosphere is vital as it blocks ultraviolet B and C rays from the sun, its presence in the troposphere is very dangerous for respiratory tract and considered as air pollution (1).Ozone has been used for a long time in sterilization, industry and medicine (1,2). The acceptance of ozone therapy as one of complementary medicine applications has led to an increase in its popularity (3).

What is medical ozone therapy?

Ozone therapy is treatment of some diseases by using medical ozone gas (combination of 3-5% O3 - 95% O2).The therapy has been started to be used after discovery of ozone generators and has increased day by day (4).

Medical ozone is produced by ozone generators with reaction of 99.9%-100% pure medical oxygen and high electrical voltage. Only 3% to 5% of this gas leaving generator is ozone, the rest is oxygen. Ozone is more suitable to use in treatments because it is more unstable than oxygen and it is easier to give reaction. Also ozone is ten times more soluble than oxygen in biological fluids. Therefore, the risk of embolism is less than oxygen. %95 of medical ozone is oxygen because it is a good solvent for ozone.Liquids for medical purposes that we can dissolve ozone in are plasma, physiological saline, water and natural oils such as olive oil.As it is in gaseous form factors such as heat, pressure, potential hydrogen (PH) and ions and antioxidant capacity affect ozone solubility (5,6).

Which mechanisms work when we apply ozone therapy?

Ozone is the third most potent antioxidant agent after fluorine and persulfate. Once ozone is applied to the body, it is no longer gas. Because of unstability, ozone turns into more stable O2 and ozonoid (OX) molecules by reacting with other molecules within seconds. Than OX molecules turn into reactive oxygen species (ROS) and lipid oxydation products (LOPs) (7). Among the ozonoids, molecules that create the most effective biological effects are peroxide. Ozonoids effect by starting mutipl biological response in the body. ROS are responsible for the early reactions of ozone therapy and LOPs are responsible for the later reactions. ROS facilitates tissue oxygenation by acting on

erythrocytes and releasing oxygen to the tissues. They stimulate immune system by acting on leukocytes and increase release of growth factors by acting on platelets. LOS effect endothelium and increase nitric oxide (NO) release. They stimulate oxidative stress resistant erythrocyte production and stem cell activation. Also they increase amount of antioxidant enzymes. After reaction between ozone and polyunsaturated Fatty Acids (PUFA), hydrogen peroxide (H2O2) and LOPs, products which are important secondary messengers occure (7,8). H2O2 actually starts many biological and therapeutic reactions as a secondary messenger. As plasma concentration of H2O2 increases, it diffuses easily into cells and increases production of various cytokines, growth factors in other cells such as erythrocytes, leukocytes and endothelial cells. O3also activates antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) from glutathione transferase (GST), glutathione (GSH) and glutathione reductase (GR) (8).

Oxidative stres theory

ROS were found to be dose-dependent secondary messengers as well as their oxidant properties. Decreased oxidative damage and/or elevated oxidative stress resistance were found in animals that lived longer. It has been shown that products of oxidation/reduction (redox) reactions at low concentrations (physiological levels) may have a role of intracellular messenger and cause positive effects on biological mechanisms. These findings caused a big change in the view of the oxidants are totally harmfull (9,10).

Hormesis Theory

Hormesis is a term used for a condition that an agent that is harmful at high doses may have a beneficial effect at low doses (11). Because of this mechanism ozone therapy was named as "therapeutic acute oxidative stress shock" by Bocci at all (9). Therefore, we may say that ozone therapy is a kind of "controlled oxidative stress" given to the body. Amount of oxidative stress should be enough to activate physiological mechanisms however, it should not be so much that may overcome intracellular antioxidant systems (AOS) and cause damage (12-14).

Clinical effects of ozone therapy

Ozone can show its oxidation effect on all organic or inorganic compounds. It can react with microorganisms and have an antimicrobial or disinfection effect. Due to its strong oxidation potential, high concentration of ozone gas may have bactericidal, viricidal and fungicidal effects. Although ozone is an

disinfectant agent, it does not have such an effect in vivo. Since tissues have their own AOS, O3 does not have any damaging or irritating effect on them (15).

Ozone therapy increases oxygen carrying capacity of erythrocytes to tissues by increasing 2,3 diphosphoglycerate (2,3-DPG) enzyme and flexibility of erythrocytes. H2O2 activates the oxidation / reduction chain, activates 2-3 DFG and glucose 6 phosphate dehydrogenase (G6PDH). H2O2 stimulates glycolysis and increases adenosine triphosphate (ATP) production. The oxygen saturation curve shifts to the right and oxygenation of tissues are increased. Erythrocytes convert most of H2O2 into water and oxygen with help of catalase and glutathione peroxidase enzymes.Reaction between O3 and PUFAs in erythrocyte membranes increase erythrocyte's elasticity and mobility. This changes modify erythrocyte membrane and vascular wall structure, decrease viscosity of the blood and increase microcirculation (16-18).

H2O2 level decreases in chronic inflammation and infectious diseases. After infectious disease or tumor agents, macrophages and granulocytes are stimulated and ROS agents are produced as a result. In chronic diseases, adhesion molecules attract inflammatory cells to the area affected. During this process, ozone increases leukocyte productionand help the process (19,20).

Nuclear factor kapa B (NFkB), which is responsible for gene expression of inflammatory molecules, increases with low doses of H2O2 diffusing into leukocytes, while it is suppressed at high doses. In presence of low doses of H2O2, tyrosine kinase is activated by NFkB phosphorylation in monocytes and lymphocytes. NFkB is activated and reaches the nucleus and regulates dose-dependently some genes responsible for immune and inflammatory response in T helper one (Th1) and T helper two (Th2) cells (19,20).

Since leukocytes have low levels of antioxidant enzymes such as GSH, they are vulnerable to ROS formed during phagocytosis. With ozone therapy formation of H2O2 in fagocytosis is activated and it ensures the completion of all broken down stages of fagocytosis correctly. Ozone regulates function of B lymphocytes which turn into plasma cells and produce immunoglobulin.Dose-dependently ozone normalizes release of cytokines such as IL-2, IL-6, IL-8, TNF-alpha and TGF, which are effective in wound healing (19,20).

Platelets, which are sensitive to acute stress, increase release of thrombocyte-induced growth factors such as platelet derived growth factor (PDGF) and transforming growth factor(TGF-β1) with hormesis effect, depending on ozone and H2O2 concentration. Due to this effect ozone is used in PRP treatment as a thrombocyte activator.Ozone also decreases aggregation of blood cells by reducing fibrinogen. Due to this effect ozone at therapeutic

doses does not cause coagulation and has a positive effect on intravascular thrombosis. However the clinician should be carefull about complications that may occur due to hemolysis during ozone therapy (21,22).

Ozone may be used as a complementary therapy in vascular disorders and circulation problems. O3 makes this effect by increasing NO levels and preventing oxidative damage. Ozone gas may react with lipids and act on cell membrane or atherosclerotic plaque. O3 provides reduction in cholesterol and atherogenic lipoprotein fractions by reacting with lipids directly and lipolysis. Also it reduces the level of membrane cholesterol (23-29).

Ozone increases G6PD enzyme activity. It activates the pentose phosphate cycle and aerobic glycolysis. It decreases blood glucose level by increasing the penetration of glucose into the cell membrane. In addition, it also reduces the level of glycosylated hemoglobin (HbA1c) (30).

As ozone may react with amino acids containing sulfur (SH) group and plasma albumin level may decrease. It was determined that ozone therapy did not cause any serious changes in protein fractions and protein metabolism. It was shown that ozone application at therapeutic doses did not affect protein structures (31).

Ozone accelerates krebs cycle and ATP production, which is important in glycolysis of carbohydrates to obtain energy. This causes an increase in metabolic rate. Ozone activates antioxidant enzymes, glutathione and inreases detoxification process. By facilitating deoxyribo nucleic acid (DNA) replication, increasing oxygenation, regulating microcirculation, increasing production of ATP in Krebs cycle, ozone activates cell regeneration. It has been suggested that it may have an antitumoral effect on the tumor cell or neutralize toxins by oxidizing them. (32-34).

Its major effect on the gastrointestinal tract is thought to be dramatic normalization in transaminases, alkaline phosphatase and bilirubin levels in liver parenchyma (35).

Ozone gas is known toxic to the lung. Inhalation of low concentration may cause irritation of throat and cough. Inhalation of high concentrations may cause bronchial mucosa and pneumocyte cell damage and pulmonary edema. Athough it has harmfull efects with inhalation, it has benefits when the therapy is applied intravenously. When it is applied with major autohemotherapy method, it resolves bronchial spasm by increasing production of NO from endothelial cells and causes relaxation in smooth muscles. Ozone increases release of cytokines by increasing the number of T lymphocytes and T helper cells in the bronchial mucosa and peripheral blood. In addition it stimulates proliferation of B lymphocytes and formation of antibodies. IgA and IgM levels

increases and phagocytes are activated. Secretory IgA supplies bronchial contanents to gain their normal structure (12).

Ozonetherapy has anti-inflammatory effect by modulating prostaglandins. Also it reduces pain by inhibiting catabolic cartilage enzymes. It decreases edema by inhibiting bradykinin release and prostoglandin synthesis. It increases the level of cytokines that neutralize proinflammation such as interleukins (IL-1, IL-8, IL-12, IL-15) and tumor necrosis factor (TNF). It normalizes release of immunosuppressive cytokines such as IL-10, and promotes synthesis of matrix proteins such as collagen and glycosaminose. Ozone gas is absorbed from the synovial fluid and increases antioxydant enzymes. The therapy inactivates endogenous ROS and inhibits proteolytic enzymes. It alkalizes the acidity in the synovial fluid. Increased levels of H2O2 stimulates proliferation of chondrocytes and activates synthesis of matrix and cartilage tissue (36-38).

Ozone reacts with amino acids and acts as a neuromediator in central nervous system. Increased aerobic glycolysis, enzyme activation and ATP synthesis positively affects neuroplasty and neuron functions (39).

Also the treatment may be chosen as a complementary therapy in patients diagnosed chronic cystitis after antibiotic-resistant cystitis treatment or open bladder surgery. In gynecology, use of ozonetherapy in first trimester is not recommended due to its possible mutagenic effects. It may be preferred in the treatment of infection-based diseases such as bacterial vaginosis. It has intraocular applications in ophthalmology. It can also be used in dermatology for antiaging, wound healing and treatment of infectious diseases. Asimpressive results in mandibular necrosis and femoral head avascular necrosis have been reported the therapy may be preffered in otorhinolaryngology, dentistry and orthopedics (40-44).

Dosage and Methods for ozone therapy application

Gamma is used for application or expression of the dosage in ozone therapy. Gamma (μg/ml) is O3 concentration in micrograms (μg) in 1 mililiter (ml) gas volume. While calculating these doses, ozone concentration is measured with a photometer. For this process, 254 nanometer (nm) band, which is close to the ultraviolet wavelength, is used. One gamma ozone means there is one microgram ozone in one mililiter of ozone/oxygen mixture. The total dose is calculated in micrograms by multiplying dosage and volume administered. Since everyone's antioxidant capacity may be different, total dose applied should be cared, and individual dose selection should be preffered (45).

Ozone therapy applications may be performed at low, medium and high doses. 0-20 gamma is classified low dose, 20-30 gamma is classified medium

dose, and 30-40 gamma is classified high dose range. Each dose range has different effects. Low-dose applications are thought to have activating, moderate-dose applications to have modulating, and high-dose applications to have suppressing effect. Appropriate dose selection should be performed according to patient, disease and result we want to achieve. For new patients it is safest to start with low dose and increasing slowly (45).

There are lots of application methods such as intravenous, intramuscular, intraarticular, periarticular, myofascial, intradiscal, intraforaminal, periradicular, paravertebral, dental, intralesional, subcutaneous, rectal, vaginal, intraperitoneal, intrapleural, intra-bladder and urethral method. Below the most frequently used application methods will be discussed (45).

Major autohemotherapy method

Ozone-resistant citrate bottles, serum sets and injectors, are used for major autohemotherapy. 100cc of blood is taken from the patient to citrated bottle, 100ml of ozone gas is injected into bottle. Then the bottle is turned upside down and blood is given back to the patient. The procedure is applied 2-3 times a week for a total of 10 sessions. Patients may continue their sessions once a month or have ten more sessions a year later acording to their prefer. In Russian protocol, physiological saline may also be ozonated, but recently ozonation of blood in ozone- resistant citrated bottles is much more recommended. Low doses may be used to activate the immune system, medium doses may be used for bioregulation or fibromyalgia patients, and high doses may be preffered to suppress immune system in infectious or rheumatic diseases (45).

Figure 1: A photo of ozone major autohemotherapy application in Baskent University Alanya Hospital Physical Medicine and Rehabilitation Department

Minor autohemotherapy method

2-10 ml of blood is taken from patient and mixed with an equal volume of ozone gas. It is given immediately by deep intramuscular injection into deltoid or gluteal region. It is thought that blood coagulates at the injection site, and mediator release occurs with local inflammation in this area.Generally, 5-10 gamma doses are preferred. A total of 5 sessions are applied once a week. It is often preferred for allergic disesases or prevention of respiratory infections (45,46).

Local application method

Intramuscular, subcutanous or intratendinous application may be performed with 10 cc ozone-resistant injectors. The muscles in which spasm or myofascial pain syndrome was detected may be injected or in patients with epicondylitis, intratendinous injection may be performed. 5-10 gamma doses are preferred, once a week totally 5 sessions are applied (45).

Intraarticular application method

It is generally aplieed to knee, shoulder and hip joints by intra-articular injection method. Several studies have shown that it reduces inflammation and pain and activates regeneration. Since the application increases growth factor release by reacting with platelets, it may also be used in combination with platelet-rich plasma. 5-10 gamma doses are preferred, once a week totally 5 sessions are applied (45).

Dermal application method

Dermal applications are often applied with ozone sauna. The patient is taken to an ozone tank, leaving head area outside of the device. It may be preferred for cosmetic purposes, skin infections or bacterial vaginosis. The application lasts for 40 minutes and patients are offered to care about water intake after application. Once or twice a week totally ten sessions are applied (45).

Bagging method

It is generally preferred in patients with uninfected open skin wounds, arterial ulcers and diabetic foot. Extremity of patient with lesion is debrided before the procedure. Extremity is taken into an ozone bag moistened with ozonated water and the bag is vacuumed. Afterwards, ozone gas is given into bag at desired dosage for 10 minutes. In this application, high doses such as 30-40 gamma are generally preferred. The bag is clamped and extremity is kept in the ozone-filled bag for 30 minutes. The ozone gas is then vacuumed back from the bag. Extremity of the patient is removed from bag and wound is closed with ozonated creams. The application is applied once a day for total of 10 sessions (45).

Rectal application method

During rectal administration, ozone reacts with water, secreted antioxidants, mucoproteins and glycocalyx in intestinal mucosa and produce ROS and LOPs. While ROS are detoxified in a short time, LOPs and absorbed O2 enter the systemic circulation. For this reason, this application method is considered to have local and systemic effects together. Generally, high dose range (30-40 gamma) is preferred to suppress disease activity in inflammatory bowel diseases such as ulcerative colitis or crohn's disease. The application is performed with special rectal sets, 2-3 times a week for total of 10 sessions (45,47).

Conditions where ozone application is contraindicated

In patients who have acute hemorrhagic diseases, thrombocytopenia, severe bleeding, high grade heart failure and who use mutiple anticoagulants, the application is not recommended. In patients with G6PDH enzyme deficiency the application is contraindicated. Because ozone also works with G6PDH enzyme, it has been shown that general condition of these patients worsens after the application. In pregnancy especially in first trimester because of mutagenicity risk, the therapy is not recommended. As the therapy increases metabolic rate, it is contraindicated in patients with uncontrolled hyperthyroidism because of the risc of hyperthyroid crisis. Also the application is not recommended in patients with organ transplantation history (2).

Side effects and complications of the application

The avarage percentage of complication after ozone therapy is 0,1% (48). In addition to simple complications such as vasovagal syncope, hypotension, and hypoglycemia, rare but serious complications such as anterior spinal cord syndrome, acute myocardial infarction (49), cardiopulmonary arrest (50), vertebrobasilar stroke (51), acute bilateral vitreo-retinal hemorrhages(52), spondylodiscitis (53), fulminating septicemia (54), spinal epidural abscess (55), septic arthritis (56), pneumocephalus (57), stroke (58)have been reported. For this reason, the application should be performed by certified clinicians in centers where all kinds of emergency interventions may be performed.

Worsening of symptoms, also known as a convalescent crisis, may also occur during treatment. Since hemolysis may develop during the procedure, if hemolyzed blood is transfused to the patient, various wide range of symptoms such as pain, low back pain to disseminated intravascular coagulation may occur. For this reason, the patient should be kept under constant observation and transfusion should be ended as soon as hemolysis is detected (45, 48).

Musculoskeletal diseases ozone application is indicated

Acording to the literature, the most common diseases in which ozone therapy is used are knee diseases (such as gonarthrosis, meniscus tear) and lumbar disc hernias. Anti-inflammatory effect, decrease in pain level, inhibition of cartilage catabolic enzymes and regeneration effect are used in knee pathologies. It may be combined with prp or used alone. It is thought to increase release of growth factors by providing platelet activation when it is combined with platelet rich plasma (PRP). There are studies suggesting that the duration of action is short when applied alone (59-64).

It has been suggested that injection of high-dose ozone into disc in spinal cord causes glycosaminoglycan lysis, decrease in proteoglycan level, dehydration and reduces the size of disc herniation. Paravertebral injection supplies pain palliation by providing an anti-inflammatory effect around facet joints and spinal nerve root, and relaxes spinal muscles. For this reasons, ozone therapy has recently been started to be preferred by neurosurgeons (65-66).

Ozone therapy is also frequently preferred in fibromyalgia. Also it has been used in chronic fatigue syndrome, rheumatic diseases and myofascial pain syndrome (67,70).

Studies comparing ozone therapy with steroid injeciton in patients diagnosed with carpal tunnel syndrome have not been shown the therapy superior to steroid injection (71,72). Also it has been shown effective in plantar fasciitis but not süperior to corticosteroids (73). Steroids have been found süperior to ozone injection in patients with shoulder impingement syndrome (74).

It is suggested that the method is also effective in neurological diseases, but the level of evidence is extremely low (75).

Usage of ozone therapy in covid pandemic

It has been suggested that ozonotherapy may be used both for recovery during covid infection and in patients who develop post-covid syndrome after infection (76,77). There is limited evidence regarding its usage but recently number of studies about the topic have been increased.

As a result there is not a concensus for musculoskeletal ozone applications and dose-volume-number standardization. There is much more evidence for low back pain and knee pain but is not enough research on other local applications. The most important reason of lack of evidence is insufficient number of studies. Ozone therapy is often preferred as a complementary treatment in musculoskeletal diseases and it is thought to take a better place in the future.

References:

1. Bocci V. A. (2006). Scientific and medical aspects of ozone therapy. State of the art. *Archives of medical research*, 37(4), 425-435.doi: 10.1016/j.arcmed.2005.08.006.
2. Nogales C.G, Ferrari P.H, Kantorovich E.O, Lage-Marques, J.L. (2008). Ozone therapy in medicine and dentistry. *J Contemp Dent Pract*, 9(4), 75-84.
3. Bocci V. (1999). Biological and clinical effects of ozone. Has ozone therapy a future in medicine? *British journal of biomedical science,* 56(4), 270.
4. Bocci V, Aldinucci C, Borrelli E, Corradeschi F, Diadori A, Fanetti G, Valacchi G. (2001). Ozone in medicine. *Ozone: science & engineering*, 23(3), 207-217.
5. Di Paolo N, Bocci V, Gaggiotti E. (2004). Ozone therapy. *The International journal of artificial organs*, 27(3), 168-175.doi: 10.1177/039139880402700303.
6. Zanardi I, Borrelli E, Valacchi G, Travagli V, Bocci V. (2016). Ozone: a multifaceted molecule with unexpected therapeutic activity. *Current medicinal chemistry*, 23(4), 304-314.doi: 10.2174/0929867323666151221150420.
7. Bocci V, Valacchi G, Corradeschi F, Aldinucci C, Silvestri S, Paccagnini E, Gerli R. (1998). Studies on the biological effects of ozone: 7. Generation of reactive oxygen species (ROS) after exposure of human blood to ozone. *Journal of biological regulators and homeostatic agents,* 12(3), 67-75.
8. Larini A, Bianchi L, Bocci V. (2003). The ozone tolerance: I) Enhancement of antioxidant enzymes is ozone dose-dependent in Jurkat cells. *Free radical research*, 37(11), 1163-1168.doi: 10.1080/10715760310001604170.
9. Bocci V. (2012). How a calculated oxidative stress can yield multiple therapeutic effects. *Free Radical Research*, 46(9), 1068-1075.doi: 10.3109/10715762.2012.693609.
10. Sagai M, Bocci V. (2011). Mechanisms of action involved in ozone therapy: is healing induced via a mild oxidative stress?. *Medical gas research*, 1(1), 1-18.doi: 10.1186/2045-9912-1-29.
11. Santoro A, Martucci M, Conte M, Capri M, Franceschi C, Salvioli S. (2020). Inflammaging, hormesis and the rationale for anti-aging strategies. *Ageing Research Reviews,* 64, 101142.doi: 10.1016/j.arr.2020.101142.

12. Bocci V, Borrelli E, Travagli V, Zanardi I. (2009). The ozone paradox: ozone is a strong oxidant as well as a medical drug. *Medicinal research reviews*, 29(4), 646-682.doi: 10.1002/med.20150
13. Bocci V. (2006). Is it true that ozone is always toxic? The end of a dogma. *Toxicology and applied pharmacology*, 216(3), 493-504.doi: 10.1016/j.taap.2006.06.009
14. Bocci V. (2004). Ozone as Janus: this controversial gas can be either toxic or medically useful. *Mediators of inflammation,* 13(1), 3-11.doi: 10.1080/0962935062000197083.
15. Franke G, Knobling B, Brill F. H, Becker B, Klupp E. M, Campos C. B, Knobloch J. K. (2021). An automated room disinfection system using ozone is highly active against surrogates for SARS-CoV-2. *Journal of Hospital Infe*ction, 112, 108-113.doi: 10.1016/j.jhin.2021.04.007
16. Akbudak I. H, Kucukatay V, Kilic-Erkek O, Ozdemir Y, Bor-Kucukatay M. (2019). Investigation of the effects of major ozone autohemotherapy application on erythrocyte deformability and aggregation. *Clinical hemorheology and microcirculation,* 71(3), 365-372.doi: 10.3233/CH-180417.
17. Mudd J. B, Dawson P. J, Adams J. R, Wingo J, Santrock J. (1996). Reaction of ozone with enzymes of erythrocyte membranes. *Archives of biochemistry and biophysics,* 335(1), 145-151.doi: 10.1006/abbi.1996.0492.
18. Bocci V, Luzzi E, Corradeschi F, Paulesu L, Rossi R, Cardaioli E, Di Simplicio P. (1993). Studies on the biological effects of ozone: 4. Cytokine production and glutathione levels in human erythrocytes. *Journal of biological regulators and homeostatic agents,* 7(4), 133-138.
19. Bocci V, Paulesu L. (1990). Studies on the biological effects of ozone 1. Induction of interferon gamma on human leucocytes. *Haematologica,* 75(6), 510-515.
20. Bocci V, Valacchi G, Corradeschi F, Fanetti G. (1998). Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production. *Mediators of Inflammation*, 7(5), 313-317.doi: 10.1080/09629359890820.
21. Bocci V. A, Valacchi G, Rossi R, Giustarini D, Paccagnini E, Pucci A. M, Simplicio P. D. (1999). Studies on the biological effects of ozone: 9. Effects of ozone on human platelets. *Platelets,* 10(2-3), 110-116.doi: 10.1080/09537109976167.
22. Valacchi G, Bocci V. (1999). Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets. *Mediators of inflammation*, 8(4-5), 205-209.doi: 10.1080/09629359990360.

23. Bocci V, Travagli V, Zanardi I. (2009). May oxygen-ozone therapy improves cardiovascular disorders? *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders),* 9(2), 78-85.doi: 10.2174/187152909788488681.
24. Valacchi G, Bocci V. (2000). Studies on the biological effects of ozone: 11. Release of factors from human endothelial cells. *Mediators of inflammation,* 9(6), 271-276.doi: 10.1080/09629350020027573
25. Bocci V, Zanardi I, Travagli V. (2011). Ozone: a new therapeutic agent in vascular diseases. *American Journal of Cardiovascular Drugs,* 11(2), 73-82.doi: 10.2165/11539890-000000000-00000.
26. Di Paolo N, Bocci V, Salvo D. P, Palasciano F, Biagioli M, Meini S, Gaggiotti E. (2005). Extracorporeal blood oxygenation and ozonation (EBOO): a controlled trial in patients with peripheral artery disease.doi: 10.1177/039139880502801012.
27. Monte A. D, Der Zee H. V, Bocci V. (2005). Major ozonated autohemotherapy in chronic limb ischemia with ulcerations. *Journal of Alternative & Complementary Medicine,* 11(2), 363-367.doi: 10.1089/acm.2005.11.363.
28. Bocci V, Zanardi I, Huijberts M. S, Travagli V. (2011). Diabetes and chronic oxidative stress. A perspective based on the possible usefulness of ozone therapy. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews,* 5(1), 45-49.doi: 10.1016/j.dsx.2010.05.014.
29. Anzolin A. P, da Silveira-Kaross N. L, Bertol C. D. (2020). Ozonated oil in wound healing: what has already been proven? *Medical gas research,* 10(1), 54.doi: 10.4103/2045-9912.279985.
30. Bocci V, Zanardi I, Huijberts M. S, Travagli V. (2014). It is time to integrate conventional therapy by ozone therapy in type-2 diabetes patients. *Annals of Translational Medicine,* 2(12).doi: 10.3978/j.issn.2305-5839.2014.07.07.
31. Bocci V, Aldinucci C. (2006). Biochemical modifications induced in human blood by oxygenation-ozonation. *Journal of biochemical and molecular toxicology,* 20(3), 133-138.doi: 10.1002/jbt.20124.
32. Bocci V, Zanardi I, Travagli V. (2010). Potentiality of oxygen-ozonetherapy to improve the health of aging people. *Current Aging Science*, 3(3), 177-187.doi: 10.2174/1874609811003030177
33. Costanzo M, Cisterna B, Vella A, Cestari T, Covi V, Tabaracci, Malatesta M. (2015). Low ozone concentrations stimulate cytoskeletal organization,

mitochondrial activity and nuclear transcription. *European journal of histochemistry: EJH,* 59(2).doi: 10.4081/ejh.2015.2515
34. Bocci V. (2008). Does ozone really "cure" cancer? *International journal of cancer,* 123(5), 1222-1222.
35. Li J, Zeng T, Tang S, Zhong M, Huang Q, Li X, He X. (2021). Medical ozone induces proliferation and migration inhibition through ROS accumulation and PI3K/AKT/NF-κB suppression in human liver cancer cells in vitro. Clinical and Translational Oncology, 23(9), 1847-1856.
36. Xu W, Zhao X, Sun P, Zhang C, Fu Z, Zhou D. (2020). The effect of medical ozone treatment on cartilage chondrocyte autophagy in a rat model of osteoarthritis. *American Journal of Translational Research,* 12(9), 5967.
37. Manoto S. L, Maepa M. J, Motaung S. K. (2018). Medical ozone therapy as a potential treatment modality for regeneration of damaged articular cartilage in osteoarthritis. *Saudi journal of biological sciences*, 25(4), 672-679.
38. Rezuş E, Burlui A, Cardoneanu A, Macovei L. A, Tamba B. I, Rezuş C. (2021). From pathogenesis to therapy in knee osteoarthritis: bench-to-bedside. *International Journal of Molecular Sciences*, 22(5), 2697.doi: 10.3390/ijms22052697.
39. Kızılay Z, Kahraman Çetin N, Aksel M, Abas B. İ, Aktaş S, Erken H. A, Yenisey C. (2019). Ozone partially decreases axonal and myelin damage in an experimental sciatic nerve injury model. *Journal of Investigative Surgery*, 32(1), 8-17.doi: 10.1080/08941939.2017.1369606.
40. Travagli V, Zanardi I, Valacchi G, Bocci V. (2010). Ozone and ozonated oils in skin diseases: a review. *Mediators of inflammation*, 2010.doi: 10.1155/2010/610418.
41. Zeng J, Lu J. (2018). Mechanisms of action involved in ozone-therapy in skin diseases. *International immunopharmocology*, 56, 235-241.
42. Borrelli E, Diadori A, Zalaffi A, Bocci V. (2012). Effects of major ozonated autohemotherapy in the treatment of dry age related macular degeneration: a randomized controlled clinical study. *International journal of ophthalmology*, 5(6), 708.doi: 10.3980/j.issn.2222-3959.2012.06.11.
43. Ragab A, Shreef E, Behiry E, Zalat S, Noaman M. (2009). Randomised, double-blinded, placebo-controlled, clinical trial of ozone therapy as treatment of sudden sensorineural hearing loss. *The Journal of Laryngology & Otology*, 123(1), 54-60.doi: 10.1017/S0022215108003770.
44. Tricarico G, Rodrigues Orlandin J, Rocchetti V, Ambrosio C. E, Travagli V. (2020). A critical evaluation of the use of ozone and its derivatives in

dentistry. European *Review for Medical and Pharmacological Sciences*, 24(17), 9071-9093.doi: 10.26355/eurrev_202009_22854.
45. United Nations Environment Programme. Ozone Secretariat. (2006). Handbook for the Montreal protocol on substances that deplete the ozone layer. UNEP/Earthprint.
46. Gracer R. I, Bocci V. (2005). Can the combination of localized "proliferative therapy" with "minor ozonated autohemotherapy" restore the natural healing process? *Medical hypotheses,* 65(4), 752-759.doi: 10.1016/j.mehy.2005.04.021.
47. Bocci V, Zanardi I, Travagli V. (2011). Important details to be clarified about the effect of rectal ozone on the portal vein oxygenation. *British journal of clinical pharmacology,* 72(2), 350-351.doi: 10.1111/j.1365-2125.2011.03974.x.
48. Steppan J, Meaders T, Muto M, Murphy K. J. (2010). A metaanalysis of the effectiveness and safety of ozone treatments for herniated lumbar discs. *Journal of vascular and interventional radiology,* 21(4), 534-548.
49. He R, Huang Q, Yan X, Liu Y, Yang J, Chen X. (2019). A case of paradoxical embolism causing anterior spinal cord syndrome and acute myocardial infarction following the intradiscal oxygen-ozone therapy. *Frontiers in Neurology*, 10, 137. Doi: 10.3389/fneur.2019.00137.
50. Beyaz S. G, Sayhan H. (2018). Six-month results of cervical intradiscal oxygen-ozone mixture therapy on patients with neck pain: preliminary findings. *Pain physician*, 21(4), E449.
51. Corea F, Amici S, Murgia N, Tambasco N. (2004). A case of vertebrobasilar stroke during oxygen-ozone therapy. *Journal of stroke and cerebrovascular diseases*, 13(6), 259-261.doi: 10.1016/j.jstrokecerebrovasdis.2004.08.004.
52. Giudice G. L, Valdi F, Gismondi M, Prosdocimo G, de Belvis V. (2004). Acute bilateral vitreo-retinal hemorrhages following oxygen-ozone therapy for lumbar disk herniation. *American journal of ophthalmology*, 138(1), 175-177. Doi: 10.1016/j.ajo.2004.02.059
53. Andrés-Cano P, Vela T, Cano C, García G, Vera J. C, Andrés-García J. A. (2016). Cervical spondylodiscitis after oxygen–ozone therapy for treatment of a cervical disc herniation: a case report and review of the literature. *HSS Journal®,* 12(3), 278-283.doi: 10.1007/s11420-016-9500-1.
54. Gazzeri R, Galarza M, Neroni M, Esposito S, Alfieri A. (2007). Fulminating septicemia secondary to oxygen-ozone therapy for lumbar disc herniation: case report. *Spine,* 32(3), E121-E123.doi: 10.1097/01.brs.0000254125.85406.6e.

55. Yang C. S, Zhang L. J, Sun Z. H, Yang L, Shi F. D. (2018). Acute prevertebral abscess secondary to intradiscal oxygen–ozone chemonucleolysis for treatment of a cervical disc herniation. *Journal of International Medical Research*, 46(6), 2461-2465.doi: 10.1177/0300060518764186.
56. Seyman D, Ozen N. S, Inan D, Ongut G, Ogunc D. (2012). Pseudomonas aeruginosa septic arthritis of knee after intra-articular ozone injection. *New microbiologica*, 35(3), 345-348.
57. Toman H, Özdemir U, Kiraz H. A, Lüleci N. (2017). Severe headache following ozone therapy: Pneumocephalus. *Agri,* 29(3), 132-136.
58. Vaiano A. S, Valente C, De Benedetti G, Caramello G. (2016). Transient cortical blindness after intradiscal oxygen–ozone therapy. *Indian Journal of Ophthalmology*, 64(12), 944.doi: 10.4103/0301-4738.198858.
59. Raeissadat S. A, Ghazi Hosseini P, Bahrami M. H, Salman Roghani R, Fathi M, Gharooee Ahangar A, Darvish M. (2021). The comparison effects of intra-articular injection of Platelet Rich Plasma (PRP), Plasma Rich in Growth Factor (PRGF), Hyaluronic Acid (HA), and ozone in knee osteoarthritis; a one year randomized clinical trial. *BMC Musculoskeletal Disorders,* 22(1), 1-14.doi: 10.1186/s12891-021-04017-x
60. Anil U, Markus D. H, Hurley E. T, Manjunath A. K, Alaia, M. J, Campbell K. A, Strauss E. J. (2021). The efficacy of intra-articular injections in the treatment of knee osteoarthritis: A network meta-analysis of randomized controlled trials. *The Knee,* 32, 173-182.doi: 10.1016/j.knee.2021.08.008.
61. Noori-Zadeh A, Bakhtiyari S, Khooz R, Haghani K, Darabi, S. (2019). Intra-articular ozone therapy efficiently attenuates pain in knee osteoarthritic subjects: A systematic review and meta-analysis. *Complementary therapies in medicine,* 42, 240-247.doi: 10.1016/j.ctim.2018.11.023.
62. Oliviero A, Giordano L, Maffulli N. (2019). The temporal effect of intra-articular ozone injections on pain in knee osteoarthritis. *British medical bulletin*, 132(1).doi: 10.1093/bmb/ldz028.

63. Arias-Vázquez P. I, Tovilla-Zárate C. A, Hernández-Díaz, Y, González-Castro T. B, Juárez-Rojop I. E, López-Narváez M. L, Legorreta-Ramírez G. (2019). Short-Term Therapeutic Effects of Ozone in the Management of Pain in Knee Osteoarthritis: A Meta-Analysis. *Pm&r,* 11(8), 879-887.doi: 10.1002/pmrj.12088
64. Wang B, Dong G. Z, Ju Y. X, Yan C. S. (2014). Case-control study on therapeutic effects of ozone and triamcinolone acetonide on the treatment of

meniscal injury. *Zhongguo gu Shang= China Journal of Orthopaedics and Traumatology,* 27(4), 295-298.
65. Rimeika G, Saba L, Arthimulam G, Della Gatta L, Davidovic K, Bonetti M, Muto M. (2021). Metanalysis on the effectiveness of low back pain treatment with oxygen-ozone mixture: Comparison between image-guided and non-image-guided injection techniques. *European journal of radiology open*, 8, 100389.doi: 10.2147/DDDT.S74518.
66. Bocci V, Borrelli E, Zanardi I, Travagli V. (2015). The usefulness of ozone treatment in spinal pain. *Drug design, development and therapy,* 9, 2677.
67. Türkyılmaz G. G, Rumeli Ş, Bakır M. (2021). Effects of Major Ozone Autohemotherapy on Physical Functionality and Quality of Life in Fibromyalgia Syndrome: A Prospective Cross-sectional Study. *Altern Ther Health Med*, 27(5), 8-12.
68. Moreno Fernández A. M, Macías García L, Valverde Moreno R, Ortiz T, Fernández Rodríguez A, Moliní Estrada A, De-Miguel M. (2019). Autohemotherapy with ozone as a possible effective treatment for Fibromyalgia. *Acta Reumatologica Portuguesa,* 44 (3), 244-249.
69. Bocci V, Zanardia I, Valacchi G, Borrelli E, Travagli V. (2015). Validity of oxygen-ozone therapy as integrated medication form in chronic inflammatory diseases. *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders),* 15(2), 127-138.
70. Raeissadat S. A, Rayegani S. M, Sadeghi F, Rahimi-Dehgolan S. (2018). Comparison of ozone and lidocaine injection efficacy vs dry needling in myofascial pain syndrome patients. *Journal of pain research*, 11, 1273.doi: 10.2147/JPR.S164629.
71. Bahrami M. H, Raeissadat S. A, Nezamabadi M, Hojjati F, Rahimi-Dehgolan S. (2019). Interesting effectiveness of ozone injection for carpal tunnel syndrome treatment: a randomized controlled trial. *Orthopedic research and reviews,* 11, 61.doi: 10.2147/ORR.S202780.
72. Forogh B, Mohamadi H, Fadavi H. R, Madani S. P, AflakianN, Ghazaie F, Babaei-Ghazani A. (2021). Comparison of ultrasound-guided local ozone (O2-O3) injection versus corticosteroid injection in patients with mild to moderate carpal tunnel syndrome. *American Journal of Physical Medicine & Rehabilitation,* 100(2), 168-172.
73. Babaei-Ghazani A, Karimi N, Forogh B, Madani S. P, Ebadi S, Fadavi H. R, Eftekharsadat B. (2019). Comparison of ultrasound-guided local ozone (O2-O3) injection vs corticosteroid injection in the treatment of chronic plantar

fasciitis: a randomized clinical trial. *Pain Medicine*, 20(2), 314-322.doi: 10.1093/pm/pny066.
74. Babaei-Ghazani A, Fadavi H. R, Eftekharsadat B, Ebadi S, Ahadi T, Ghazaei F, Khabbaz M. S. (2019). A randomized control trial of comparing ultrasound-guided ozone (O2-O3) vs corticosteroid injection in patients with shoulder impingement. *American journal of physical medicine & rehabilitation,* 98(11), 1018-1025.doi: 10.1093/pm/pny066.
75. Masan J, Sramka M, Rabarova D. (2021). The possibilities of using the effects of ozone therapy in neurology. *Neuroendocrinol. Lett*, 42, 13-21.
76. Yousefi B, Banihashemian S. Z, Feyzabadi Z. K, Hasanpour S, Kokhaei P, Abdolshahi A, Eslami M. (2022). Potential therapeutic effect of oxygen-ozone in controlling of COVID-19 disease. Medical Gas Research, 12(2), 33.doi: 10.4103/2045-9912.325989.
77. Tirelli U, Franzini M, Valdenassi L, Pisconti S, Taibi R, Torrisi C, Chirumbolo S. (2021). Fatigue in post-acute sequelae of SARS-CoV2 (PASC) treated with oxygen-ozone autohemotherapy-preliminary results on 100 patients. Eur. Rev. Med. Pharmacol. Sci, 25, 5871-5875.

Next Generation Sequencing: Areas of use in Medicine

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Next-generation sequencing (NGS) or massively parallel sequencing, is a method of sequencing millions of DNA fragments (or cDNA). NGS has started widely use in the clinical laboratory just because of its ability to simultaneous analysis of custom design gene panels, whole exome (WES) or whole genome (WGS) with a single run. In many clinical laboratories, NGS is an established tool for germline and somatic mutation detection. Germline mutation testing includes targeted large clinical exome gene panel, WES, WGS, custom design small gene panel or mitochondrial DNA (mtDNA) sequencing (1,2). Depending on the laboratory's requirements, with targeted panel testing, it is possible to sequence several genes related to inherited disorders such as nephropathies, cardiomyopathies, immune deficiencies, neurologic disorders, retina dystrophies, bone marrow failure syndromes, deafness, mitochondrial disorders, encephalopathies, myopathies, connective tissue disorders, and cancer predisposition syndromes (3-12).

NGS Technologies are not only used for germline mutation testing but also cell-free fetal DNA (cffDNA) testing in prenatal diagnosis and circulating tumor DNA (ctDNA) testing. Blood contains various types of biological materials including cell-free DNA. A portion of cfDNA released from tumor cells can be detected in the blood samples of cancer patients and this DNA is called ctDNA. The non-invasive technique that enables ctDNA mutation analysis from blood samples of cancer patients is referred to as liquid biopsy (13). In prenatal diagnosis, chromosomal deletions and duplications can be detected using cffDNA samples (14). cffDNA in maternal plasma derives from the placenta (15) and high-throughput technologies can accurately detect fetal trisomies (Trisomy 13,18 and 21) and chromosomal abnormalities in a short time.

Besides its role in germline and somatic mutation analysis, NGS can be used in RNA sequencing/expression, methylation studies, HLA typing, and blood group typing.

Generic Workflow of NGS

There are four main steps in a wet lab including DNA extraction, library preparation, target enrichment, and sequencing (16,17). First, a sufficient amount of DNA should be extracted from samples. For the measurement of DNA quantitation, the use of intercalating dyes (Qubit) is required rather than standard spectrophotometry (18,19).

Second, the DNA is processed for library preparation. Different sequencing platforms use similar steps for library preparation. There are mainly five steps in library preparation including *mechanicalfragmentation* to produce 350-550

base pair fragments; *enzymaticreactions* for end repair, adenylation and adapter ligation (molecular barcodes, universal PCR primers, hybridization probes); *size selection and clean-up* for creating DNA fragments of defined length, magnetic beads are used for this purpose; *amplification* for attaching sequencing adapters and to increase DNA concentration; *quantification* for quality assessment before sequencing run (16).

Third, target enrichment which is generally combined with the library preparation step based on multiplex PCR amplification (20), DNA hybridization to specific biotinylated probes (21) or DNA capture via molecular inversion probe circularisation (22).

As the last step sequencing run can be performed on different platforms such as Illumina, Ion Torrent, and MGI/BGI sequencers. A comparison of the sequencing platforms was given in

Table 1. Comparison of NGS platforms

Platforms	**Illumina**	**Ion Torrent**	**BGI**	**MGI**
Sequencing chemistry	Sequencing by synthesis	Sequencing by synthesis	DNA nanoball (DNB) and probe-anchor synthesis (cPAS)	DNA nanoball (DNB) and probe-anchor synthesis (cPAS)
Detection chemistry	Fluorescent	pH	Fluorescent	Fluorescent
Starting Material	1-5 µg DNA	1 µg DNA	25 ng DNA	25 ng DNA
Read length (bp)	2*250 Miseq	100	100	100
	2*100 Hiseq2500			
Run Time	39 hours Miseq	4 hours	24 hours	48 hours
	27 hours Hiseq2500			
Reads per run (million)	~1 Miseq	1	1	1
	~3 Hiseq2500			
Run throughput (GBp)	~8 Miseq	~0,1	80	150
	~120 Hiseq2500			

The raw data reads undergo bioinformatic analysis including FASTQ file generation, quality analysis, mapping to reference genome and annotation of the variants (23,24). American College of Medical Genetics and Genomics (ACMG) guideline is used to classify the variants as pathogenic, likely pathogenic, with unknown significance, likely benign or benign. Different in silico prediction algorithms are implemented to annotate disease-causing mutations. These algoritms are based on information of the sequence homology, pathogenicity frequency, protein structure, evolutionary and etc (25). Frequently used public databases for the interpretation of NGS data are given in Table 2.

Widely used Gene Panels for NGS

1. Exome sequencing

There are two types of exome panels widely used in NGS platform. One of them is clinical exome sequencing (CES) which is a test for the detection of disease-causing variations within 1% of the genome enabling rapid genome scaling of DNA with a reduced cost compared to Sanger sequencing (26,27). Clinical exome sequencing panels cover 4500-6000 genes. With the advance of whole exome sequencing (WES), approximately 180000 exons of human DNA (1-2% of the genome) can be sequenced in a short time (28). The aim of using WES is to identify genetic alterations in all exons of the genome.

2. Whole Genome sequencing

Exome sequencing only allows the detection of alterations in % of the genome that span for coding regions but 99% remaining of the genome is not covered. Therefore, the structural and non-coding variants can be detected with whole genome sequencing (WGS). WGS has been found to be more powerful compared to WES for the identification of potential disease-causing mutations (29).

Table 2. In silico Algorithms and online databases

Name of Datase	Website
ClinVar	https://www.ncbi.nlm.nih.gov/clinvar
dbSNP	https://www.ncbi.nlm.nih.gov/projects/SNP
NCBI genetic testing registry	https://www.genetests.org
MutationTaster	https://www.mutationtaster.org/
Varsome	https://varsome.com/
Franklin	https://franklin.genoox.com/clinical-db/home
SIFT	https://sift.bii.a-star.edu.sg/
PolyPHEN	http://genetics.bwh.harvard.edu/pph2/
Leiden open variant database	https://databases.lovd.nl/shared/genes
gnomAD browser	https://gnomad.broadinstitute.org/
1000 Genomes	https://www.internationalgenome.org/1000-genomes-browsers/index.html

Although NGS is a method used in laboratories of the Genetics department, it is in collaboration with many other departments in medicine because of the genetic basis of many diseases. Rare diseases, cancers, haematologic malignancies, endocrine disorders, neurologic diseases and rheumatologic diseases and etc. can be caused by mutations in a single gene. Therefore there is a review of clinical applications of next-generation sequencing in the practice of clinical Medicine. The Human Phenotype Ontology (HPO) browser is a standardized vocabulary of phenotypic abnormalities associated with more than7000 diseases (30). HPO contains 13,000 terms and more than 156,000 annotations from Orphanet, DECIPHER, and OMIM.

Neurology

The Human genome contains approximately 20000 genes of which 80% are actively expressed in the brain (31). Whole exome sequencing can be used for mutation analysis of Mendelian neurological disorders including intellectual disabilities,polyneuropathy, cerebellar ataxias, neuromuscular diseases, Alzheimer, ALS, dystonia, epilepsy and etc. The whole exome sequencing approach also allows the detection of novel causal mutations and with proband-parents studies, named as WES-Trio, genotype comparison can be performed between affected children and parents (32). The number of disease-associated genes for many neurological diseaseshas been identified after the introduction of next-generation sequencing (33). Neurological disease-associated genes are represented in Table 3.

Table 3. Genes related to Neurologic phenotypes

Alzheimer	ABCA7,APOE,APP,CACNA1G,GATA1,HFE,MPO,NOS3,PLAU,PSEN1,PSEN2
ALS	ALS2,ANG,ANXA11,ATXN2,C9orf72,CCNF,CFAP410,CHCHD10,CHMP2B,CYLD,DAO,DCTN1,EPHA4,ERBB4,FIG4,FUS,GLE1,GLT8D1,HNRNPA1,HNRNPA2B1,KIF5A,MAPT,MATR3,NEFH,NEK1,OPTN,PFN1,PON1,PON2,PON3,PPARGC1A,PRPH,PSEN1,SETX,SIGMAR1,SOD1,SPG11,SPTLC1,SQSTM1,TAF15,TARDBP,TBK1,TIA1,TREM2,TRPM7,TUBA4A,UBQLN2,UNC13A,VAPB,VCP
Ataxia	AAAS, AARS1, ABCA2, ABCB7, ABHD12, ACO2, ADGRG1, ADPRS, AFG3L2, ALAS2, ALDH5A1, AMPD2, ANO10, AP1S2, APTX, ARSA, ATCAY, ATM, ATN1, ATP1A3, ATP2B3, ATP8A2, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8, ATXN10, B3GALNT2, B4GAT1, BBS1, BEAN1, BRF1, CA8, CACNA1A, CACNA1G, CACNB4, CAD, CAMTA1, CASK, CCDC88C, CDK5, CHMP1A, CLCN2, CLN5, CLN6, CLP1, CLPP, COA7, COASY, COG5, COQ8A, COX20, CP, CRPPA, CSTB, CTBP1, CWF19L1, CYP2U1, CYP27A1, DAB1, DAG1, DARS2, DCC, DDHD2, DKC1, DMXL2, DNAJC5, DNAJC19,DNMT1, DOCK3, DYNC1H1, EBF3, EEF2, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, ELOVL4, ELOVL5, EPM2A, EXOSC1, EXOSC3, EXOSC5, EXOSC8, FA2H, FBXL4, FGF14, FKRP, FKTN, FLVCR1, FMR1, FOLR1, FRMD4A, FXN,GBA2, GFAP, GJC2, GLS, GMPPB, GOSR2, GPAA1, GRID2, GRM1, HARS1, HEXA, HEXB, IRF2BPL, ITPR1, KCNA1, KCNA2, KCNC3, KCND3, KCNJ10, KCNN2, KIF1C, LAMA1, LARGE1, LARS2, MARS2, MINPP1, MMACHC, MORC2, MRE11, MSTO1, MT-ATP6, MTCL1, MTFMT, MTPAP, MTTP, MVK, NAGLU, NHLRC1, NKX2-1, NKX6-2, NMNAT2, NOP56, NPC1, NPC2, OPA1, OPA3, OPHN1, PAX2, PAX6, PCLO, PDYN, PEX16, PHGDH, PI4KA, PIK3R5, PITRM1, PLA2G6, PMPCA, PMPCB, PNKP,PNPLA6, POLG, POLR3A, POLR3B, POMGNT1, POMGNT2, POMK, POMT1, POMT2, PPP2R2B, PRICKLE1, PRKCG, PRNP, PRRT2, PTF1A, PTRH2, RARS2, RELN, RNF170, RNF216, ROBO3, RORA, RUBCN, RXYLT1, SACS, SAR1B, SCN1A, SCN2A, SCN8A, SCYL1, SEPSECS, SETX, SIL1, SLC1A3,SLC2A1,SLC9A1, SLC9A6, SLC17A5, SLC25A46, SLC44A1, SLC52A2, SMPD4, SNAP25, SNX14, SPR, SPTBN2, SQSTM1, SRD5A3, STUB1, SVBP, SYNE1, SYT14, TBC1D23, TBP, TDP1, TDP2, TERT, TGM6, THG1L, TINF2, TMEM106B, TMEM240, TOE1, TPP1, TSEN2, TSEN15, TSEN34, TSEN54, TTBK2, TTC19, TTPA, TUBA1A, TUBA8, TUBB, TUBB2B, TUBB3, TUBB4A, TWNK, UBR4, UBTF, UCHL1, VAMP1, VLDLR, VPS13D, VPS53, VRK1, WDR73, WDR81, WFS1, WWOX, XRCC1, ZFYVE26, ZNF592, SPG7

Polyneuropathy	AARS1, ABCA1, ABCC9, ABHD12, ACOX1, ACTC1, ACTN2, AGTPBP1, AGXT, AIFM1, ALDH3A2,ANKRD1, AP1 APOA1, APTX, ARHGEF10, ARL6IP1, ARSA, ATL1, ATL3, ATM, ATP ATP7A,B4GALNT1, BAG3, BCKDHB, BRAF, BSCL2, C1orf194, C19orf12, CACNB4, CAS Q2, CAV3, CCT5, CD59, CHCHD10, CLTCL1, CNTNAP1, COA7, COQ8A, COX6A1, CPOX, CRYAB, CSRP3, CTDP1, CYP27A1, DARS2, DCAF8, DCTN1, DEGS1, DES, DGUOK, DHH, DHTKD1, DHX9, DMD, DNAJB2, DNAJC3, DNM2, DNMT1, DRP2, DSC2, DSG2, DSP, DST, DSTYK, DTNA, DYNC1H1, EGR2, ELP 1, EMD,EMILIN1, ERBB3, ERCC6, ERCC8, ETFDH, FA2H, FAH, FAM126A, FBLN5, FBXO38, FGD4, FGF14, FIG4,FKTN, FLVCR1, FXN, GAA, GALC, GAN, GART, GATAD1, GBA2, GBF1, GDAP1, GJB1, GJC2, GLA, GLE1,GNB4, HADHA, HADHB, HARS1, HINT1, HK1, HMBS, HOXD10, HRAS, HSPB1, HSPB3, HSPB8, IARS2,IGHMBP2, INF2,ITPR1, ITPR3, JAG1, JPH2, JUP, KARS1, KCNA1, KCNA2, KCNC3, KIF1A, KIF1B, KIF5A, KLC2, KRAS, L1CAM, LAMA4, LAMP2, LAS1L, LDB3, LITAF, LMNA, LRSAM1, LYST, MAP2K1, MAP2K2, MCM3AP, MED25, MFN2, MMACHC, MME,MORC2, MPV17, MPZ, MRE11, MT-ATP6, MT-RNR1, MT-TL1, MTMR2, MTRFR, MTTP, MYBPC3, MYH6, MYH7, MYH14, MYL2, MYL3, MYOZ2, MYPN, NAGA, NAGLU, NDRG1, NEBL, NEFH, NEMF, NEXN, NGF, NIPA1, NRAS, NTRK1, NUDT2,OPA1, OPA3, PDHA1, PDK3, PDLIM3, PDYN, PEX7, PEX10, PHYH, PKP2, PLEKHG5, PLN, PLP1, PMM2, PMP2, PMP22, PNKP, PNPLA6,POLG, POLR3A, POLR3B, PPOX, PRDM12, PRKAG2, PRKCG, PRNP, PRPS1, PRX, PTEN, PTPN11, PTRH2,RAB7A, RAF1, RBM20, REEP1, RETREG1, RIT1, RYR2, SACS, SBF1, SBF2, SCARB2, SCN5A, SCN9A, SCN10A, SCN11A, SCP2, SCYL1, SELENOI, SEPTIN9, SETX, SGCD, SH3TC2, SIGMAR1, SIL1, SLA2, SLC1A3, SLC5A7, SLC12A6, SLC25A19,SLC25A46, SLC52A1, SLC52A2, SLC52A3, SMN1, SORD, SOS1, SOX10, SPART, SPAST, SPG11, SPG21, SPTAN1, SPTBN2, SPTBN4, SPTLC1, SPTLC2,SUCLA2, SURF1, SYT2, TCAP, TDP1, TFG, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TRIM2,TRPA1, TRPV4, TTBK2, TTN, TTPA, TTR, TUBB3, TWNK, TYMP, UBA1, UBA5, VAPB, VCL, VCP, VPS13A, VRK1, VWA1, WARS1, WASHC5, WNK1, WWTR1, XK, XPA, XRCC1, YARS1, ZFYVE26, ZFYVE27, NEFL, SCO2, SPG7
Myopathy	ABCC9,ABHD5,ACACA,ACAD9,ACADM,ACADS,ACTA1,ACTC1,ACTN2,ADGRG6,ADSS1,AGK,AGL,AGRN,AK9,AKT1,ALDOA,ALG2,ALG14,ALPL,AMPD1,ANKRD1,ANO5,AP1S2,B3GALNT2,B4GALT1,BAG3,BAG5,BAZ1B,BCL7B,BIN1,BSCL2,BUD23,C1QBP,CACNA1S,CAP2,CAPN3,CASQ1,CASZ1,CAV3,CCDC174,CDON,CENPF,CFL2,CHAT,CHCHD10,CHKB,CHRNA1,CHRNB1,CHRND,CHRNE,CHST14,CISD2,CLIP2,COL6A1,COL6A2,COL6A3,COL9A1,COL9A2,COL9A3,COL12A1,COL13A1,COLQ,COMP,CPT2,CRPPA,CRYAB,CSRP3,CTNS,DES,DHX16,DISP1,DLL1,DMD,DNA2,DNAJB6,DNAJC30,DNM1L,DNM2,DOK7,DOLK,DPAGT1,DPM3,DSE,DSG2,DSP,DYSF,EIF4H,ELN,EMD,EPG5,ERGIC1,FGF8,FGFR1,FHL1,FHL2,FKBP6,FKBP14,FKRP,FKTN,FLAD1,FLNC,FOXH1,FXR1,G6PC3,GABRD,GAS1,GATAD1,GCLC,GFER,GFPT1,GIPC1,GK,GLI2,GMPPB,GNE,GTF2I,GTF2IRD1,GTF2IRD2,GYG1,HADHA,HADHB,HAND2,HNRNPA1,HNRNPA2B1,HNRNPDL,HSPG2,INPP5K,ISCU,ITGA7,JAG2,KBTBD13,KCNAB2,KLHL9,KLHL40,KLHL41,LAMA4,LAMB2,LAMP2,LARGE1,LDB3,LIMK1,LIPE,LMNA,LMNB2,LMOD3,LRP4,LRP12,LUZP1,MAN2B1,MEGF10,METTL27,MG

	ME1,MLXIPL,MMP23B,MSTO1,MT-ATP6,MT-CO1,MT-CO2,MT-CO3,MT-CYB,MT-ND1,MT-ND2,MT-ND4,MT-ND4L,MT-ND5,MT-ND6,MT-RNR1,MT-TC,MT-TE,MT-TF,MT-TH,MT-TI,MT-TK,MT-TL1,MT-TP,MT-TQ,MT-TS1,MT-TS2,MT-TT,MT-TV,MT-TW,MTAP,MTMR14,MTTP,MUSK,MYBPC1,MYBPC3,MYF6,MYH2,MYH6,MYH7,MYH14,MYMK,MYO9A,MYO18B,MYOD1,MYOT,MYPN,NALCN,NARS2,NCF1,NDUFA11,NDUFAF1,NDUFAF4,NDUFB3,NDUFS2,NEB,NEFH,NEFL,NEXN,NODAL,NUBPL,OPA1,ORAI1,PABPN1,PANK2,PDPN,PGAM2,PGK1,PLEC,PLN,PLOD1,PNPLA2,POLG,POLG2,POLRMT,POMGNT1,POMT1,POMT2,PPARG,PPCS,PRDM16,PRKAG2,PRKCZ,PSEN1,PSEN2,PTCH1,PTEN,PUS1,RAF1,RAPSN,RBM20,RERE,RET,RFC2,RILPL1,RMND1,RRM2B,RYR1,SAR1B,SCN4A,SCN5A,SDHA,SDHAF1,SDHB,SDHD,SELENON,SGCA,SGCB,SGCD,SGCG,SHH,SIL1,SIX3,SKI,SLC5A7,SLC18A3,SLC22A5,SLC25A1,SLC25A3,SLC25A4,SNAP25,SPEN,SQSTM1,STIM1,STX1A,SUFU,SVIL,SYNE1,SYNE2,SYT2,TAF1A,TANGO2,TAFAZZIN,TBCE,TBL2,TCAP,TDGF1,TGIF1,TIA1,TK2,TKFC,TMEM43,TMEM270,TMPO,TNNC1,TNNI3,TNNT1,TNNT2,TNPO3,TPI1,TPM1,TPM2,TPM3,TRAPPC11,TRIM32,TRIP4,TRMU,TTN,TWNK,TXNRD2,UBA1,UBE4B,UNC45B,UNC80,VAMP1,VCL,VCP,VMA21,VPS13A,VPS37D,VWA1,WFS1,XDH,XK,YARS2,ZBTB20,ZIC2,ACACA,ACACA,GTF2I,GTF2I,NEFL,ORAI1,TBCE,TBCE,TBCE,GTF2I,ACACA
Epilepsy	ABCC8, ALDH5A1, ALDH7A1, ALG2, ALG3, AMACR, ARFGEF2, ARHGEF9, ARX, ASNS, ATP1A2, ATP1A3, BOLA3, CACNA1A, CACNA1H, CACNB4, CACNG2, CASK, CDH15, CDKL5, CHD2, CHRNA2, CHRNA4, CHRNB2, CLCN2, CLN8, CNTNAP2, COG8, COQ2, CPA6, CSTB, CTSD, DEAF1, DNM1, DOCK7, DOCK8, DOLK, DYNC1H1, DYRK1A, EFHC1, EPB41L1, EPM2A, FH, FIG4, FLNA, FOXG1, GABRA1, GABRB3, GABRD, GABRG2, GCK, GLYCTK, GOSR2, GRIN1, GRIN2A, GRIN2B, HCFC1, HCN1, IER3IP1, INS, JRK, KCNAB2, KCNJ11, KCNMA1, KCNQ2, KCNQ3,KCNT1, KCTD7, KIF1A, KIRREL3, LGI1, LMNB2, MAPK10, MBD5, MECP2, MED17, MEF2C, NF1, NHLRC1, NRXN1, NTNG1, PAFAH1B1, PCDH19, PDSS2, PDX1, PHGDH, PIGA, PIGL, PIGO, PIGV, PLCB1, PNKP, POLG, PRDM16, PRICKLE1, PRICKLE2, PRODH, PRRT2, RELN, ROGDI, SCARB2, SCN1A, SCN1B, SCN2A, SCN8A, SCN9A, SETBP1, SKI, SLC1A3, SLC2A1, SLC6A1, SLC25A22, SNIP1,SPTAN1, SRPX2, ST3GAL3, ST3GAL5, STRADA, STXBP1, SYN1, TBC1D24, TCF4, TSC1, TSC2, UPB1, WWOX, YWHAE, ADRA2B, AARS

Rheumatology

Rheumatologic diseases have their own genetic background and many risk loci corresponding to susceptibility and rheumatic disease progression are identified up to now (34,35). Disease-causing gene mutations of Rheumatoid arthritis, systemic lupus erythematosus (SLE), osteoarthritis, ankylosing spondylitis and autoinflammatory diseases can be identified by CES and WES. Genes related to rheumatologic diseases are given in Table 4.

Table 4. Genes related to Rheumatic phenotypes

Rheumatoid	ACP5,ANKRD55,CD244,CD247,CIITA,DCLRE1C,GCH1,HLADRB1,IGHG2, IGKC,IL2RA,IL2RB,IL6, IL10,IMPDH2,LACC1,MIF,NFKBIL1,PTPN2,PTPN22,SLC22A4,STAT4
SLE	ACP5,C1QA,C1QB,C1QC,C1S,C2,C3,C4A,C8A,CASP10,CFTR,CTLA4,DDX 41,DNASE1,DNASE1L3,FAS,FASLG,FCGR2A,FCGR2B,IGHG2,IGKC,IRAK 1,MASP2,PEPD,PNP,PRKCD,PTPN22,RASGRP1,SEMA6B,SERPING1,SMP D1,SOCS1,SPP1,STAT4,TLR7,TPP2,TREX1
Osteoarthritis	ACAN,AEBP1,AIP,ANKH,ANKRD55,ASPN,ATP7B,CANT1,CCN6,CD247,C LCN7,COL1A1,COL2A1,COL3A1,COL5A1,COL5A2,COL9A1,COL9A2,COL 9A3,COL11A1,COL11A2,COMP,DDRGK1,F8,F9,FBN1,FGFR3,FRZB,GBA, GDF5,GHR,GNAS,GPR101,HGD,HLAB,HPGD,IL2RA,IL2RB,KIF22,LMNA, MATN3,MEFV,MIR140,MMP13,PHEX,PTPN2,PTPN22,SCARB2,SLC26A2,S LC40A1,SMAD2,SMAD3,STAT4,STT3A,TGFB3,TNFRSF11B,TRAPPC2,TR PS1,TRPV4,UFSP2,ZMPSTE24,ZNF687
Autoinflammator	ADA2, ELANE, HAX1, IL1RN, IL10, IL10RA, IL10RB, IL36RN, LPIN2, MEFV, MVK, NLRP3, NLRP7, NLRP12, NOD2, PLCG2, PSTPIP1, TNFRSF1A

Ophthalmology

Inherited eye diseases are rere and shows genetic heterogeneity. OMIM results in 2798 descriptions for the search “eye”term (36). There are over 350 hereditary eye diseases including retinitis pigmentosa, color blindness, retina dystrophies, Leber congenital amaurosis and etc. (37). The diagnostic capacity of the NGS testing in ophthalmological diseases is about 50-70%.WES and targeted sequencing are started widely used for identifying genetic alterations in clinical and research fields of retinal diseases (38). More than 240 genes have been associated with retinal disorders but underlying mechanisms are not still enlightened yet (39). A list of genes related to eye diseases are shown in Table 5.

Table 5. Genes associated with eye-diseases

Choroideremia	BEST1,CHM,EPHA2,IMPG1,IMPG2,POU3F4,PRPH2,UBE3B
Retinitis Pigmentosa	ABCA4,ABHD12,ACBD5,ACOX1,ADGRV1,AHR,AIPL1,ALG6,AMACR,AP3B2,ARL2,ARL2BP,ARL3,ARL6,ODAD2,ASPA,ATXN2,BBIP1,BBS1,BBS2,BBS4,BBS5,BBS7,BBS9,BBS10,BBS12,BCS1L,BEST1,C1QTNF5,CFAP418,CC2D2A,CCDC28B,CCDC39,CCDC40,CCDC65,CCDC103,ODAD1,ODAD3,CCNO,CDH23,CDHR1,CEP290,CERKL,CFAP221,CFAP298,CFAP300,CFAP410,CIB2,CLDN19,CLN3,CLRN1,CNGA1,CNGB1,COQ2,CRB1,CRX,CTSD,CWC27,DHDDS,DHX38,DNAAF1,DNAAF2,DNAAF3,DNAAF4,DNAAF5,DNAH1,DNAH5,DNAH9,DNAH11,DNAI1,DNAI2,DNAJB13,DNAL1,DPAGT1,DRC1,EXOSC2,EYS,FAM161A,FDXR,FLVCR1,FOXJ1,FSCN2,GAS2L2,GAS8,GATA3,GGCX,GNAT1,GRK1,GUCA1B,HGSNAT,HK1,HMX1,HSD17B10,HSPD1,HYDIN,IDH3B,IFT27,IFT74,IFT140,IFT172,IMPDH1,IMPG2,IQCB1,KIF3B,KIF5A,KIZ,KLHL7,LRAT,DNAAF11,LRRC56,LZTFL1,MAK,MAPKAPK3,MCIDAS,MDH2,MERTK,MFRP,MKKS,MKS1,MT-ATP6,MT-ND1,MT-ND2,MT-ND3,MT-ND4,MT-ND5,MT-ND6,MT-TK,MTTL1,MTTV,MTTW,MTTP,MVK,MYO6,MYO7A,NDUFA9,NEK2,NEK10,NME8,NPHP4,NR2E3,NRL,OFD1,PANK2,PCARE,PCDH15,PDE6A,PDE6B,PDE6G,PDZD7,PEX1,PEX2,PEX3,PEX5,PEX6,PEX7,PEX10,PEX11B,PEX12,PEX13,PEX14,PEX16,PEX19,PEX26,PHYH,DNAAF6,PMM2,POGZ,PPP2R3C,PRCD,PROM1,PRPF3,PRPF4,PRPF6,PRPF8,PRPF31,PRPH2,PRPS1,RBP3,RDH11,REEP6,RGR,RHO,RLBP1,ROM1,RP1,RP2,RP9,RPE65,RPGR,RPL10,RRM2B,RSPH1,RSPH3,RSPH4A,RSPH9,SAG,SDCCAG8,SEMA4A,SH2B1,SLC7A14,SLC35A2,SNRNP200,SPAG1,SPEF2,SRD5A3,STK36,TELO2,TMEM67,TOPORS,TRAF3IP1,TRNT1,TTC8,TTC12,ODAD4,TULP1,USH1C,USH1G,USH2A,WARS2,WDR19,DYNC2I2,WHRN,ZMYND10,ZNF408,ZNF513,CCDC39,CLN3,GAS2L2,GAS2L2,NR2E3,NR2E3,RBP3,NR2E3,GAS2L2

Night Blindness	ABCA4,ADAM9,ADGRV1,AGBL1,AGBL5,AHI1,AHR,AIPL1,ARHGEF18,ARL2BP,ARL3,ARL6,ARSG,ATF6,BBS1,BBS2,BBS4,BEST1,C1QTNF5,CFAP418,CA4,CABP4,CACNA1F,CACNA2D4,CCDC28B,CDH23,CDHR1,CEP78,CERKL,CFAP410,CHM,CIB2,CLCC1,CLEC3B,CLRN1,CNGA1,CNGA3,CNGB1,CNGB3,CNNM4,COG4,COL8A2,CRB1,CRX,CWC27,CYP4V2,DHDDS,DHX38,DPAGT1,DRAM2,ELOVL4,ESPN,EYS,FAM161A,FGFR2,FLVCR1,FSCN2,GGCX,GNAT1,GNB3,GNS,GPR179,GRK1,GRM6,GUCA1A,GUCA1B,GUCY2D,HADHA,HARS1,HGSNAT,HK1,HKDC1,HSD3B7,IDH3A,IDH3B,IDS,IFT88,IFT140,IFT172,IMPDH1,IMPG1,IMPG2,INPP5E,ITM2B,KCNJ13,KCNV2,KIAA1549,KIF3B,KIZ,KLHL7,LCA5,LRAT,LRIT3,MAK,MERTK,MFN2,MFRP,MMP19,MTTS2,MTTP,MVK,MYO6,MYO7A,NEK2,NMNAT1,NR2E3,NRL,NYX,OAT,OFD1,OPN1LW,OPN1MW,PCARE,PCDH15,PDE6A,PDE6B,PDE6G,PDZD7,PEX1,PEX2,PEX3,PEX5,PEX6,PEX7,PEX10,PEX11B,PEX12,PEX13,PEX14,PEX16,PEX19,PEX26,PHYH,PITPNM3,POC1B,POMGNT1,POU3F4,PRCD,PROM1,PRPF3,PRPF4,PRPF6,PRPF8,PRPF31,PRPH2,RAB28,RAX2,RBP3,RDH5,RDH11,RDH12,REEP6,RGR,RHO,RIMS1,RLBP1,RNU4ATAC,ROM1,RP1,RP1L1,RP2,RP9,RPE65,RPGR,RPGRIP1,SAG,SCAPER,SEMA4A,SLC4A11,SLC7A14,SLC24A1,SNRNP200,SPATA7,STIM1,TCF4,TIMP3,TLCD3B,TOPORS,TRAPPC9,TRNT1,TRPM1,TTC8,TTLL5,TTPA,TUB,TULP1,UNC119,USH1C,USH1G,USH2A,VPS13B,WDR19,WHRN,YARS1,ZEB1,ZNF408,ZNF513,ZPR1,AGBL1,AGBL1,ARHGEF18,GPR179,GPR179,GUCA1A,NR2E3,NR2E3,OPN1MW,OPN1MW,RBP3,NR2E3,AGBL1,GPR179,OPN1MW

Retina Degeneration	AARS1,ABCA4,ABCC6,ACTL6A,ACTL6B,AGO2,AIPL1,ALDH3A2,ALG6,ALMS1,ANKRD17,ANO10,AP3B2,APOB,APOE,ARL2BP,ARL6,ARSG,ARV1,ASAH1,ASXL1,ATF6,ATP1A2,ATP1A3,ATP6V1A,ATXN7,BAP1,BBS1,BBS2,BBS4,BBS9,BCAS3,BCORL1,BEST1,BPTF,C1QTNF5,CFAP418,C9,CA2,CACNA1A,CACNA1B,CCDC28B,CCDC32,CDH3,CDHR1,CDK19,CELF2,CEP78,CEP164,CFAP410,CFH,CFHR1,CFHR3,CFI,CLCC1,CLCN3,CLN3,CLN5,CLN6,CLTC,CNGA3,CNGB1,CNGB3,CNKSR2,CNOT3,COL2A1,COL18A1,CP,CRX,CST3,CTNNB1,CTSD,CWC27,CYFIP2,CYP4V2,DALRD3,DDX6,DHDDS,DHX38,DLAT,DLG4,DNM1,DOCK3,DPYSL5,DRAM2,EEF1A2,EFEMP1,ELOVL4,EMC10,ERCC2,ERCC3,ERCC6,ERCC8,FBLN1,FBLN5,FBN2,FBXW11,FGF12,FGF13,FKRP,FKTN,GABRA2,GABRA5,GABRB2,GABRG2,GBA,GNAT2,GNB5,GNPTAB,GRIA4,GRIN2D,GRK1,GTF2E2,GTF2H5,GUCA1A,GUCA1B,GUCY2D,HCN1,HGSNAT,HK1,HLAA,HMCN1,HPDL,HS2ST1,HSD17B10,HTT,HUWE1,IDH3A,IDUA,IFT140,IFT172,IMPDH1,IMPG2,INPP5E,JARID2,KAT8,KCNA2,KCNB1,KCNJ13,KCNMA1,KCNV2,KDM4B,KIAA1549,KMT2E,LMA1,LARGE1,LCA5,LIG3,LMBRD2,LMNA,LRAT,LZTFL1,MAB21L1,MADD,MAN2B1,MAN2C1,MAPK8IP3,MAPKAPK3,MCOLN1,MED13,MED27,MERTK,MFRP,MIR204,MMACHC,MPLKIP,PALS1,MTTP,MYT1L,NECAP1,NMNAT1,NR2E3,NRL,NTNG1,NTNG2,NTRK2,NUS1,OPA1,PANK2,PARS2,PAX2,PCYT1A,PDE6C,PDE6H,PEX7,PHYH,PISD,PITPNM3,PLK4,PNPLA6,POMGNT1,POMK,POMT1,POMT2,POU3F4,PPP3CA,PPT1,PRCD,PROM1,PRPF4,PRPF8,PRPF31,PRPH2,PSMD12,PTPN23,PUS7,RAX2,RBP4,RDH5,RDH11,REEP6,RHO,RIMS1,RLBP1,RLIM,RNF2,RNF113A,RP1L1,RP9,RPE65,RPGR,RPGRIP1,RS1,SAG,SATB1,SCAPER,SCN3A,SCN8A,SDCCAG8,SEMA4A,SETD1A,SH3BP2,SIX6,SLC1A2,SLC6A6,SLC7A14,SLC12A2,SLC13A5,SLC19A2,SNRNP200,SPATA7,SPG11,SPTBN1,SRCAP,STUB1,SUMF1,SVBP,SYNCRIP,SYNGAP1,SYNJ1,SZT2,TANC2,TARS1,TBR1,TCF7L2,TCF20,TIMP3,TLCD3B,TMEM67,TNFRSF11B,TNRC6B,TOPORS,TPP1,TRAF3IP1,TRAK1,TRAPPC4,TRIP12,TRMT1,TRNT1,TTC8,TTC21B,TUB,TUBB4B,TULP1,UBA5,UBR7,USP45,VCAN,WASF1,WDFY3,WDR19,WRN,WWOX,XYLT1,XYLT2,YARS1,YWHAG,ZFYVE26,ZMIZ1,ZNF423,ZNF513,CLN3,GUCA1A,NR2E3,NR2E3,SUMF1,NR2E3

Obstetrics and Gynecology

NGS has been improved forfetal mutational status and aneuploidy screening. The advance of the use of NGS in obstetrics and gynecology is to perform NGS for preimplantation genetic testing, a comprehensive testing for 23 chromosome pairs and trophectoderm (TE) biopsy at the blastocyst stage (40); prenatal whole genome sequencing (41); Non-invasive prenatal diagnosis for aneuploidy testing (42), and the Guthrie test, also named as phenylketonuria test (43). Whole-exome sequencing identifies fetal abnormalities and fetal DNA can be obtained from amniotic liquid, chorionic villi, umbilical cord blood, or abortion material(44). Maternal plasma samples can be used in non-invasive prenatal screening for aneuploidy and For fetal screening of mutational status Trio

sequencing (fetus and parents) is recommended in order to filter uninformative genomic variants (45). Besides fetal screening, NGS can be used to identify common gynecological problems in women. The genes associated with related disorders are shown in Table 6.

Table 6. Genes associated with common gynecological diseases

Primary ovarian insufficiency	AARS2,AIRE,ANAPC1,ANAPC7,B4GALNT1,BLM,BMP15,BNC1,C14orf39,CASP10,CEP164,CEP290,CHP1,COL25A1,DCAF17,DIAPH2,EIF2B1,EIF2B2,EIF2B3,EIF2B4,EIF2B5,ERAL1,ERCC4,FAS,FASLG,FIGLA,FMR1,FOXL2,FSHR,GALK1,GALT,GGPS1,HFM1,HSF2BP,INVS,IQCB1,LARS2,LMNA,MCM3AP,MCM8,MRPS22,NBN,NOBOX,NPHP1,NPHP3,NPHP4,NR5A1,NUP107,PMM2,POF1B,POLG,POLG2,POLR3H,PRKCD,PSMC3IP,RASGRP1,RCBTB1,RIN2,SDCCAG8,SPIDR,STAG3,THOC6,TRAF3IP1,TTI2,TWNK,WDR19,XRCC2,ZFHX4,ZSWIM7
Thrombophilia	AGGF1,ANK1,DLD,EPB42,F5,F9,GATA2,HBB,HRG,MYD88,PROC,PROS1,SLC4A1,SPTA1,SPTB,THBD
Polycystic ovaries	APAT2,AKT1,AKT2,ALMS1,ANTXR2,ATM,BAZ1B,BCL7B,BSCL2,BUD23,CAV1,CAVIN1,CBX2,CIDEC,CLIP2,CORIN,CYB5A,CYP11B1,CYP17A1,CYP19A1,DHH,DHX37,DMRT1,DNAJC30,EIF4H,ELN,ESR1,FKBP6,FLT1,FOS,FOXL2,FSHR,GTF2I,GTF2IRD1,GTF2IRD2,HNF1A,KLLN,LIMK1,LIPE,LMNA,LMNB2,MAP3K1,METTL27,MLXIPL,MMP2,MMP14,MSX1,MT-CYB,NCF1,NR5A1,NR0B1,PHKA2,PHKB,PHKG2,PIK3CA,PLIN1,POR,PPARG,PTEN,RFC2,SDHB,SDHC,SDHD,SEC23B,SETD2,SLC37A4,SOX3,SOX9,SRY,STK11,STOX1,STX1A,TBL2,TMEM270,USF3,VPS37D,WT1,GTF2I,GTF2I,GTF2I

Abnormality of the menstrual cycle	AGGF1,AGPAT2,AIP,AKT1,AKT2,ALMS1,ANOS1,ANTXR1,AR,ARMC5,ATP7B,AXL,BAP1,BBS9,BCOR,BLOC1S3,BLOC1S5,BMP2,BMP15,BMPR1B,BNC1,BPTF,BRD4,BSCL2,C14orf39,CAV1,CAVIN1,CCDC141,CDH23,CDKN1A,CDKN1B,CDKN1C,CDKN2B,CDKN2C,CDON,CHD7,CIDEC,CISD2,CLPP,CPE,CTDP1,CYB5A,CYP11B1,CYP17A1,CYP19A1,DCC,DHH,DHX37,DIAPH1,DIAPH2,DMRT3,DNM1L,DUSP6,EIF2B1,EIF2B2,EIF2B3,EIF2B4,EIF2B5,ERAL1,ERCC6,ERCC8,ESR1,ESR2,F2,F5,F7,F8,F10,F11,F13A1,F13B,FANCM,FEZF1,FGA,FGB,FGF8,FGF17,FGFR1,FGG,FIGLA,FIP1L1,FLI1,FLRT3,FMR1,FOS,FOXA2,FOXL2,FSHB,FSHR,GALT,GATA3,GATA4,GDF9,GHR,GLI2,GNAS,GNRH1,GNRHR,GP1BA,GP1BB,GP6,GP9,GPR101,GPR161,H6PD,HAMP,HARS2,HDAC8,HERC2,HESX1,HFE,HFM1,HJV,HMGA2,HPS4,HPS5,HS6ST1,HSD17B4,HSF2BP,IGF2,IL17RD,IPW,IRF2BP2,ITGA2B,ITGA8,ITGB3,KISS1,KISS1R,LARS2,LEP,LEPR,LHB,LHX4,LIG4,LIPE,LMAN1,LMNA,MAGEL2,MAP3K1,MCFD2,MCM8,MCM9,MEN1,MKRN3,MRPS22,MSH4,MSH5,MSTO1,MYH9,NABP1,NBEAL2,NDN,NDNF,NF2,NIN,NIPBL,NOBOX,NPAP1,NPM1,NR3C1,NR5A1,NR0B1,NSMF,NUMA1,NUP107,OCA2,OTX2,PAPSS2,PCSK1,PDE4D,PDE8B,PDE11A,PDGFB,PEX1,PEX6,PHKA2,PHKB,PHKG2,PIK3CA,PLAG1,PLAU,PLIN1,PML,POF1B,POLG,POLG2,POLR3H,POR,POU1F1,PPARG,PRKACA,PRKACG,PRKAR1A,PRLR,PROK2,PROKR2,PROP1,PRORP,PSMB8,PSMC3IP,PSMD12,PTPN11,PWAR1,PWRN1,RAD21,RARA,RCBTB1,RNF216,ROBO1,SEMA3A,SEMA3E,SERPINE1,SETD2,SIM1,SLC25A13,SLC29A3,SLC37A4,SLFN14,SMARCB1,SMARCE1,SMC1A,SMC3,SMCHD1,SMO,SNORD115-1,SNORD116-1,SNRPN,SOHLH1,SOST,SOX3,SOX9,SOX10,SPIDR,SPRY4,SRA1,SRY,STAG3,STAT3,STAT5B,STUB1,SUFU,SYCE1,TAC3,TACR3,TBL1X,TBL1XR1,TERT,TFR2,TKT,TP63,TRAF7,TRMT10A,TWNK,USP8,VAMP7,VWF,WAS,WDR11,WIPF1,WNT4,WNT7A,WRN,WT1,WWOX,YARS1,ZBTB16,ZFPM2,ZMPSTE24,ZSWIM7,PDE8B,PDE11A,PRORP

Psychiatry

Psychiatric disorders are not monogenic but polygenic. Genome-wide association studies revealed that there were more than 100 loci associated with psychiatric disorders (46). It has been observed that rare indels/SNVs, loss of function variants are enriched in neurodevelopmental disorders including schizophrenia, autism, intellectual disability and etc (47). Genes related to neurodevelopmental diseases are shown in Table 7.

Table 7. Genes associated with neuropsychiatric disorders

Schizophrenia	ADGRV1,AMACR,APOL2,APOL4,ARSA,ARSG,ARVCF,ATG7,ATP2A2,CAT,CDH23,CEP78,CHI3L1,CHRNA7,CIB2,CLRN1,COMT,DAOA,DISC2,DNAJC13,DNMT3A,DRD3,DSG4,EIF4G1,ESPN,FLI1,FTSJ1,GBA,GIGYF2,GJA5,GJA8,GP1BB,HARS1,HIRA,HTR2A,JMJD1C,KRT81,KRT83,KRT86,LRRK2,MED12,MSTO1,MT-TE,MT-TS2,MTHFR,MYO7A,NIPA1,NIPA2,NKX2-1,PCDH15,PDZD7,PRODH,PSAP,RBM12,RREB1,RTN4R,SEC24C,SETD1A,SHANK3,SNCA,SYN2,TBX1,TUBG1,UFD1,UPF3B,USH1C,USH1G,USH2A,VPS35,WHRN,ZBTB20,ZDHHC9,GJA5,GJA5,GJA5
Bipolar	ARVCF,ATP2A2,CEP85L,CHRNA7,CLCN4,COMT,DNMT3A,FA2H,FLI1,GP1BB,GRIA1,HIRA,JMJD1C,MECP2,MT-CO1,MT-CO2,MT-CO3,MT-ND1,MT-ND4,MT-ND5,MT-ND6,MT-TF,MT-TH,MT-TL1,MT-TQ,MT-TS1,MT-TS2,MT-TW,POLG,POLG2,RPS6KA3,RREB1,RRM2B,SEC24C,SLC25A4,SMPD1,TBCK,TBX1,TWNK,UFD1,VPS16
Anorexia	ACAT1,ALPL,AQP2,ARG1,ASXL1,ATM,ATP1A2,ATP1A3,ATRX,AVPR2,BCOR,BRCA1,BRCA2,C4A,CACNA1A,CALR,CBL,CBS,CCND1,CCR1,CD3D,CD3E,CD247,CDKN1A,CDKN1B,CDKN2A,CDKN2B,CDKN2C,DAXX,ERAP1,FAS,FIP1L1,FLI1,GBA,GLA,HLA-B,HLA-DRB1,HLCS,HMGCL,IFNGR1,IL10,IL12A,IL12A-AS1,IL12B,IL23R,IRF2BP2,IRF4,JAK2,KLRC4,KRAS,MC2R,MECP2,MEFV,MEN1,MLX,MMADHC,MMUT,MPL,MRAP,MYD88,NABP1,NFS1,NNT,NPM1,NUMA1,P4HA2,PALB2,PALLD,PKHD1,PML,PRKAR1A,PTPN3,PTPN22,RABL3,RARA,RUNX1,SCARB2,SLC1A3,SLC4A1,SLC19A2,SLC39A4,SLC46A1,SMAD4,SRSF2,STAR,STAT3,STAT4,STAT5B,SYK,TBL1XR1,TET2,TGFB1,TLR4,TP53,TXNRD2,UBAC2,ZBTB16,RUNX1
Obsessive-compulsive behavior	ADCY5,ADGRV1,ADNP,AFF2,AP1G1,ARID1B,ASH1L,AUTS2,BAZ1B,BCL7B,BCR,BRD4,BUD23,C9orf72,CARS1,CHD5,CHD7,CHMP2B,CLCN3,CLCN4,CLIP2,CLN5,COASY,CREBBP,CRKL,DMPK,DNAJC30,DPF2,DRD2,EHMT1,EIF4H,ELN,EP300,FKBP6,FMO3,FMR1,FOXP1,GABRA1,GABRD,GABRG2,GATAD2B,GCH1,GRIA1,GRIA2,GRN,GTF2I,GTF2IRD1,GTF2IRD2,HCN1,HDAC4,HDAC8,HDC,HNRNPH2,HTR2A,HTT,IMPDH2,KCTD17,KDM4B,LIMK1,MAGEL2,MAP1B,MAPK1,MAPK8IP3,MAPT,MED12,METTL27,MLXIPL,NCF1,NDN,NIPBL,NKX2-1,NLGN1,NR2F1,OCA2,OCRL,PANK2,PCDH19,PDZD8,PITRM1,POGZ,PPM1D,PSEN1,RAD21,RFC2,SCN1A,SCN1B,SCN2A,SCN8A,SCN9A,SEMA3E,SETD5,SGCE,SIN3A,SIN3B,SLC2A3,SLC6A4,SLC35C1,SLC45A1,SLITRK1,SMC1A,SMC3,SNRPN,SPTBN1,SQSTM1,SRCAP,STX1A,STX1B,SYNGAP1,TBL2,TBX1,TCF20,TIAM1,TKT,TLK2,TMEM106B,TMEM270,TOR1A,TREM2,TRIO,TRRAP,TTC19,UBE2A,UBE3A,USP7,USP9X,VCP,VPS13A,VPS16,VPS37D,XK,YWHAG,GTF2I,GTF2I,GTF2I

Depression	AARS2,ABCA7,ABCB4,ABCB11,ADGRV1,ADH1C,AFG3L2,AIMP1,AIP,ALAD,ALDH4A1,ALKBH8,AMACR,ANG,ANXA11,AOPEP,AP1G1,AP2S1,AR,ARMC5,ARSA,ARSG,ARVCF,ATP1A3,ATP7B,ATP8B1,ATP13A2,ATRX,ATXN2,ATXN8OS,ATXN10,B3GALNT2,BAZ1B,BCL7B,BCR,BCS1L,BMPR1A,BUD23,C9orf72,C12orf4,C19orf12,CABP4,CACNA1G,CACNA1H,CARS1,CASR,CBS,CC2D1A,CCNF,CDH23,CDKN1A,CDKN1B,CDKN2B,CDKN2C,CDON,CEP78,CEP85L,CFAP410,CHCHD10,CHD7,CHMP2B,CHRNA2,CHRNA4,CHRNB2,CIB2,CISD2,CLCN4,CLIP1,CLIP2,CLN6,CLRN1,COASY,COL7A1,COMT,COQ2,CPOX,CRADD,CRBN,CRH,CRKL,CSF1R,CTSF,CYP27A1,DAO,DCPS,DCTN1,DEPDC5,DGUOK,DISP1,DLL1,DNA2,DNAJC5,DNAJC6,DNAJC13,DNAJC30,DNMT1,DRD2,DUSP6,EDC3,EHMT1,EIF4G1,EIF4H,ELN,EPCAM,EPHA4,EPM2A,ERBB4,ESPN,EZR,FA2H,FAN1,FBXO31,FGF8,FGF14,FGF17,FGFR1,FIG4,FKBP5,FKBP6,FLT4,FMN2,FMO3,FMR1,FOXH1,FRRS1L,FUS,GABRA1,GABRB3,GABRG2,GALT,GAS1,GBA,GCH1,GDAP2,GIGYF2,GLA,GLE1,GLI2,GLT8D1,GLUD2,GNA11,GNAS,GNRH1,GNRHR,GP1BB,GPR35,GPR101,GRIK2,GRIN2A,GRM7,GRN,GSN,GTF2I,GTF2IRD1,GTF2IRD2,HARS1,HIRA,HLAB,HLADQA1,HLADQB1,HLADRB1,HMBS,HNMT,HNRNPA1,HS6ST1,HTR2A,HTRA2,HTT,IDUA,IFNG,IMPDH2,IQSEC1,IRF4,JMJD1C,JPH3,JRK,KCNJ2,KCNT1,KCTD17,KDM5B,KISS1,KISS1R,KRAS,LGI1,LIMK1,LINS1,LMAN2L,LMNB1,LRRK2,MAMLD1,MAN1B1,MAN2B1,MAPK1,MAPT,MATR3,MBOAT7,MECP2,MED23,MED25,MEN1,METTL23,METTL27,MLH1,MLH3,MLXIPL,MMP1,MSH2,MSH6,MST1,MSTO1,MT-CO1,MT-CO2,MT-CO3,MT-ND1,MT-ND4,MT-ND5,MTND6,MTTF,MTTH,MTTL1,MTTL2,MTTN,MTTQ,MTTS1,MTTS2,MTTT,MTTW,MYO7A,NAA20,NCDN,NCF1,NDST1,NEFH,NEK1,NEMF,NHLRC1,NODAL,NOTCH3,NR1H4,NR4A2,NSMF,NSUN2,OCRL,OPTN,P4HA2,PAH,PANK2,PARK7,PCDH15,PDCD1,PDE8B,PDE11A,PDGFB,PDGFRB,PDZD7,PER2,PER3,PFN1,PGAP1,PIGC,PIK3CA,PINK1,PLA2G6,PLP1,PMS1,PMS2,PODXL,POLG,POLG2,PON1,PON2,PON3,PPARGC1A,PPP2R2B,PPT1,PRKACA,PRKAR1A,PRKCG,PRKN,PRNP,PROK2,PROKR2,PRPH,PRSS12,PSAP,PSEN1,PTCH1,PTPN22,PTS,RELN,RFC2,RPS6KA3,RPS20,RREB1,RRM2B,RSRC1,SARS1,SDHA,SEC24C,SEMA4A,SEMA4D,SGCE,SHH,SIX3,SLC2A1,SLC2A3,SLC6A4,SLC7A6OS,SLC12A2,SLC20A2,SLC25A4,SLC45A1,SMC1A,SMPD1,SNCA,SNCAIP,SOD1,SPAST,SPRY4,SPTBN1,SQSTM1,SRPX2,ST3GAL3,STAG2,STIL,STUB1,STX1A,STX16,SYNJ1,TAC3,TACR3,TAF15,TARDBP,TBC1D7,TBK1,TBL2,TBP,TBX1,TCF4,TDGF1,TECR,TET3,TGFBR2,TGIF1,THOC2,TK2,TMEM106B,TMEM270,TNIK,TOR1A,TPH2,TRAPPC9,TREM2,TREX1,TRHR,TSC1,TSC2,TSHB,TTC5,TTC19,TUSC3,TWNK,UBE4A,UBQLN2,UCHL1,UFD1,UFSP2,UNC13A,UQCRC1,USH1C,USH1G,USH2A,USP8,VAPB,VCP,VPS13A,VPS13C,VPS35,VPS37D,WARS2,WASHC4,WDR11,WFS1,WHRN,XK,XPR1,ZC3H14,ZIC2,GTF2I,GTF2I,MATR3,PDE8B,PDE11A,TAF15,TAF15,TBC1D7,GTF2I,TAF15

Hyperactivity	AARS1,AASS,ABCD1,ACSL4,ACTL6A,ACTL6B,ACY1,ADAT3,ADH5,ADNP,ADSL,AFF2,AGO2,AGTR2,AIMP1,ALDH5A1,ALG13,ALG14,ALKBH8,AMT,ANAPC1,ANK3,ANKRD11,ANKRD17,AP1G1,AP2M1,AP3B2,APC2,ARF1,ARG1,ARHGEF6,ARID1A,ARID1B,ARID2,ARV1,ARVCF,ARX,ASH1L,ASPM,ATP1A1,ATP1A2,ATP1A3,ATP6V1A,ATP6V0A1,ATP10A,ATR,ATRX,AUTS2,B3GALNT2,BAP1,BAZ1B,BCAP31,BCAS3,BCL7B,BCORL1,BCR,BMPR1A,BPTF,BRD4,BSCL2,BUD23,C12orf4,CABP4,CACNA1A,CACNA1B,CACNA1H,CAMTA1,CARS1,CASK,CC2D1A,CCBE1,CCDC32,CDC42BPB,CDH2,CDH11,CDK8,CDK19,CDKL5,CDKN1C,CDON,CELF2,CHD2,CHD5,CHD7,CHRNA2,CHRNA4,CHRNA7,CHRNB2,CIC,CLCN3,CLCN4,CLIP1,CLIP2,CLN5,CLTC,CNKSR2,CNOT3,CNTNAP2,COMT,CORO1A,CRADD,CRBN,CREBBP,CRH,CRKL,CSGALNACT1,CSNK2A1,CUL3,CUL4B,CUX2,STEEP1,CYFIP2,CYP27A1,DALRD3,DCDC2,DCPS,DDB1,DDX3X,DDX6,DEAF1,DEPDC5,DHCR7,DHDDS,DHTKD1,DISP1,DLG3,DLG4,DLL1,DMD,DMXL2,DNAJC12,DNAJC21,DNAJC30,DNM1,DOCK3,DPF2,DPH1,DPP6,DPYD,DPYSL5,DRD4,DRD5,DYM,DYNC1I2,DYRK1A,EBP,EDC3,EEF1A2,EIF2AK1,EIF4H,ELN,EMC10,EP300,EPCAM,EZR,FAN1,FANCD2,FANCL,FBXO11,FBXO31,FBXW11,FGD1,FGF8,FGF12,FGF13,FGFR1,FGFR3,FKBP6,FLCN,FLI1,FLII,FMN2,FMR1,FOXH1,FOXP1,FRMPD4,FRRS1L,FTSJ1,GABRA1,GABRA2,GABRA5,GABRB2,GABRB3,GABRG2,GALC,GALT,GAMT,GAS1,GATA4,GATAD2B,GCSH,GDI1,GLDC,GLI2,GLRA2,GLUD1,GNAO1,GNAQ,GNB1,GNB5,GNE,GNS,GP1BB,GRIA1,GRIA4,GRIK2,GRIN1,GRIN2A,GRIN2D,GRM7,GTF2I,GTF2IRD1,GTF2IRD2,H3-3A,H4C5,HAL,HCFC1,HCN1,HDAC4,HDAC8,HDC,HERC2,HGSNAT,HIRA,HIVEP2,HMGA2,HNMT,HNRNPH2,HOXA2,HPDL,HS2ST1,HSPG2,HTT,HUWE1,IFNG,IGF1,IGF2,IKBKG,IL1RAPL1,INPP5E,IPW,IQSEC1,IQSEC2,JARID2,JMJD1C,JRK,KANSL1,KAT5,KAT8,KCNA1,KCNA2,KCNB1,KCNC2,KCNK9,KCNMA1,KCNN2,KCNT1,KDM3B,KDM4B,KDM5B,KDM5C,KDM6B,KIF11,KIF14,KIF15,KMT2A,KMT2B,KMT2E,KMT5B,KRAS,LGI3,LHCGR,LIG4,LIMK1,LINS1,LMAN2L,LMBRD2,LNPK,MADD,MAGEL2,MAN1B1,MAN2C1,MANBA,MAP1B,TRAPPC14,MAPK1,MAPK8IP3,MAPK10,MBD5,MBOAT7,MCTP2,MECP2,MED12,MED12L,MED13,MED13L,MED23,MED25,MED27,METTL5,METTL23,METTL27,MICU1,MID2,MKRN3,MLH1,MLH3,MLXIPL,PALS1,MSH2,MSH6,MTOR,MYT1L,NAA15,NAA20,NAGLU,NBEA,NBN,NCDN,NCF1,NDP,NDST1,NECAP1,NEMF,NEUROD2,NEXMIF,NF1,NFIX,NIPA1,NIPA2,NIPBL,NKAP,NKX21,NLGN1,NODAL,NOP56,NPAP1,NR2F1,NSD1,NSD2,NSDHL,NSUN2,NTNG1,NTNG2,NTRK1,NTRK2,NUS1,OCA2,OCRL,ODC1,OPHN1,PAH,PAK3,PANK2,PARS2,PCDH19,PCGF2,PCNT,PDCD6IP,PDE4D,PDZD8,PGAP1,PHF21A,PHIP,PIDD1,PIEZO2,PIGC,PIGP,PIGQ,PIK3CA,PLA2G6,PLAG1,PMS1,PMS2,PNKP,PNP,POGZ,POLA1,PPM1D,PPP1R12A,PPP1R21,PPP2R1A,PPP3CA,PRKAR1A,PRKAR1B,PRKCG,PRNP,PRODH,PRR12,PRSS12,PSMB1,PSMD12,PTCH1,PTCHD1,PTPN23,PUF60,PUS7,PWAR1,PWRN1,RAB39B,RAC1,RAD21,RAI1,RERE,RFC2,RIC1,RLIM,RNF2,RPS6KA3,RPS20,RREB1,RSRC1,RUSC2,SARS1,SATB1,SATB2,SCN1A,SCN1B,SCN2A,SCN3A,SCN8A,SEC24C,SEMA3E,SEMA4A,SETBP1,SETD1A,SETD2,SETD5,SGSH,SH2B1,SH3KBP1,SHANK3,SHH,SHMT2,SHOC2,SHROOM4,SIK1,SIM1,SIN3A,SIX3,SLC1A2,SLC1A4,SLC2A1,SLC6A1,SLC6A8,SLC7A6OS,SLC9A7,SLC12A2,SLC13A5,SLC25A13,SLC25A22,SLC45A1,SLITRK1,SMARCA2,SMARCA4,SMARCB1,SMARCC2,SMARCD1,SMARCE1,SMC1A,SMC3,SMPD1,SNORD115,SNORD116,SNRPN,SOX4,SOX5,SOX6,SOX11,SPEN,SPG7,SPR,SPRED1,SPTBN1,SRCAP,SRPX2,ST3GAL3,STAG2,STIL,STS,STX1A,SVBP,SYNCRIP,SYNGAP1,SYNJ1,SYP,SZT2,TAF1,TAF6,TANC2,TAOK1,TBL1X,TBL2,TBR1,TBX1,TCF7L2,TCF20,TDGF1,TECR,TET3,TGFBR2,TGIF1,THRB,TIAM1,TIMM8A,TKT,TLK2,TMCO1,TMEM67,TMEM222,TMEM270,TNIK,TNPO2,TNRC6B,TPH2,TRAK1,TRAPPC4,TRAPPC9,TRIM8,TRIO,TRIP12,TRMT1,TSC1,TSC2,TSHB,TSHR,TSPAN7,TTC5,TTI2,TUBB2B,TUBG1,TUSC3,UBA5,UBE3A,UBE4A,UBR7,UBTF,UFD1,UFSP2,UPF3B,UQCC2,USP7,USP9X,USP27X,VPS13A,VPS37D,WAC,WASF1,WASHC4,WBP11,WDFY3,WDR62,WWOX,YME1L1,YWHAG,YY1,ZC3H14,ZDHHC9,ZIC2,ZMIZ1,ZMYM2,ZNF41,ZNF81,ZNF292,ZNF711,ZSWIM6,GTF2I,GTF2I,H4C5,H4C5,IKBKG,IKBKG,KMT2B,PCGF2,PCGF2,SPG7,USP27X,USP27X,H4C5,GTF2I,PCGF2,USP27X,IKBKG

Cardiology

Cardiovascular diseases with mendelian inheritance encompass multiple syndromes such as Familial cardiomyopathies (idiopathic or familial Dilated Cardiomyopathy, Arrhythmogenic right ventricular cardiomyopathy, Hypertrophic Cardiomyopathy), Familial arrhythmias (Long QT syndrome, Short QT Syndrome, Brugada syndrome, Catecholaminergic Polymorphic Ventricular Tachycardia)familial hypercholesterolemia, Marfan syndrome, and Sudden Arrhythmic Death Syndrome. The ratio for identification of causative variations by NGS-based platforms is 80% for long QT syndromes and 20-40% for dilated cardiomyopathy (48). Table 8 represents the cardiovascular disease-associated genes.

Table 8. Genes associated with cardiac diseases

Cardiomyopathy	AARS2,ABCC6,ABCC8,ABCC9,ABHD5,ACAD8,ACAD9,ACADS,ACADVL,ACTA1,ACTC1,ACTN2,ADA2,ADAR,ADCY5,ADORA2A,AGK,AGL,AGPAT2,AHCY,AIP,ALG1,ALG3,ALMS1,ALPK3,ANK1,ANKRD1,ANKRD11,ANKS6,ANO5,ARSB,ATAD3A,ATP1A2,ATP1A3,ATP5F1D,ATP5F1E,ATP5MK,ATP6V1A,BAG3,BAG5,BAZ1B,BBS2,BCL7B,BCS1L,BMP2,BOLA3,BRAF,BRCA1,BRCA2,BRCC3,BRIP1,BSCL2,BUD23,C1QBP,CACNA1A,CAP2,CASQ2,CASZ1,CAV1,CAV3,CAVIN1,CCND1,CDKN1C,CENPE,CHKB,CISD2,CLIP2,CLN3,CLPB,COA5,COA6,COA8,COL7A1,COQ2,COQ4,COX6B1,COX7B,COX10,COX14,COX15,CPT1A,CPT2,CRYAB,CSRP3,D2HGDH,DCAF8,DEF6,DES,DHCR7,DLD,DLK1,DMD,DNAJC19,DNAJC30,DOLK,DPM3,DSC2,DSG2,DSP,DTNA,ECHS1,EIF4H,ELAC2,ELN,EMD,ENPP1,EPB42,EPG5,ERBB3,ERCC2,ERCC3,ERCC4,ERCC6,ERCC8,EYA4,FAH,FANCA,FANCB,FANCC,FANCD2,FANCE,FANCF,FANCG,FANCI,FANCL,FANCM,FASTKD2,FBXL4,FHL1,FHL2,FHOD3,FIG4,FKBP6,FKRP,FKTN,FLAD1,FLNC,FNIP1,FOS,FOXRED1,FTO,FXN,GAA,GABRD,GATA4,GATA5,GATAD1,GATC,GBE1,GJA5,GLA,GLB1,GMPPB,GNE,GNPTAB,GNS,GPC3,GPC4,GPR101,GSN,GTF2E2,GTF2H5,GTF2I,GTF2IRD1,GTF2IRD2,GTPBP3,GUSB,GYG1,GYS1,HADH,HADHA,HADHB,HAMP,HAND2,HBB,HCCS,HFE,HGSNAT,HJV,HLA-B,HMGCL,HNRNPA1,HNRNPA2B1,HPS1,HRAS,HSD17B10,HSPG2,IDH2,IDS,IDUA,IFIH1,IGF2,IL12B,INSR,ITPA,JAG2,JPH2,JUP,KANSL1,KAT6B,KBTBD13,KCNAB2,KCNH1,KCNJ2,KCNJ5,KCNJ8,KCNJ11,KCNQ1,KCNQ1OT1,KIF20A,KLF1,KLHL41,KRAS,LAMA2,LAMA3,LAMA4,LAMB3,LAMC2,LAMP2,LDB3,LIAS,LIMK1,LIMS2,LIPT1,LMNA,LMOD2,LRP12,LRPPRC,LTBP4,LUZP1,LZTR1,MAD2L2,MAP2K1,MAP2K2,MC2R,MCM10,MEFV,MEG3,MEN1,METTL27,MGME1,MIB1,MICOS13,MIPEP,MLX,MLXIPL,MLYCD,MMACHC,MMP1,MMP23B,MMUT,MPLKIP,MRAP,MRAS,MRPL3,MRPL44,MRPS14,MRPS22,MT-ATP6,MT-CO1,MT-CO2,MT-CO3,MT-ND1,MT-ND2,MT-ND3,MT-ND4,MT-ND5,MT-ND6,MT-TE,MT-TF,MT-TH,MT-TK,MT-TL1,MT-TN,MT-TQ,MT-TS1,MT-TS2,MT-TT,MT-TV,MT-TW,MTFMT,MTO1,MYBPC3,MYH6,MYH7,MYL2,MYL3,MYLK2,MYO18B,MYOCD,MYOT,MYOZ2,MYPN,MYSM1,NAGA,NAGLU,NAXD,NBAS,NCF1,NDUFA1,NDUFA2,NDUFA4,NDUFA6,NDUFA9,NDUFA10,NDUFA11,NDUFA12,NDUFA13,NDUFAF1,NDUFAF2,NDUFAF3,NDUFAF4,NDUFAF5,NDUFAF6,NDUFAF8,NDUFB3,NDUFB8,NDUFB9,NDUFB10,NDUFB11,NDUFS1,NDUFS2,NDUFS3,NDUFS4,NDUFS6,NDUFS7,NDUFS8,NDUFV1,NDUFV2,NEB,NEK8,NEU1,NEXN,NF1,NFS1,NNT,NONO,NPPA,NRAS,NUBPL,NUP107,OPA1,PALB2,PARS2,PCCA,PCCB,PDGFRA,PDHA1,PDPN,PET100,PEX1,PEX2,PEX3,PEX5,PEX6,PEX7,PEX10,PEX11B,PEX12,PEX13,PEX14,PEX16,PEX19,PEX26,PGM1,PHKA2,PHKG2,PHYH,PIGT,PKP2,PLN,PMM2,PNPLA2,POLG,POLG2,POMGNT1,POMK,POMT1,POMT2,PPA2,PPARG,PPCS,PPP1CB,PPP1R21,PRDM16,PRKAG2,PRKCZ,PSEN1,PSEN2,PTPN11,PYGL,PYGM,QRSL1,RAB3GAP2,RAD51,RAD51C,RAF1,RBCK1,RBM20,RERE,RFC2,RFWD3,RIT1,RMND1,RMRP,RNASEH1,RNASEH2A,RNASEH2B,RNASEH2C,RNF113A,RNF220,RNU4ATAC,RPL3L,RRM2B,RTL1,RYR1,RYR2,SAMHD1,SARDH,SCN5A,SCO2,SDHA,SDHAF1,SDHB,SDHD,SELENON,SGCA,SGCB,SGCD,SGSH,SHMT2,SHOC2,SKI,SLC1A3,SLC2A10,SLC4A1,SLC5A6,SLC6A6,SLC19A2,SLC19A3,SLC22A5,SLC25A3,SLC25A4,SLC25A20,SLC30A10,SLC40A1,SLX4,SMC1A,SOS1,SPEG,SPEN,SPRED2,SPTA1,SPTB,STAR,STX1A,SUCLG1,SUFU,SURF1,SYNE1,SYNE2,TACO1,TAF1A,TANGO2,TAPT1,TARS1,TAFAZZIN,TBL2,TCAP,TERT,TFR2,TGFB1,TGFB3,TIMMDC1,TK2,TKFC,TMEM43,TMEM70,TMEM126A,TMEM126B,TMEM270,TMPO,TNNC1,TNNI3,TNNI3K,TNNT2,TOP3A,TPI1,TPM1,TPM2,TPM3,TRDN,TREX1,TRIP4,TRMT5,TRNT1,TSFM,TTN,TTPA,TTR,TULP3,TWNK,TXNRD2,UBE2T,UBE4B,UBR1,UCP2,UQCRFS1,USP9X,VAC14,VCL,VCP,VHL,VPS13A,VPS33A,VPS37D,WARS2,WFS1,XK,XRCC2,XRCC4,XYLT1,XYLT2,YARS2,CLN3,GJA5,GJA5,GTF2I,GTF2I,RMRP,RMRP,SCO2,SCO2,GJA5,GTF2I,RMRP,SCO2

Hypercholesterol	ABCB4,ABCG5,ABCG8,AGPAT2,ALB,APOB,APOC2,APOC3,APOE,APTX,ATP6AP1,BSCL2,CAV1,CAV3,CAVIN1,CCDC115,CEP19,CETP,COG4,CREB3L3,CYP7A1,DEAF1,DGAT1,DIO1,DLK1,DYRK1B,FLII,FOS,GALK1,GHR,IFT172,IQSEC2,JAG1,KIF12,LDLR,LDLRAP1,LIPA,LIPC,LMNA,LPL,LRP6,MEF2A,MEG3,MYO5B,NUP107,OCRL,PCSK9,PHKA2,PHKB,PHKG2,PIK3R5,PPARG,PYGL,RAI1,RSPO1,RTL1,SETX,SLC7A7,SLC25A13,SLC37A4,TBL1X,TDP1,TMEM199,TSHB,TTC26,TTPA
Cardiac arrest	ABCA3,ABCA12,ABCC6,ABCC9,ABCG5,ABCG8,ACAD9,ACADVL,ACTC1,ACTN2,AKAP9,AKT1,ALG10B,ALPK3,ANK2,APOB,BAZ1B,BCL7B,BUD23,CACNA1C,CACNA2D1,CACNB2,CALM1,CALM2,CALM3,CASQ2,CAV3,CLCF1,CLIP2,CPT1A,CRLF1,CSRP3,DES,DNAJC19,DNAJC30,DPP6,DSC2,DSG2,DSP,DTNA,EIF4H,ELN,EMD,ENPP1,EYA4,FHL1,FHOD3,FKBP6,FLNC,GBA,GNAI2,GPD1L,GTF2I,GTF2IRD1,GTF2IRD2,HCN4,HLA-B,HLA-DRB1,HMGCL,IKZF1,JUP,KCND3,KCNE1,KCNE2,KCNE3,KCNE5,KCNH2,KCNJ2,KCNJ5,KCNJ8,KCNQ1,LDB3,LDLR,LDLRAP1,LEMD2,LIMK1,LMNA,LRP6,METTL27,MLXIPL,MRPL12,MYBPC3,MYH7,MYL2,MYL3,MYOZ2,NCF1,NDUFB11,NOS1AP,NUP155,P4HA2,PCSK9,PGM1,PKP2,PPA2,PRKAG2,PTEN,PTPN22,RAB3GAP2,RANGRF,RBM20,RFC2,RYR2,SCN1B,SCN2B,SCN3B,SCN4B,SCN5A,SCN10A,SCNN1A,SEMA3A,SFTPB,SFTPC,SGCD,SLC2A10,SLC4A3,SLC19A2,SLC25A20,SLMAP,SNTA1,STX1A,SYNE1,SYNE2,TANGO2,TBL2,TBX5,TECRL,TGFB3,TGFBR1,TGFBR2,TMEM43,TMEM270,TNNC1,TNNI3,TNNI3K,TNNT2,TOR1A,TPM1,TRDN,TRPM4,TSPYL1,TTC26,VPS37D,WAS,WIPF1,GTF2I,GTF2I
Sudden Cardiac Death	ABCA12,ABCC6,ABCG5,ABCG8,ACAD9,ACADVL,AKAP9,AKT1,ALG10B,ANK2,APOB,BAZ1B,BCL7B,BUD23,CACNA1C,CACNA2D1,CALM1,CALM2,CALM3,CASQ2,CAV3,CLCF1,CLIP2,CPT1A,CRLF1,CSRP3,DES,DNAJC19,DNAJC30,DPP6,DSC2,DSG2,DSP,DTNA,EIF4H,ELN,EMD,ENPP1,EYA4,FHL1,FHOD3,FKBP6,FLNC,GNAI2,GPD1L,GTF2I,GTF2IRD1,GTF2IRD2,HLA-B,HLA-DRB1,IKZF1,JUP,KCNE1,KCNE2,KCNH2,KCNJ2,KCNJ5,KCNQ1,LDB3,LDLR,LDLRAP1,LEMD2,LIMK1,LMNA,LRP6,METTL27,MLXIPL,MYBPC3,MYH7,MYL2,MYL3,MYOZ2,NCF1,NOS1AP,NUP155,P4HA2,PCSK9,PGM1,PKP2,PPA2,PRKAG2,PTEN,PTPN22,RBM20,RFC2,RYR2,SCN4B,SCN5A,SCN10A,SGCD,SLC4A3,SNTA1,STX1A,SYNE1,SYNE2,TBL2,TBX5,TECRL,TGFB3,TMEM43,TMEM270,TNNC1,TNNI3,TNNT2,TPM1,TRDN,VPS37D,WAS,WIPF1,GTF2I,GTF2I,GTF2I

Microbiology

Besides their roles in identifying mutations in inherited diseases, NGS platforms are also very effective tools for the sequencing of bacterial genomes. 16S rRNA gene is conserved in almost all bacteria required for microbial identification. NGS-based Technologies hypervariable regions of 16S rRNA provide a time-consuming and cost-effective approach for microbial identification (49-51). Besides, it gives rise to the characterization of the niches of microbial communities in the human body (52).

NGS technologies have also some advantages for the detection of foodborne pathogens by enabling to the characterization of culturable and non-culturable taxa (53). Research on food microbiology is generally focusing on the whole genome sequencing of bacterial isolates (54) and highlights the microbial

diversity that is associated with foodstuffs (55,56) whereas some are using NGS for screening the presence of fresh produce human pathogens (57).

We have been facing a tough global threat COVID-19 pandemic duration since 2019. Recent data related to infectious diseases such as MERS, SARS, and Ebola have shown that NGS is a very powerful tool for the origin detection, spread, transmission chains and phylogenetic analysis of the outbreaks (58,59). During the SARS-COV2 pandemic using high throughput Technologiesallowed the detection of SARS-COV2 mutations in different variants (60)and phylogenetic analysis revealed a deep understanding of genetic divergence

Pharmacology

Pharmacogenomic explains the relationship between multiple genes and genomic variations affected by drugs throughout the genome whereas pharmacogenetics is a change that occurs in the body as a result of the response of the genomic structure of the individual to drugs. Recently NGS has been also implemented for comprehensive profiling of pharmacogenes related to drug pharmacokinetics and pharmacodynamics by discovering not only common and rare genetic variations for SNV/gene-drug associations but also novel targets, thus showing the clinical relevance of the variants.

Briefly, high-throughput NGS Technologies has a wide range of usage in Medicine and not restricted for germline analysis but also analysis for somatic mutations, copy number variation and gene fusions.

REFERENCES

1. Pagon R. A. (2006). GeneTests: an online genetic information resource for health care providers. Journal of the Medical Library Association: JMLA, 94(3), 343–348.
2. Platt, J., Cox, R., & Enns, G. M. (2014). Points to consider in the clinical use of NGS panels for mitochondrial disease: an analysis of gene inclusion and consent forms. Journal of genetic counseling, 23(4), 594–603.
3. Audo, I., Bujakowska, K. M., Léveillard, T., Mohand-Saïd, S., Lancelot, M. E., Germain, A., Antonio, A., Michiels, C., Saraiva, J. P., Letexier, M., Sahel, J. A., Bhattacharya, S. S., & Zeitz, C. (2012). Development and application of a next-generation-sequencing (NGS) approach to detect known and novel gene defects underlying retinal diseases. Orphanet journal of rare diseases, 7, 8.
4. Nijman, I. J., van Montfrans, J. M., Hoogstraat, M., Boes, M. L., van de Corput, L., Renner, E. D., van Zon, P., van Lieshout, S., Elferink, M. G., van der Burg, M., Vermont, C. L., van der Zwaag, B., Janson, E., Cuppen, E., Ploos van Amstel, J. K., & van Gijn, M. E. (2014). Targeted next-generation sequencing: a novel diagnostic tool for primary immunodeficiencies. The Journal of allergy and clinical immunology, 133(2), 529–534.
5. Esmail Nia, G., Fadaee, M., Royer, R., Najmabadi, H., & Akbari, M. R. (2016). Profiling Fanconi Anemia Gene Mutations among Iranian Patients. Archives of Iranian medicine, 19(4), 236–240.
6. Artuso, R., Fallerini, C., Dosa, L., Scionti, F., Clementi, M., Garosi, G., Massella, L., Epistolato, M. C., Mancini, R., Mari, F., Longo, I., Ariani, F., Renieri, A., & Bruttini, M. (2012). Advances in Alport syndrome diagnosis using next-generation sequencing. European journal of human genetics : EJHG, 20(1), 50–57.
7. Weerakkody, R. A., Vandrovcova, J., Kanonidou, C., Mueller, M., Gampawar, P., Ibrahim, Y., Norsworthy, P., Biggs, J., Abdullah, A., Ross, D., Black, H. A., Ferguson, D., Cheshire, N. J., Kazkaz, H., Grahame, R., Ghali, N., Vandersteen, A., Pope, F. M., & Aitman, T. J. (2016). Targeted next-generation sequencing makes new molecular diagnoses and expands genotype-phenotype relationship in Ehlers-Danlos syndrome. Genetics in medicine : official journal of the American College of Medical Genetics, 18(11), 1119–1127.
8. Liu, Y. T., Lee, Y. C., & Soong, B. W. (2015). What we have learned from the next-generation sequencing: Contributions to the genetic diagnoses and understanding of pathomechanisms of neurodegenerative diseases. Journal of neurogenetics, 29(2-3), 103–112.

9. Voelkerding, K. V., Dames, S., & Durtschi, J. D. (2010). Next generation sequencing for clinical diagnostics-principles and application to targeted resequencing for hypertrophic cardiomyopathy: a paper from the 2009 William Beaumont Hospital Symposium on Molecular Pathology. The Journal of molecular diagnostics : JMD, 12(5), 539–551.
10. De De Keulenaer, S., Hellemans, J., Lefever, S., Renard, J. P., De Schrijver, J., Van de Voorde, H., Tabatabaiefar, M. A., Van Nieuwerburgh, F., Flamez, D., Pattyn, F., Scharlaken, B., Deforce, D., Bekaert, S., Van Criekinge, W., Vandesompele, J., Van Camp, G., & Coucke, P. (2012). Molecular diagnostics for congenital hearing loss including 15 deafness genes using a next generation sequencing platform. BMC medical genomics, 5, 17.
11. Na, J. H., Shin, S., Yang, D., Kim, B., Kim, H. D., Kim, S., Lee, J. S., Choi, J. R., Lee, S. T., & Kang, H. C. (2020). Targeted gene panel sequencing in early infantile onset developmental and epileptic encephalopathy. Brain & development, 42(6), 438–448.
12. Gonzalez-Quereda, L., Rodriguez, M. J., Diaz-Manera, J., Alonso-Perez, J., Gallardo, E., Nascimento, A., Ortez, C., Natera-de Benito, D., Olive, M., Gonzalez-Mera, L., Munain, A. L., Zulaica, M., Poza, J. J., Jerico, I., Torne, L., Riera, P., Milisenda, J., Sanchez, A., Garrabou, G., Llano, I., … Gallano, P. (2020). Targeted Next-Generation Sequencing in a Large Cohort of Genetically Undiagnosed Patients with Neuromuscular Disorders in Spain. Genes, 11(5), 539.
13. Chen, M., & Zhao, H. (2019). Next-generation sequencing in liquid biopsy: cancer screening and early detection. Human genomics, 13(1), 34.
14. Phadke, S. R., Puri, R. D., & Ranganath, P. (2017). Prenatal screening for genetic disorders: Suggested guidelines for the Indian Scenario. The Indian journal of medical research, 146(6), 689–699.
15. Hui L. (2016). Noninvasive prenatal testing for aneuploidy using cell-free DNA - New implications for maternal health. Obstetric medicine, 9(4), 148–152.
16. Hess, J. F., Kohl, T. A., Kotrová, M., Rönsch, K., Paprotka, T., Mohr, V., Hutzenlaub, T., Brüggemann, M., Zengerle, R., Niemann, S., & Paust, N. (2020). Library preparation for next generation sequencing: A review of automation strategies. Biotechnology advances, 41, 107537.
17. Rizzo, J. M., & Buck, M. J. (2012). Key principles and clinical applications of "next-generation" DNA sequencing. Cancer prevention research (Philadelphia, Pa.), 5(7), 887–900.
18. S., Chen, L., Houghton, J., Kemppainen, J., Marko, A. C., Zeigler, R., & Latham, G. J. (2013). Functional DNA quantification guides accurate

next-generation sequencing mutation detection in formalin-fixed, paraffin-embedded tumor biopsies. Genome medicine, 5(8), 77.
19. obin, J. D., Ludlow, A. T., LaRanger, R., Wright, W. E., & Shay, J. W. (2016). Comparison of DNA Quantification Methods for Next Generation Sequencing. Scientific reports, 6, 24067.
20. Tewhey, R., Warner, J. B., Nakano, M., Libby, B., Medkova, M., David, P. H., Kotsopoulos, S. K., Samuels, M. L., Hutchison, J. B., Larson, J. W., Topol, E. J., Weiner, M. P., Harismendy, O., Olson, J., Link, D. R., & Frazer, K. A. (2009). Microdroplet-based PCR enrichment for large-scale targeted sequencing. Nature biotechnology, 27(11), 1025–1031.
21. nirke, A., Melnikov, A., Maguire, J., Rogov, P., LeProust, E. M., Brockman, W., Fennell, T., Giannoukos, G., Fisher, S., Russ, C., Gabriel, S., Jaffe, D. B., Lander, E. S., & Nusbaum, C. (2009). Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nature biotechnology, 27(2), 182–189.
22. Nilsson, M., Malmgren, H., Samiotaki, M., Kwiatkowski, M., Chowdhary, B. P., & Landegren, U. (1994). Padlock probes: circularizing oligonucleotides for localized DNA detection. Science (New York, N.Y.), 265(5181), 2085–2088.
23. Magi, A., Benelli, M., Gozzini, A., Girolami, F., Torricelli, F., & Brandi, M. L. (2010). Bioinformatics for next generation sequencing data. Genes, 1(2), 294–307.
24. DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., del Angel, G., Rivas, M. A., Hanna, M., McKenna, A., Fennell, T. J., Kernytsky, A. M., Sivachenko, A. Y., Cibulskis, K., Gabriel, S. B., Altshuler, D., & Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature genetics, 43(5), 491–498.
25. Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine : official journal of the American College of Medical Genetics, 17(5), 405–424.
26. Biesecker, L. G., & Green, R. C. (2014). Diagnostic clinical genome and exome sequencing. The New England journal of medicine, 371(12), 1170.
27. Neveling, K., Feenstra, I., Gilissen, C., Hoefsloot, L. H., Kamsteeg, E. J., Mensenkamp, A. R., Rodenburg, R. J., Yntema, H. G., Spruijt, L.,

Vermeer, S., Rinne, T., van Gassen, K. L., Bodmer, D., Lugtenberg, D., de Reuver, R., Buijsman, W., Derks, R. C., Wieskamp, N., van den Heuvel, B., Ligtenberg, M. J., … Nelen, M. R. (2013). A post-hoc comparison of the utility of sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. Human mutation, 34(12), 1721–1726.

28. Suwinski, P., Ong, C., Ling, M. H. T., Poh, Y. M., Khan, A. M., & Ong, H. S. (2019). Advancing Personalized Medicine Through the Application of Whole Exome Sequencing and Big Data Analytics. Frontiers in genetics, 10, 49.
29. Belkadi, A., Bolze, A., Itan, Y., Cobat, A., Vincent, Q. B., Antipenko, A., Shang, L., Boisson, B., Casanova, J. L., & Abel, L. (2015). Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. Proceedings of the National Academy of Sciences of the United States of America, 112(17), 5473–5478.
30. https://hpo.jax.org/app/
31. Negi, S. K., & Guda, C. (2017). Global gene expression profiling of healthy human brain and its application in studying neurological disorders. Scientific reports, 7(1), 897.
32. Cardoso, A. R., Lopes-Marques, M., Silva, R. M., Serrano, C., Amorim, A., Prata, M. J., & Azevedo, L. (2019). Essential genetic findings in neurodevelopmental disorders. Human genomics, 13(1), 31.
33. Boycott, K. M., Rath, A., Chong, J. X., Hartley, T., Alkuraya, F. S., Baynam, G., Brookes, A. J., Brudno, M., Carracedo, A., den Dunnen, J. T., Dyke, S. O. M., Estivill, X., Goldblatt, J., Gonthier, C., Groft, S. C., Gut, I., Hamosh, A., Hieter, P., Höhn, S., Hurles, M. E., … Lochmüller, H. (2017). International Cooperation to Enable the Diagnosis of All Rare Genetic Diseases. American journal of human genetics, 100(5), 695–705.
34. Yarwood, A., Han, B., Raychaudhuri, S., Bowes, J., Lunt, M., Pappas, D. A., Kremer, J., Greenberg, J. D., Plenge, R., Rheumatoid Arthritis Consortium International (RACI), Worthington, J., Barton, A., & Eyre, S. (2015). A weighted genetic risk score using all known susceptibility variants to estimate rheumatoid arthritis risk. Annals of the rheumatic diseases, 74(1), 170–176.
35. Maehlen, M. T., Olsen, I. C., Andreassen, B. K., Viken, M. K., Jiang, X., Alfredsson, L., Källberg, H., Brynedal, B., Kurreeman, F., Daha, N., Toes, R., Zhernakova, A., Gutierrez-Achury, J., de Bakker, P. I., Martin, J., Teruel, M., Gonzalez-Gay, M. A., Rodríguez-Rodríguez, L., Balsa, A., Uhlig, T., … Lie, B. A. (2015). Genetic risk scores and number of autoantibodies in patients with rheumatoid arthritis. Annals of the rheumatic diseases, 74(4), 762–768.

36. OMIM gene map statistics; [Accessed 2022Nov27]. https://www.omim.org/statistics/geneMap
37. https://disorders.eyes.arizona.edu/for-patients/handout-list
38. Chen, Y., & Palczewski, K. (2016). Systems Pharmacology Links GPCRs with Retinal Degenerative Disorders. Annual review of pharmacology and toxicology, 56, 273–298.
39. Chaitankar, V., Karakülah, G., Ratnapriya, R., Giuste, F. O., Brooks, M. J., & Swaroop, A. (2016). Next generation sequencing technology and genomewide data analysis: Perspectives for retinal research. Progress in retinal and eye research, 55, 1–31.
40. Coonen, E., van Montfoort, A., Carvalho, F., Kokkali, G., Moutou, C., Rubio, C., De Rycke, M., & Goossens, V. (2020). ESHRE PGT Consortium data collection XVI-XVIII: cycles from 2013 to 2015. Human reproduction open, 2020(4), hoaa043.
41. Donley, G., Hull, S. C., & Berkman, B. E. (2012). Prenatal whole genome sequencing: just because we can, should we?. The Hastings Center report, 42(4), 28–40.
42. Carbone, L., Cariati, F., Sarno, L., Conforti, A., Bagnulo, F., Strina, I., Pastore, L., Maruotti, G. M., & Alviggi, C. (2020). Non-Invasive Prenatal Testing: Current Perspectives and Future Challenges. Genes, 12(1), 15.
43. Tresbach, R. H., Sperb-Ludwig, F., Ligabue-Braun, R., Tonon, T., de Oliveira Cardoso, M. T., Heredia, R. S., da Silva Rosa, M. T. A., Martins, B. C., Poubel, M. O., da Silva, L. C. S., Maillot, F., & Schwartz, I. V. D. (2020). Phenylketonuria Diagnosis by Massive Parallel Sequencing and Genotype-Phenotype Association in Brazilian Patients. Genes, 12(1), 20.
44. Drury, S., Williams, H., Trump, N., Boustred, C., GOSGene, Lench, N., Scott, R. H., & Chitty, L. S. (2015). Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities. Prenatal diagnosis, 35(10), 1010–1017.
45. Green, R. C., Berg, J. S., Grody, W. W., Kalia, S. S., Korf, B. R., Martin, C. L., McGuire, A. L., Nussbaum, R. L., O'Daniel, J. M., Ormond, K. E., Rehm, H. L., Watson, M. S., Williams, M. S., Biesecker, L. G., & American College of Medical Genetics and Genomics (2013). ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genetics in medicine : official journal of the American College of Medical Genetics, 15(7), 565–574.
46. Sanders, S. J., Neale, B. M., Huang, H., Werling, D. M., An, J. Y., Dong, S., Whole Genome Sequencing for Psychiatric Disorders (WGSPD), Abecasis, G., Arguello, P. A., Blangero, J., Boehnke, M., Daly, M. J., Eggan, K., Geschwind, D. H., Glahn, D. C., Goldstein, D. B., Gur, R. E., Handsaker, R. E., McCarroll, S. A., Ophoff, R. A., … Freimer, N. B.

(2017). Whole genome sequencing in psychiatric disorders: the WGSPD consortium. Nature neuroscience, 20(12), 1661–1668.
47. Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., O'Donnell-Luria, A. H., Ware, J. S., Hill, A. J., Cummings, B. B., Tukiainen, T., Birnbaum, D. P., Kosmicki, J. A., Duncan, L. E., Estrada, K., Zhao, F., Zou, J., Pierce-Hoffman, E., Berghout, J., Cooper, D. N., … Exome Aggregation Consortium (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616), 285–291.
48. Parikh, V. N., & Ashley, E. A. (2017). Next-Generation Sequencing in Cardiovascular Disease: Present Clinical Applications and the Horizon of Precision Medicine. Circulation, 135(5), 406–409.
49. Claesson, M. J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J. R., Ross, R. P., & O'Toole, P. W. (2010). Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic acids research, 38(22), e200.
50. Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America, 108 Suppl 1(Suppl 1), 4516–4522.
51. Degnanegnan, P. H., & Ochman, H. (2012). Illumina-based analysis of microbial community diversity. The ISME journal, 6(1), 183–194.
52. Forde, B. M., & O'Toole, P. W. (2013). Next-generation sequencing technologies and their impact on microbial genomics. Briefings in functional genomics, 12(5), 440–453.
53. Nastasijevic, I., Milanov, D., Velebit, B., Djordjevic, V., Swift, C., Painset, A., & Lakicevic, B. (2017). Tracking of Listeria monocytogenes in meat establishment using Whole Genome Sequencing as a food safety management tool: A proof of concept. International journal of food microbiology, 257, 157–164.
54. Hyeon, J. Y., Li, S., Mann, D. A., Zhang, S., Li, Z., Chen, Y., & Deng, X. (2018). Quasimetagenomics-Based and Real-Time-Sequencing-Aided Detection and Subtyping of Salmonella enterica from Food Samples. Applied and environmental microbiology, 84(4), e02340-17.
55. Leff, J. W., & Fierer, N. (2013). Bacterial communities associated with the surfaces of fresh fruits and vegetables. PloS one, 8(3), e59310.
56. Aw, T. G., Wengert, S., & Rose, J. B. (2016). Metagenomic analysis of viruses associated with field-grown and retail lettuce identifies human and animal viruses. International journal of food microbiology, 223, 50–56.

57. Leonard, S. R., Mammel, M. K., Lacher, D. W., & Elkins, C. A. (2015). Application of metagenomic sequencing to food safety: detection of Shiga Toxin-producing Escherichia coli on fresh bagged spinach. Applied and environmental microbiology, 81(23), 8183–8191.
58. de Wit, E., van Doremalen, N., Falzarano, D., & Munster, V. J. (2016). SARS and MERS: recent insights into emerging coronaviruses. Nature reviews. Microbiology, 14(8), 523–534.
59. Shrivastava, S., Puri, V., Dilley, K. A., Ngouajio, E., Shifflett, J., Oldfield, L. M., Fedorova, N. B., Hu, L., Williams, T., Durbin, A., Amedeo, P., Rashid, S., Shabman, R. S., & Pickett, B. E. (2018). Whole genome sequencing, variant analysis, phylogenetics, and deep sequencing of Zika virus strains. Scientific reports, 8(1), 15843.
60. Yavarian, J., Nejati, A., Salimi, V., Shafiei Jandaghi, N. Z., Sadeghi, K., Abedi, A., Sharifi Zarchi, A., Gouya, M. M., & Mokhtari-Azad, T. (2022). Whole genome sequencing of SARS-CoV2 strains circulating in Iran during five waves of pandemic. PloS one, 17(5), e0267847.

Notes On Forensic Psychiatry Nursing
(Why is genetics important in forensic psychiatric nursing?)

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Giriş:

Forensic Psychiatric Nursing in Turkey, just like in other countries (Martin., 2001), emerges as a sub-specialty of psychiatric nursing (Polar and Karakaş., 2020). Forensic Psychiatric Nursing; It is seen as a complex field that includes some non-nursing concepts such as crime and psychiatric care. In fact, since it requires a theoretical background beyond psychiatric care, it is reported as a special sub-field of psychiatric nursing, although it is defended as an independent field (Martin., 2001; Scannell., 2018). Forensic Psychiatry: Whether a person will receive a psychiatric diagnosis after/during an action (resulting in a crime), if he gets a psychiatric diagnosis, whether he will be held responsible for his action, whether the psychiatric diagnosis he received was before the action that resulted in crime, even if he has a psychiatric diagnosis. It is a field of science that evaluates the period during the action (Scannell., 2018; Polat and Karakaş., 2020).

The earliest records we know about Forensic Psychiatry are reported as the studies of Egypt and India to understand poisonings. It is stated that the Greek civilization / Hippocrates and the Romans tried to illuminate death according to the way of injury, not swearing in poisonings. Ancient documents belonging to China, on the other hand, explain that death is explained by establishing a relationship between the data of medical science and the type of injury. The documents belonging to Forensic Psychiatric Nursing are; It is clearly mentioned in the archives of the United States from the 1950s. Early records in Forensic Psychiatric Nursing included care for a sexual assault victim (Scannell., 2018). The Forensic Psychiatry team, which includes nurses, can often encounter an individual with an abuse, mental health problem or an individual who develops a mental health problem after a crime. Considering that care covers a constantly changing process; Nurses are indispensable for forensic psychiatry because they move to the needed role and provide the care needs in this role. For example; It is known that an individual who uses substances has different care needs, such as different criminal profiles of an individual with psychotic features (Taylor., 1998; Scannell., 2018).

Crime and punishment systems see the abuse of the victim's mental health problem as aggravating for punishment, but Forensic Psychiatry and Forensic Psychiatric Nursing provides scientific data to identify the victim's basic mental health needs and provide the necessary treatment/care.

Forensic psychiatry nurses have to be careful during mental evaluations. The primary reason for this is; It is the fact that the current psychiatric condition or psychiatric history is not told "correctly" by the individuals who need care and their families. Moreover, when the social relations and environment of the

perpetrator are deducted, it is said that most of these people are "at risk" to commit a crime. However, the development of the concept of "trust" in the therapeutic relationship and avoiding the "stigmatist" approach to individuals are seen as the main purposes of care. In order to overcome it, it is important to collect and observe observation and data collection with nurse-nurse interaction (Nyman et all., 2020; Munthe et all., 2010).

Nurses working in forensic units in Turkey generally consist of individuals who have undergone general nursing education. There is no standardization in hospital units. There are no nurses who have specialized training in forensic psychiatry, providing care to forensic psychiatry patients, the therapeutic content of the care or what services are provided. For this reason, the treatment and care of these patients are carried out by nurses who lack the necessary training (B. Arabacı., 2008). It is reported that nurses generally want to work in forensic patient services, stay away from forensic psychiatry nursing-related studies, and are reluctant to attend trainings based on forensic psychiatry nursing (Arabacı and Çam., 2013). The reason for this negative opinion; Individuals with a forensic psychiatric diagnosis who have been involved in crime may be described as "frightening". When nurses do not receive adequate forensic and psychiatric training, the roles they will undertake may be too heavy for them in addition to their general roles. However, it is necessary to undertake roles other than ethical and legal dimensions and basic care/counseling/educational roles (Mason., 2002). In Turkey, only 17 theses on forensic psychiatric nursing were reported in 2019 (between 1997-2019) (Sentürk et all., 2019). As can be seen, it reaches a very limited number of researchers and publications in academic terms.

However, a forensic psychiatric nurse; how effective mental illnesses can be for crime?, what is the epidemiological data of perpetrators and victims?, how much mental illness is found proportionally between perpetrators and victims?, genetic parameters (for monitoring the family and detecting individuals with hidden mental disorders in the family) What can happen and how it will work?, to understand the genetic and environmental conditions (for maintenance tasks such as monitoring the effectiveness of pharmacological agents) that enable/facilitate having a mental illness, and to comprehend the consequences. In addition to all these, integrating basic maintenance knowledge into the service will support the expansion of their roles. Otherwise, it will become inevitable to experience task confusion within the service (Mason 2002).

Mental Illness, Genetics and Forensic Psychiatry Nurse

The brain is the most complex object of scientific research. As its development is not linear, gene-gene and gene-environment interactions direct its development. Apart from these interactions, the temporal interaction of approximately 100,000 million neurons also affects their development. This complex development and interaction process also makes it difficult to understand/solve processes such as behavior, thought, emotion, perception and memory. This phenomenon, which has more than 100 trillion synaptic connections or interactions, is accompanied by more than 100 neurotransmitters (Hyman., 2000; Hart., 2018). However, the complexity of the brain is that it is not static. A new knowledge, a new skill, or a new emotional response changes the way the neurons involved in processing the synaptic connection of the circuit in which learning occurs. This process is called neuroplasticity; in this process new synapses can be formed and old ones pruned; existing synapses can be strengthened or weakened. As a result, information processes differently in each neuron (hence the individual). Connections and structuring result in personalized neural modification (Hyman., 2000; Phelan., 2002).

The physiopathological process of brain diseases ultimately affects behavior. This process creates gaps in circuits that cross the damaged area or can kill cells that cause neural projections. Just like brain functionality, its physiopathological processes are reported to have a very complex structure. For example; Finding only a few genes associated with the phenotype, measuring neurotransmitter levels, or finding a pathological point in magnetic resonance findings is not sufficient to explain the biological basis of a disease such as schizophrenia. We need to understand how the disease process disrupts the parallel distributed processing underlying relevant aspects of thinking, emotion, and motivation. An important question is also how one version of a particular gene (allele) contributes to the risk of depression or schizophrenia while only a slightly different version does not (Hyman., 2000; Phelan., 2002). Another example of this situation is; In a study conducted with individuals diagnosed with bipolar disorder, it was stated that risky genes were almost not seen in diagnosed patients (Gordovez., 2020). Although genes cannot be a major cause in neuropsychiatric diseases, they appear as conditions with mechanisms and interactions that need to be understood (McGuffin and Murray., 2013).

Regarding the genetic background of mental illnesses; Although there is any gene that is considered to be major responsible in the individual, the intensity and form of the symptoms vary depending on the gene phenotype. For example, there are 3 different phenotypes in schizophrenia. Depending on which of the 3 phenotypes individuals are present, the intensity, shape, and direction of

schizophrenia-specific symptoms (Avramopoulos., 2018) vary (Avramopoulos., 2018; Sullivan., 2005). In a study with 1087 samples (1938), in which individuals whose ancestors were diagnosed with schizophrenia, schizophrenia emerged in 10% of individuals after 10 years of follow-up (Kallman., 1938). As can be seen, since the early 1900s, when genetics was just beginning to gain momentum, the genetics of schizophrenia offers us assumptions about phenotypes. It is now possible to continue these studies not only with individuals with schizophrenia in their ancestors, but also with individuals whose ancestors have schizophrenia and carry schizophrenia phenotypes (Golow et all., 2020; Liu et all., 2021; Twari et all., 2022).

A forensic psychiatric nurse will be able to use genetics in care, treatment and observation, as well as as an important tool in distinguishing the family's behavior and the role of the family in the exacerbation of the disease. The person's pre-care anamnesis; It can facilitate multiple hospitalizations, drug non-compliance, genetic specificity of symptoms, and family monitoring. Genetic; It not only creates a roadmap for the forensic psychiatric nurse for care, but also supports the nurse to defend herself and the person she cares for, with legal/ethical dimensions. Forensic psychiatric nurse; the aspect that should be considered separately from a psychiatric nurse or a forensic nurse; is that genetic-environmental interaction can predict the problems that will arise in the family tree of psychiatric/neuropsychiatric pathologies.

Table 1. *Roles of Psychiatric Nurse, Forensic Psychiatric Nurse, Forensic Nurse*

	Psychiatric Nurse	**Forensic psychiatric nurse**	**Forensic nurse**
Roles	Advocacy	Advocacy*	Advocacy
	----------	Discussing the Crime **Presence of crime ***	
	Observation/Consulting	Observation/Consulting ****	Observation/Consulting *****
	Empaty	Empaty******	Empaty
	Collaboration	Collaboration *******	Collaboration
	Socializing Agent	Socializing Agentwith Ethical and Legal aspects	Change Agent
	Caregiver role	Caregiver and Educational Role	Caregiver role
	Educator role	Educator role	Educator role
	Advanced Psychiatry Nursing Roles	Advanced Psychiatry Nursing Roles ----------	
	Therapeutic role	Therapeutic role ********	Therapeutic role

*Usually, the nurse does not talk about the crime, guilt and the event that caused the crime. First of all, he must get rid of his judgments.

**In this role, the forensic psychiatry nurse should proceed by evaluating whether the crime is in the presence of a mental illness, whether there is an exacerbation period of the mental illness, and the insight of the individual.

***In this context, the Forensic Nurse should be able to understand the existence of the crime. He must observe and deal with the crime with the individual.

**** While performing this role, the Forensic Psychiatric Nurse (in addition to the basic observation items of the psychiatric nurse) should observe whether there is substance use, criminological determinations, and grief problems. If necessary, he should demonstrate his advisory role. Using basic information, she must predict what symptoms are present and what family processes she should follow, as sheds light on genetics. In the light of the observations he has made in the family and the data he has collected, he should monitor the effect of the family on the exacerbations. If he/she detects data on whether the family needs genetic counseling and whether they need a psychiatric consultation, they should definitely report it.

****** The Forensic Psychiatric Nurse should be aware that the individual should not appear to be collecting data or communicating effectively in order to be convicted further. In this respect, priorities should know that they need to use "therapeutic environment/communication skills". For empathy, this therapeutic transmission will provide "confidence." It will be easier to interpret and provide the information required for maintenance and monitoring.

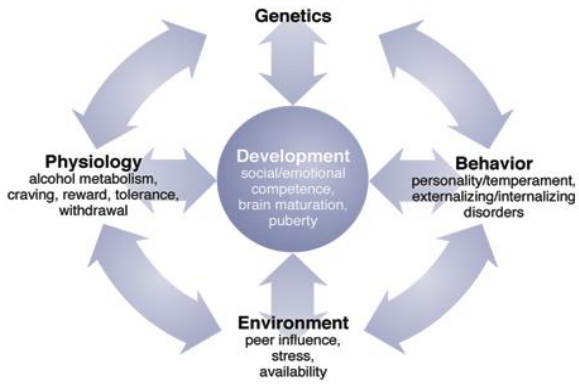
********** In this role, the nurse should give responsibilities to the individual in daily routines, completely away from stigmatist behaviors. In the same way, she should continue the care process by using common mechanisms after the necessary observations with the family.

******** This role can provide the only information for the "risk diagnoses" of the Forensic Psychiatric nurse. In this context, it can present very serious data such as the tendency towards risky behaviors and the tendency to crime/detection of the role of mental illness in this tendency. (Mason., 2002; Mason at all., 2008; Lyons., 2009)

Forensic psychiatry nurses also have roles that are specific to the behavior resulting in crime and that develop in accordance with the mental/psychic state of the individual, apart from the stated roles. The fact that Forensic Psychiatric Nurses are generally uneducated or they are prone to criminal behavior can create obstacles during the "discussion of crime". At this stage, the nurse may ignore some bad events and risks, as she wishes the patient to exhibit "good progress". The Forensic Psychiatric Nurse is also responsible for protecting the "individual under care who is prone to crime". This “protection” does not mean changing the scope of the crime, presenting false observational results, or concentrating on parameters that will show “good going”. On the contrary, it is to protect the individual who may be harmed, on the basis of "not being harmed" (Bowring., 2006; Mason at all., 2008; Lyons., 2009).

The Forensic Psychiatric Nurse who wants to fulfill these roles must know the genetic bases that must be observed, diagnosed and predicted. Although the given pharmacological agents do not give the same output in every patient, it depends on many factors depending on the individual, but the title of "genetics" should be assimilated. If a gene transfer that may pose a risk of disease is detected (if it is present in the family / ancestors), the genetic phenotype must also be known. These phenotypes may be important in the follow-up of symptoms and in monitoring the effectiveness of drugs on symptoms.

Figure 1. *Genetic, environment interaction cycle*



Reference:https://www.bricefoundation.org/single-post/2016/05/06/genes-and-environmentals-effect-on-a-persons-behavior

As can be seen in Figure 1, genetics and environment have important consequences both within and between themselves. For example, its effect on behavior and psychology is seen in this cycle.

Even mental health professionals find it "more likely" that an individual with a mental health problem commits a "crime". But; Compared to the general population, the crime rate of individuals with mental health/mental health problems seems to be lower. Even individuals with mental health problems who have committed a crime before but entered into a treatment protocol have a low crime rate in the social population (Hidays and Burns., 2010¸Ghiasi at all., 2022). Contrary to popular belief, gene, gene-environment and environment interactions that make individuals with mental problems "at risk" for "crime" do not increase the crime these individuals will commit. Although genetic transmission creates a risk of mental illness, they are not "criminal genes". Recognizing certain interactions and genetic phenotypes, knowing whether the individual has them, and observing the family in this respect can explain the risk of crime as "behavioral and mental".

Conclusion

Since the Forensic Psychiatric Nurse has to have some additional roles such as counseling for rape, sexual assault, legal and ethical dimensions apart from her main nursing roles, she has to observe the effects of treatment and pharmacological agents well. At this stage, the science of genetics; While the treatment is ongoing and whatever the legal/ethical dimension says, some of the symptoms have an important place in predicting the state of the disease and tendency to crime.

Reference

1. Martin, T. (2001). Something special: Forensic psychiatric nursing. Journal of Psychiatric and Mental Health Nursing, 8(1), 25-32.
2. Polat, H., & Karakaş, S. A. (2020). Adli psikiyatri hemşireliği. Ebelik ve Sağlık Bilimleri Dergisi, 3(3), 225-232.
3. Scannell, M. J. (2018). Fast Facts About Forensic Nursing: What You Need to Know. Springer Publishing Company.
4. Peternelj-Taylor C.A. (1998) Forbidden love: sexual exploitationin the forensic milieu. Journal of Psychosocial Nursing36, 17–23.
5. Nyman, M., Hofvander, B., Nilsson, T., & Wijk, H. (2020). Mental health nurses' experiences of risk assessments for care planning in forensic psychiatry. International journal of forensic mental health, 19(2), 103-113.
6. Munthe, C., Radovic, S., & Anckars€ater, H. (2010). Ethicalissues in forensic psychiatric research on mentally disor-dered offenders.Bioethics,24(1), 35–44.
7. Baysan-Arabacı L (2008) Adli Psikiyatri Hastalarının Bakımında Hemşirenin Rolü. Adli Psikiyatri Dergisi, 5 : 25-30.
8. Baysan Arabacı, L., & Çam, M. O. (2013). he Attitudes Nurses Working at Psychiatric Hospitals in Turkey have towards Forensic Psychiatric Patients and the associated Factors. *Turkish Journal of Psychiatry*, *24*(4).
9. Senturk, S., Karahan, B., & Topcu, E. T. (2019). Examination of Postgraduate Dissertations Including Nursing in the Field of Forensic Sciences in Turkey. International Journal of Caring Sciences, 12(3), 1725.
10. Mason, T. (2002). Forensic psychiatric nursing: A literature review and thematic analysis of role tensions. Journal of Psychiatric and Mental Health Nursing, 9(5), 511-520.
11. Hart, S. (2018). Brain, attachment, personality: An introduction to neuroaffective development. Routledge.
12. Hyman, S. E. (2000). The genetics of mental illness: implications for practice. Bulletin of the World Health Organization, 78, 455-463.
13. Gordovez, F. J. A., & McMahon, F. J. (2020). The genetics of bipolar disorder. Molecular psychiatry, 25(3), 544-559.
14. Phelan, J. C. (2002). Genetic bases of mental illness–a cure for stigma?. *TRENDS in Neurosciences*, *25*(8), 430-431.
15. McGuffin, P., & Murray, R. (Eds.). (2013). The new genetics of mental illness. Butterworth-Heinemann.
16. Avramopoulos, D. (2018). Recent advances in the genetics of schizophrenia. Complex Psychiatry, 4(1), 35-51.

17. Sullivan, P. F. (2005). The genetics of schizophrenia. PLoS medicine, 2(7), e212.
18. Kallmann, F. J. (1938). The genetics of schizophrenia.
19. Tiwari, A. K., Zai, C. C., Müller, D. J., & Kennedy, J. L. (2022). Genetics in schizophrenia: where are we and what next?. Dialogues in clinical neuroscience.
20. Golov, A. K., Kondratyev, N. V., Kostyuk, G. P., & Golimbet, V. E. (2020). Novel approaches for identifying the molecular background of schizophrenia. Cells, 9(1), 246.
21. Liu, J., Li, S., Li, X., Li, W., Yang, Y., Guo, S., ... & Luo, X. J. (2021). Genome-wide association study followed by trans-ancestry meta-analysis identify 17 new risk loci for schizophrenia. BMC medicine, 19(1), 1-15.
22. Mason, T., Lovell, A., & Coyle, D. (2008). Forensic psychiatric nursing: skills and competencies: I role dimensions. Journal of Psychiatric and Mental Health Nursing, 15(2), 118-130.
23. Lyons, T. (2009). Role of the forensic psychiatric nurse. Journal of Forensic Nursing, 5(1), 53-57.
24. Bowring-Lossock, E. (2006). The forensic mental health nurse–a literature review. Journal of Psychiatric and Mental Health Nursing, 13(6), 780-785.
25. Ghiasi, N., Azhar, Y., & Singh, J. (2022). Psychiatric illness and criminality. In *StatPearls [Internet]*. StatPearls Publishing.Ghiasi, N., Azhar, Y., & Singh, J. (2022). Psychiatric illness and criminality. In StatPearls [Internet]. StatPearls Publishing.
26. Hiday, V. A., & Burns, P. J. (2010). Mental illness and the criminal justice system. A handbook for the study of mental health: Social contexts, theories, and systems, 478-498.

Overview Of Occupational Health And Safety

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INTRODUCTION

It is clearly observed that industrialization and mechanization, which has become widespread in our country and in the world, with the technology that provides continuous development in the period we live in, has also caused many health and safety hazards. Since the industrial revolution, the need for manpower has started to increase gradually as a result of the transition to the factory order. The fact that those who settled in the regions where industrialization increased could not eat well, the unfavorable environment in the working areas, insufficient remuneration, unhealthy accommodation and working in the activity areas caused the employees to face various occupational risks. With the mechanization taking the place of the high-tempo workforce, it has become necessary to take various measures in order for employers to prevent work accidents and occupational problems that may occur. When the problems, which were ignored at first, reached the dimensions that would cause bigger problems day by day, various rules and laws were created in order to prevent these problems, and steps were taken to carry out activities related to occupational health and safety. In this study, the importance of occupational health and safety is given by considering employers, employees, economies of countries and social aspects. In addition, the objectives of occupational health and safety are mentioned under the titles of protecting employees, ensuring production safety, ensuring operational safety.

1. GENERAL INFORMATION

Work health; It is defined as the protection of workers from diseases and accidents that may arise due to their working environment and the healthy doing of the work (Anonim, 2012). Occupational safety, on the other hand, is a general name of the studies carried out to prevent situations that may impair health due to different reasons during the execution of works in enterprises.

The concept of occupational health and safety includes all actions that can be taken to prevent risks, as well as to detect and evaluate potential hazards in advance, and to eliminate these risks or reduce them to an acceptable level.

The formation of occupational safety and occupational health awareness in the workplace is important for the company's interests as it will contribute to production. Considering that with the precautions to be applied in the workplace, the situations that may cause danger from work accidents or unsafe working environments will be eliminated, it will ensure that the working environment becomes safe.

Protecting the safety and health of personnel is a legal requirement as well as a human requirement. It is even more possible to prevent occupational accidents

that may occur by ensuring occupational health and safety, compared to paying the resulting financial expenses. The main purpose of occupational health and safety activities is to ensure that the life of the personnel is secured. When the scientific studies are examined, it is seen that the damage caused by occupational accidents is much higher than the costs incurred for ensuring occupational health and safety.

2. OBJECTIVES OF OCCUPATIONAL HEALTH AND SAFETY

In contemporary life, the most basic goals of all countries and communities have been to bring business life to a humane state by giving importance to the individual and the worker, to approach their goals by improving their living standards. In this context, the first target for employers; minimizing job losses caused by diseases and accidents, and achieving high productivity with a more favorable working environment at the workplace. The target for the personnel is; is to maintain his life by working when he can trust and stay healthy (Bilir, 2011).

2.1 Employee Protection

Occupational health and safety constitute the main purpose of the personnel. Ensuring that the personnel are not affected by occupational ailments and accidents that may occur in the works, ensuring their mental and physical well-being, will undoubtedly increase the happiness of the workers over time. OHS ensures healthy progress in the development of personnel by making it normal for individuals to operate in an area free from occupational risks. In this sense, the creation of a safe and healthy area of activity for the personnel is seen as a fundamental right for the personnel (Okan, 1991).

There are two important groups of factors in terms of individual and environment that are effective in maintaining worker health and safety in workplaces. Individual factors; age, gender, can be given as examples. Environmental factors are; physical, chemical, biological factors, ergonomic and psycho-social factors can be given as examples. In this context, it is necessary to carry out researches in different fields against the protection of the employees by taking security measures in the enterprises, increasing the personnel to the state of holistic well-being, which is the maximum point in terms of physical, mental and medical aspects, eliminating the factors that adversely affect health in the enterprise, and creating harmony between the jobs and the workers.

2.2 Ensuring Production Safety

When occupational diseases and accidents at work are reduced to the lowest levels, loss of labor and workdays will also decrease to a minimum level. As a result of this, production will be preserved and the morale of the employees will increase and the level of productivity will increase in a healthy and trustworthy environment. The opposite situation will be faced in places that produce without exhibiting preventive approaches. As workdays and labor losses will increase, they will have difficulties in production (Coşkun, 2007).

2.3 Ensuring Business Security

Workplaces have become more complex as a result of developing technologies and industrialization day by day. Workplaces have to take precautions to separately determine the risks in the systems that may put the safety of not only the employees, but also the workplace at risk. Thanks to the possible precautions that can be taken in the establishments where the production works continue, it will be possible to avoid the risks in health and the accidents that may occur in the works, as machine malfunctions, failures, events that may cause explosions, fires, which may be caused by the fields of activity that are not sufficient in terms of safety and health, will be prevented. (Çelik, 2011).

3. THE IMPORTANCE OF OCCUPATIONAL HEALTH AND SAFETY

The importance of OHS is not handled unilaterally. The importance of OHS is multifaceted; It is important in terms of employers, employees, economies of countries and social aspects. These topics are discussed in detail in the sub-headings.

3.1. The Importance of OHS for Employees

The main goal of the health and safety of the work is to prevent injuries, loss of limbs and tissue or loss of life, and to prevent accidents and diseases that may occur as a result. The reason for this is that individuals are at the top of the production work (Görücü, 2004).

Individuals earn the income that they can provide for daily subsistence with working. In the event that employees are affected by occupational diseases and accidents that may occur at work, first of all, the income level of the employee and the household will decrease. This situation will create undesirable negative consequences on the employee and the people of his household. An employee who is disabled by losing his tissues or organs does not only suffer financial

difficulties, but also faces serious moral damages in terms of spirituality. It is one of the most important objectives of the work to be healthy and to be carried out in safety, to prevent the employees from the negative effects of the workplace, to ensure that they carry out their activities in a safe and comfortable environment, to protect the employees from accidents and to make their physical and mental health safe (Durdu, 2006).

3.2 The Importance of OHS for Employers

Responsible supervisors are responsible for keeping employees and personnel away from possible negativities that may occur in the workplace and creating an area of activity that is free from hazards. This responsibility is not only a legal obligation but also a humanitarian duty for employers. The fact that the work is carried out in a healthy and safe manner by employers is also important because it can be an expense. The expenses incurred by the companies in order to ensure that the work is carried out in a healthy and safe manner will undoubtedly be integrated into the production, leading to an increase in production prices. However, when it is spread over a long period of time, the return of those spent on the healthy and safe execution of the work may be more than the expenses caused by accidents and occupational disorders that may occur in the works. After an accident or illness occurs, the costs of treatments can be very high. However, protective measures can mostly be tolerated with low costs (Bilir, 2005).

Direct costs of accidents and diseases to employers (Durdu, 2006); First aid costs applied at the time of the accident, hospital costs after the accident, costs of incapacity paid to the employee for a certain period of time or permanent incapacity for work, courthouse payments. Indirect payments are (Durdu, 2006); These are the losses that may occur due to labor losses, losses due to the absence of employees, examination of the accident, losses due to the injured employee and the work being brought back to the same order, losses that will pass due to legal procedures and similar reasons, loss of production, and failure to meet orders on time.

3.3 The Importance of OHS in Social Care

Increasing the working speed due to raising the productivity level of the workplaces and providing more income, overwork, day and night working order and inappropriate work environments have caused the reactions of the employees and non-governmental organizations (Gerek, 2000). Reactions have led to the emergence of legal and illegal regulations related to the reduction of working hours, the fulfillment of day and night working order under certain

conditions, and the reduction of the shift system and the hours worked at night to a reasonable level, and taking the necessary measures to carry out the work in a health and safety manner.

In many business lines where there are difficult working conditions, it is aimed to reduce the salary costs and to try to reduce the objections that may arise against the working environment by employing people from different nationalities. Employees of workplaces that consider occupational health and safety important spread the training they have received to their families and close circles outside the workplace. For example, earthquake, fire, first aid etc. at work. At the end of the exercises and trainings, they share the information they have learned with their relatives and create a consciousness in the society (Karadağ, 2010).

3.4 The Importance of OHS in terms of Countries Economies

By understanding the importance of carrying out the work in health and safety, and by taking the legal measures that may be required for this code, the states that seriously manage the process will be very profitable in terms of economy and social aspects. It has been determined that the costs of work accidents and occupational diseases in states with an increased industrialization rate are at the level of 1-3% of the gross national product. Based on the comprehensive research results of the ILO and WHO, it is seen that the situation in Turkey is at the top of the list of European countries and 3rd in the world in terms of accidents that may occur at work (Karadeniz, 2012). It is assumed that the cost figures of accidents and occupational diseases that may occur in the works can be almost 4 billion Turkish liras over the years (Karadağ, 2010).

Financial losses caused by work accident and occupational disease are important for the economy of the country. It is imperative that important measures regarding occupational health and safety are carried out flawlessly. Along with the decrease in production values related to occupational health and safety, it also affects major economy indicators on the opposite side.

The parameters affecting the indicator values related to the economy are listed as follows (Fikri, 2001);

1) Since payments are made to individuals whose bodily integrity is impaired as a result of a work accident or occupational disease and their deceased relatives, such payments bring a serious burden in terms of SSI,

2) The expenses required for diagnosis, examination or treatment as a result of accidents that may occur at work or occupational diseases and incapacity expenses are paid by the security institution, these expenses are also important,

3) Losses that cannot be compensated by the security institution are recourse to those who caused them in return for material and moral compensation or deprivation of income by the victims,

4) Those who die or become disabled as a result of accidents at work or occupational diseases may not be able to enter business life again, and these people may be deprived of the contributions that could be added to the production and gross national product they could have provided if they could work, and the insurance premium income they could pay if they worked.

4. CONCLUSION

It is necessary for employers and employees to make OHS a lifestyle, to act with the understanding that priority is human, priority is life, and safety culture should be adopted by all employees. Laws and regulations enacted on behalf of OHS are a set of rules and laws enacted for the adoption of the concept of safety culture throughout society.

Protecting the safety and health of personnel is a legal requirement as well as a human requirement. It is even more possible to prevent work accidents that may occur by ensuring occupational health and safety, compared to paying the resulting financial expenses. The main purpose of occupational health and safety activities is to ensure that the life of the personnel is secured. When the scientific studies are examined, it is seen that the damage caused by occupational accidents is much higher than the costs incurred for ensuring occupational health and safety.

REFRENCES

1. Anonim (2012) TMMOB Makine Mühendisleri Odası: 67; Ekim, 2010: 21-24
2. Anonim (2012), İş Sağlığı ve Güvenliği Kanunu. TC Resmi Gazete, RG No:28339, Tarih: 30.06.2012
3. Bilir, N. (2005). İş sağlığı ve güvenliğinde çağdaş bir yaklaşım: risk değerlendirilmesi ve risk yönetimi. İş Sağlığı ve Güvenliği Dergisi, 25(5): 9-12.
4. Bilir, N. (2011). Meslek Hastalıkları (Tanı, Tedavi Ve Korunma İlkeleri), Hacettepe Tıp Dergisi, 42,147-152.
5. Coşkun, B., (2007) "Türkiye' de İşçi ve İşverenlerin, İş Sağlığı ve Güvenliği Açısından Görev ve Sorumlulukları", Beykent Üniversitesi(BEÜ), SBE, Yayınlanmamış Yüksek Lisans Tezi (YYLT), İstanbul.
6. Demir, A. (2017) İş Sağlığı ve Güvenliği Ders Notları.
7. Durdu, A. (2006) İşçi Sağlığı Ve İş Güvenliği Düzenlemeleri İle İlgili İşgörenlerin Tutumlarını Belirlemeye Yönelik Bir Araştırma.
8. Fikri Ş., (2001) "İş Sağlığı ve İş Güvenliği", İşçi Sağlığı ve Güvenliği Dergisi, Ankara, S:1.
9. Gerek, N. (2000), İşçi Sağlığı ve İş Güvenliği, Eskişehir, Anadolu Üniversitesi Yayın No:967.
10. Görücü N, (2004) Türk ve İngiliz İş Sağlığı ve Güvenliği Teftiş SistemlerininKarşılaştırılması, yayımlanmamış Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, İstanbul.
11. Karadağ, S., E. (2010). Türk İnşaat Sektörü 'nün İş Güvenliği Açısından Risk Analizi (Yüksek Lisans Tezi). Ege Üniversitesi, Fen Bilimleri Enstitüsü, İzmir.
12. Karadeniz, O., (2012) "Dünya'da ve Türkiye'de İş kazaları ve Meslek Hastalıkları ve Sosyal Koruma Yetersizliği", Çalışma ve Toplum Dergisi, C.8, S.34, İstanbul.

Covid-19 Pandemic as a Biological Disaster and Biosensor Studies in the Disease Detection

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INTRODUCTION

From the past to the present, populations have faced many biological threats that have caused epidemics and pandemics. These intentional, incidental, or natural events have caused various diseases and deaths. Biological disasters are caused by infectious pathogens [1]. Especially recent mass migrations, climate changes, environmental pollution, Chemical, Biological, Radiological, and Nuclear incidents (CBRN), and terrorist attacks make us encounter disasters more frequently and with high impact rates [2]. Studies define disasters as events that threaten the health of all living things, disrupt the functioning of regular life and the health system, and cause material and moral losses [3]. Biological disasters emerging as an example of these risks, usually occur because of the contamination of some harmful microorganisms with natural epidemics and seriously affect society. But these biological agents can be deliberately and artificially released onto society and have severe effects [4].

These pathogens might develop into epidemics that pose severe risks to public health. In addition, many factors shown in Table 1 contribute to the rapid spread of these pathogens among the population. Against the risk of contagion and spread of the illness, measures such as reducing contagion-triggering contacts, restricting international and inter-provincial transportation, and reducing interaction between people come to the fore [5]. In this context, the literature identifies epidemics as biological disasters. Increasing antimicrobial resistance, ecological changes, chronic diseases, malnutrition, and poor economic situation are the dynamic impacts on the range and interaction of epidemics. In addition, infection risk, climate change, weak immune systems, and poor primary health care services are active factors in the risk of a new epidemic [6].

Table 1. Factors affecting the spread of infections

Microbial adaption and transformation	Global tourism and trade
Infection vulnerability	Technology and industry
Atmospheric Conditions	Community health policies
Varying Ecosystem	Deprivation and social imbalance
Human demographics and attitudes	War and starvation
Financial developments	Political conditions

Biological threats have naturally or deliberately affected many civilizations. According to historical records, the plague of Athens in classical Greece was one of the first known epidemics and caused 40,000 deaths. An infectious

disease called the Black Death, which emerged in China in the fourteenth century and spread to western countries, cost the lives of approximately 50 million people [7]. One of the significant biological elements of public health is pandemic viruses. Historical records also report that viruses, which spread rapidly by changing hosts, caused epidemics that resulted in death in ancient civilizations. The smallpox virus has affected societies for centuries by producing epidemics. Influenza also caused severe cases and epidemics historically with its high pandemic effect. The deadliest outbreak in history was the 1918 "*Spanish Flu"* pandemic, in which 500,000,000 people became ill and 50,000,000 died [1]. The Ebola virus epidemic between 2013 and 2016 also spread from West African countries to European countries. A total of 28,646 cases and 11,323 dyings took place worldwide [8].

Viruses do not fall into the class of living organisms. They are biological materials multiplying in host cells and eventually causing disease with their protein and nucleic acid structure (DNA or RNA) [9]. In other words, viruses manipulate the host's cellular machinery to produce more of themselves. This situation also indicates that a virus infecting a person has previously replicated and developed itself in another human or animal host. This virus transmission is also called host jumping or spillover. Numerous infectious diseases exist–viral or non-viral–such as smallpox, cholera, flu, and HIV, transmitted by "animal-to-human host jumping" events [7]. The transmission of pathogens has various determinants, such as the distance between the host and the pathogen, the structure of the host cell surface receptors, the bio-environmental conditions of the host and the virus, and the nucleic acid type in the virus. For example, RNA viruses use different virions when changing hosts, which leads to the emergence of many virion types depending on the same pathogen (virus) source [9]. In other words, during spread, viruses, which play a chief role in many epidemics, mutate because of rapid replications and numerous factors (host barrier, encountering diseases, etc.) in different hosts and thus cause viral diversity [10].

Coronaviruses

The coronavirus disease, which caused the death of approximately four million people worldwide as of 2021, was also caused by an enveloped, positive single-stranded RNA virus called coronavirus-2 (SARS-CoV-2), dispersed from many animal species [11, 12]. SARS-CoV-2 is one of seven members of the β coronavirus family known to contaminate humans, causing extreme respiratory syndromes. While four types of coronavirus (229E, NL63, OC43, and HKU1) cause symptoms similar to the common cold in people, the other three types,

SARS-CoV-2, SARS-CoV, and MERS-CoV with severe symptoms have death rates of 5%, 10%, and 37%, respectively [13].

In the phylogenetic taxonomic classification, human coronaviruses belonging to the order Nidovirales, family Coronaviridae, subfamily Coronavirinae has four genera: α (alpha), β (beta), γ (gamma), and δ (delta) (Figure 1). The most important of these are α and β [14]. β coronaviruses consist of two subspecies: Sarbecovirus (SARS-like viruses, ARS-CoV, and SARS-CoV-2) and Merbecovirus (MERS-like viruses, MERS-CoV) [9].

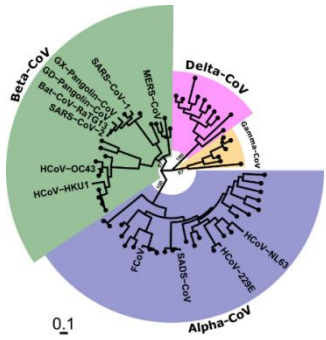

Figure 1. Phylogenetic classification of some coronaviruses [9]

Some studies have investigated the zoonotic transfer pathway of the pandemic-causing SARS-CoV-2. Many vertebrate species are a reservoir for coronaviruses (e.g., bats, pangolins, snakes, and mice) [15]. Studies have shown the characteristics of this human-infectious virus. The 29,881 bp long genome of the virus encoding 9860 amino acids encodes structural and non-structural proteins [13]. While the S, E, M, and N gene fragments encode structural proteins, the ORF region encodes non-structural proteins such as 3-chymotrypsin-like protease, papain-like protease, and RNA-dependent RNA polymerase [16]. The spike (S) protein of SARS-CoV-2 plays a crucial role in recognizing the receptor on the host cell's surface and in the virus penetration into the cell membrane. The S protein consists of two subunits, S1 and S2. However, three coronaviruses (HCoV-NL63, SARS-CoV, and SARS-CoV-2) use angiotensin-converting enzyme 2 (ACE2) as a receptor [17]. The renin-angiotensin system (RAS) is crucial in regulating vascular tone and fluid-electrolyte homeostasis in the cardiovascular system [18]. ACE converts angiotensin-I to angiotensin-II. High angiotensin-II level increases hypertension development and renal fluid and electrolyte absorption [19]. As a carboxymonopeptidase enzyme, ACE2, in contrast, hydrolyzes the cleavage of a C-terminal moiety from Angiotensin-II to convert it back to Angiotensin-(1-

7), a vasodilator. ACE2 is a receptor located on the cell membranes of various tissues [18]. As a result, ACE2 creates a vasodilative response by lowering blood pressure and regulating fluid balance, unlike ACE. The S1 subunit of SARS-CoV-2 holds a receptor-binding domain familiar to this host's ACE2 receptor, while the S2 subunit ensures the adsorption of the virus to the host cell membrane (Figure 2). As a result, the S protein provides the SARS-CoV-2 virus penetration into the host cell [13].

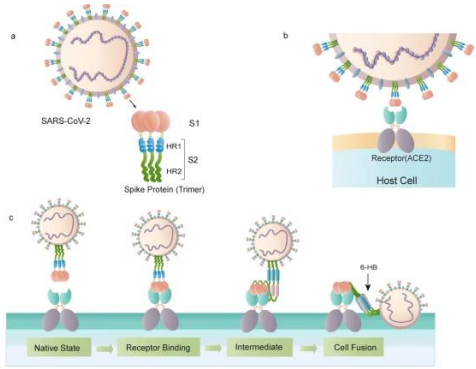

Figure 2. Adsorption of the coronavirus into the cell [13].

Previous studies considering the structural similarities between SARS-CoV-2 and SARS-CoV coronavirus strains in RaTG13 bats and Malayan pangolins (Manis javanica) have claimed that the virus that gains new genomic features through adaptation, spread primarily as "animal-to-human" and then "human-to-human" through zoonotic transfer [12]. Before the 2019 pandemic, some scientists conducted studies on coronavirus in 2007 and emphasized that some bat species were naturally large reservoir hosts of SARS-CoV-like virus and that this situation could pose perilous risks [15].

The coronavirus-19 (COVID-19) pandemic has gained its place in the top three global diseases affecting public health. Besides, the World Health Organization announced it as the sixth international health crisis. Previous studies have discovered that COVID-19 causes several diseases, including severe acute respiratory syndromes [20], intravascular coagulation (DIC), thrombotic microangiopathy [21] post-traumatic anxiety, and depression [22].

COVID-19 is mainly spread from human to human by breathing in droplets from an infected person or touching contaminated surfaces, then touching the eyes, nose, or mouth [23]. Although the incubation time for COVID-19 is within 14 days of exposure, overall, incidents occur between four and five days after exposure [24].

COVID-19 Detection

Diagnosis is essential in taking precautions against the spread of the epidemic among the public and in controlling the disease. Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) with high sensitivity, Reverse Transcription Polymerase Chain Reaction (RT-PCR), and Real-time RT-PCR are the most employed techniques in coronavirus diagnosis [25-27]. The application times of these techniques are short, and their analytical performance is satisfactory. However, the insufficiency of kits might delay the diagnosis. Here, computed tomography (CT) technology can also be utilized. CT results of patients presenting with a COVID-19 complaint display bilateral pulmonary parenchymal ground-glass morphology [28, 29].

In addition, serological tests such as ELISA or rapid antibody tests that provide IgM/IgG already serve in the COVID-19 diagnosis. In this context, literature studies show that serological tests not only allow for inspecting the ongoing epidemic but also produce a retrospective evaluation of the attack rate and epidemic severity [30].

COVID-19 and Biosensors

In addition to various preventive measures and prediction models to diminish the spread of the epidemic, pharmacological approaches are also improved. The early detection and social isolation of diseased individuals are the most effective methods for the limited transmission of the epidemic [31-33]. Today's relatively insufficient early diagnosis test kits might produce false-negative results because the sample values taken during the virus incubation period are outside the detection limits of the diagnostic methods [31, 33, 34].

Biosensors, which are analytical techniques created by merging physicochemical analysis approaches and biological matters, are more sensitive than traditional diagnostic systems such as PCR and ELISA and can detect analytes with higher sensitivity than these systems. In biosensors, the increased precision of the biological method and the diagnostic sensitivity of the physical analysis system are combined [35].

Today's literature covers many different biosensor studies detecting potential neuro and biological agents. Biosensors that use antigen and antibody interaction are called immunosensors. Since there is high specific molecular recognition and affinity between the antibody and its antigen, the specificity and sensitivity of immunosensors are generally higher than other sensors. The affinity constant of the antibody and antigen is usually 10^8 M^{-1} and can climb to 10^{15} M^{-1}[36].

Immunosensors are systems in which antigens and antibodies, the immune system elements, are used in the recognition layer. The signals from this layer are transformed into appropriate data in the converter part and transferred to the digital environment. In this system, which utilizes antigen-antibody interaction, devices that can convert molecular interaction into detectable signals in different ways are employed. Through antibody-antigen interaction, specific physicochemical changes, such as mass, temperature, and electric potential, emerge. These changes are translated into interpretable signals through the transducer part of the immunosensor [37].

Early diagnosis is vital to reduce the critical complications and mortality rate caused by COVID-19 transmission. In these diagnostic methods, problems, such as applicability, high sensitivity, and technical personnel, should be overcome. In this context, many biosensor development studies previously conducted on the COVID-19 diagnosis have aimed to detect antigens, antibodies, or nucleic acids in patient samples such as sputum, saliva, and blood [38, 39]. Biosensor methods used in diagnosis are fluorescent, electrochemical, optical, or colorimetric based. In addition, paper-based-chipped Point of care sensors is also being developed [40].

COVID-19 in terms of Disaster Management

In disaster management, the preparation, mitigation, and response stages of COVID-19 differ according to disaster type, and the process requires full coordination between institutions. In the "disaster management process of the COVID-19 period," which is considered a disaster, the authority that provided health coordination and an integrated disaster management chain was the Ministry of Health. The Ministry of Health published the "Pandemic Influenza National Preparedness Plan" in 2009 and aimed to establish a communication and information network by providing coordination between all institutions and organizations. In addition, by distributing the duty and responsibilities between institutions, the ministry targeted to complete the in-house shortcomings against such disasters and determined the following what-to-do list:

- Completion of the shortcomings against the disasters and pandemics to be experienced and the readiness of the institutions
- Planning the work to be done against the pandemic and working on the measures to be taken by evaluating the risk before the pandemic
- Ensuring national coordination during the pandemic, determining the duties and responsibilities of all institutions and organizations
- Guidance to institutions and organizations during the preparation phase

- Providing support and solutions for the positive outcome of the influenza preparation phase .

As seen, ensuring coordination and conducting studies in the fight against the pandemic has been planned within the Ministry of Health. However, in the current situation, the task of providing all coordination and inter-institutional communication within the Turkiye Disaster Response Plan has been assigned to the Disaster and Emergency Management Presidency (AFAD) [42].

Every day, research provides more and more information on how the spread and impact of COVID-19 affect different demographic groups. This information brings relevant factors such as gender, age, chronic illness, and health services' accessibility to the fore in the COVID-19 risk assessment. COVID-19-related risk factors vary between groups. When people are exposed to disease, their risk perceptions change, and they adopt restrictions. Besides these situations, radical evaluations should be made by considering the economic dimensions of disaster management measures [43].

CONCLUSION AND RECOMMENDATIONS

Traditional test kits may be insufficient to detect the virus in the incubation period because sample values may be outside the kit detection range, and thus the test may give false-negative results. Biosensors, which are analytical systems, are more sensitive than traditional diagnostic systems such as PCR and ELISA and can detect the analyte of smaller value. Biosensors are the combination of the great particularity of the biological technique and the diagnostic sensitivity of the physical analysis method. Therefore, the need for advanced biosensors for early diagnosis of COVID-19 and low-cost sensors for electrode immobilization is growing day by day.

REFERENCES

1. Narayanan, N., et al., *Disaster Preparedness: Biological Threats and Treatment Options.* Pharmacotherapy, 2018. **38**(2): p. 217-234.
2. EKŞİ, A., *KBRN TERÖRİZMİNDE RİSK DEĞERLENDİRMESİ VE YÖNETİMİ.* Journal of International Social Research, 2016. **9**(42).
3. Özmen, B. and T. Özden, *Türkiye'nin afet yönetim sistemine ilişkin eleştirel bir değerlendirme.* İstanbul Üniversitesi Siyasal Bilgiler Fakültesi Dergisi, 2013(49).
4. Narayanan, N., et al., *Disaster preparedness: biological threats and treatment options.* Pharmacotherapy: The journal of human pharmacology and drug therapy, 2018. **38**(2): p. 217-234.
5. Lu, H., C.W. Stratton, and Y.W. Tang, *Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle.* Journal of medical virology, 2020. **92**(4): p. 401.
6. Şimşek, Z., *Biyolojik Afet Olarak Covid 19 Pandemisi Özelinde Mevsimlik Tarım İşgücü ve Ailelerine Yönelik Temel Sağlık Hizmetlerinin Sunumu››.* Sağlık ve Toplum (Özel Sayı), 2020: p. 103-111.
7. Morens, D.M., G.K. Folkers, and A.S. Fauci, *Emerging infections: a perpetual challenge.* Lancet Infect Dis, 2008. **8**(11): p. 710-9.
8. Spengler, J.R., et al., *Perspectives on West Africa Ebola Virus Disease Outbreak, 2013-2016.* Emerg Infect Dis, 2016. **22**(6): p. 956-63.
9. Morens, D.M., et al., *The Origin of COVID-19 and Why It Matters.* Am J Trop Med Hyg, 2020. **103**(3): p. 955-959.
10. Poirier, E.Z. and M. Vignuzzi, *Virus population dynamics during infection.* Curr Opin Virol, 2017. **23**: p. 82-87.
11. Khan, W.H., et al., *COVID-19 Pandemic and Vaccines Update on Challenges and Resolutions.* Front Cell Infect Microbiol, 2021. **11**: p. 690621.
12. Asselah, T., et al., *COVID-19: Discovery, diagnostics and drug development.* J Hepatol, 2021. **74**(1): p. 168-184.
13. Huang, Y., et al., *Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19.* Acta Pharmacol Sin, 2020. **41**(9): p. 1141-1149.
14. Corman, V.M., et al., *Hosts and Sources of Endemic Human Coronaviruses.* Adv Virus Res, 2018. **100**: p. 163-188.
15. Andersen, K.G., et al., *The proximal origin of SARS-CoV-2.* Nat Med, 2020. **26**(4): p. 450-452.

16. Chan, J.F., et al., *Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan.* Emerg Microbes Infect, 2020. **9**(1): p. 221-236.
17. Beacon, T.H., G.P. Delcuve, and J.R. Davie, *Epigenetic regulation of ACE2, the receptor of the SARS-CoV-2 virus(1).* Genome, 2021. **64**(4): p. 386-399.
18. Li, Q., et al., *ACE2 as therapeutic agent.* Clin Sci (Lond), 2020. **134**(19): p. 2581-2595.
19. Gottschalk, G., K. Knox, and A. Roy, *ACE2: At the crossroad of COVID-19 and lung cancer.* Gene Rep, 2021. **23**: p. 101077.
20. Wen, Z., et al., *Tongue and pulse features of 668 asymptomatic patients infected with the severe acute respiratory syndrome coronavirus 2 omicron variant in Shanghai.* J Tradit Chin Med, 2022. **42**(6): p. 1006-1011.
21. Levi, M., et al., *Coagulation abnormalities and thrombosis in patients with COVID-19.* Lancet Haematol, 2020. **7**(6): p. e438-e440.
22. Li, W., et al., *Network Analysis of Depression, Anxiety, Posttraumatic Stress Symptoms, Insomnia, Pain, and Fatigue in Clinically Stable Older Patients With Psychiatric Disorders During the COVID-19 Outbreak.* J Geriatr Psychiatry Neurol, 2022. **35**(2): p. 196-205.
23. Wu, D., et al., *The SARS-CoV-2 outbreak: what we know.* International Journal of Infectious Diseases, 2020. **94**: p. 44-48.
24. Li, Q., et al., *Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia.* New England journal of medicine, 2020.
25. Zhai, P., et al., *The epidemiology, diagnosis and treatment of COVID-19.* International journal of antimicrobial agents, 2020. **55**(5): p. 105955.
26. To, K.K.-W., et al., *Consistent detection of 2019 novel coronavirus in saliva.* Clinical Infectious Diseases, 2020. **71**(15): p. 841-843.
27. Huang, P., et al., *A rapid and specific assay for the detection of MERS-CoV.* Frontiers in microbiology, 2018. **9**: p. 1101.
28. Xie, X., et al., *Chest CT for typical 2019-nCoV pneumonia: relationship to negative RT-PCR testing.* Radiology, 2020.
29. Ai, T., et al., *Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases.* Radiology, 2020.
30. Bakanliği, T.S., *COVID-19 (SARS-CoV2 Enfeksiyonu) Rehberi.* Erişim (Erişim Tarihi: July 16, 2020), 2020.

31. Li, Q., et al., *Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia.* N Engl J Med, 2020. **382**(13): p. 1199-1207.
32. Wu, J.T., K. Leung, and G.M. Leung, *Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study.* Lancet, 2020. **395**(10225): p. 689-697.
33. Rothe, C., et al., *Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany.* N Engl J Med, 2020. **382**(10): p. 970-971.
34. Phan, L.T., et al., *Importation and Human-to-Human Transmission of a Novel Coronavirus in Vietnam.* N Engl J Med, 2020. **382**(9): p. 872-874.
35. Kokbas, U., L. Kayrın, and A. Tuli, *Biosensors and their Medical Applications.* Archives Medical Review Journal 2013.
36. Vaughan, R.D. and G.G. Guilbault, *Piezoelectric immunosensors, Piezoelectric Sensors.* Springer Berlin Heidelberg, 2007: p. 237-280.
37. Byrne, B., et al., *Antibody-based sensors: principles, problems and potential for detection of pathogens and associated toxins.* Sensors, 2009. **9**(6): p. 4407-4445.
38. Choi, J.R., et al., *based sample-to-answer molecular diagnostic platform for point-of-care diagnostics.* Biosensors and Bioelectronics, 2015. **74**: p. 427-439.
39. Tang, R., et al., *based device with on-chip reagent storage for rapid extraction of DNA from biological samples.* Microchimica Acta, 2017. **184**(7): p. 2141-2150.
40. Choi, J.R., *Development of point-of-care biosensors for COVID-19.* Frontiers in chemistry, 2020. **8**: p. 517.
41. Bakanlığı, S., *Sağlık Bakanlığı 2019-2023 Stratejik Planı.* 2019.
42. Duruel, M.C. and D.Y. Kurumu, *Afet ve Acil Durum Yönetimi Başkanlığı (AFAD) Stratejik Planlarının Değerlendirilmesi.*
43. Ryan, B.J., et al., *COVID-19 community stabilization and sustainability framework: an integration of the Maslow hierarchy of needs and social determinants of health.* Disaster medicine and public health preparedness, 2020. **14**(5): p. 623-629.

Nursing Care in Geriatric Patients Undergoing Muscle-Skeleton Surgery

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INTRODUCTION

With aging, some changes occur in the musculoskeletal system. With aging, bone loss occurs, bone mineral density decreases, and bone structure deteriorates. The cartilage structure in the joints becomes thinner; Some substances in the structure of cartilage lose its flexibility, become hard and become more rigid and brittle. Ligaments and tendons become prone to tearing, and the tears that occur are difficult to heal. The fluid content of the discs in the spine decreases, their nutrition is disturbed, cracks and abrasions occur. The number and size of muscle fibers gradually decreases, which leads to a decrease in skeletal muscle mass and strength. As a result of these and similar changes in the musculoskeletal system, elderly people often; osteoporosis, osteoarthritis, degenerative joint diseases, rheumatoid arthritis, pelvic fracture and fall fractures are seen (Çopuroğlu C, Heybeli, 2011).

Orthopedic problems seen in advanced ages are generally associated with osteoporosis and degeneration. While osteoporosis is a source of discomfort in itself, it leads to increased fragility of bones and prepares the ground for fractures that can occur even with simple falls or very minor traumas. Secondary problems developing after fracture; Wound healing problems, bedsores, lung infections, prolonged immobility and many subsequent complications are the main elements of a vicious circle that begins as a result of osteoporosis. In addition, factors such as decreased vision, hearing ability and balance control with aging, the necessity of using many drugs, and walking disorders due to musculoskeletal diseases such as osteoarthritis increase the risk of falling in the elderly. With aging, the decrease in bone mineral density accelerates and the resulting osteoporosis causes fractures and especially hip fractures, which can lead to disability or death, as a result of a fall that does not cause bone fractures in normal people (Çopuroğlu et al., 2011).

EVALUATION OF GERIATRIC PATIENTS BEFORE ORTHOPEDIC SURGERY

It is known that hip fractures are one of the most common orthopedic problems, especially in the ranking of hospitalization in elderly individuals (Della Rocca et al., 2013). In our country, it has been reported that patients who apply to the emergency department due to trauma have various fractures, mainly femur fractures, and 66% of them are hospitalized (Kara et al., 2014).

Due to musculoskeletal problems, elderly patients hospitalized in Orthopedics and Traumatology clinics are often accompanied by chronic diseases. Care for the majority of the problems experienced by elderly individuals is included in nursing practices, and nurses have to provide care to

an increasing number of elderly individuals in Orthopedics and Traumatology Clinics, as in various fields. Orthopedic nurses fulfill the roles of caregiver, trainer, consultancy and care coordinator in care for the elderly. In order to carry out these roles effectively; They need to have sufficient knowledge and experience on aging, physiological, cognitive, psychosocial and economic changes related to aging, chronic diseases in the elderly, and health and social support services for the elderly, and develop their knowledge and experience through continuous training (Kaptan, 2013; Şeliman et al., 2010).). The main purpose in the care of elderly individuals; minimizing dependency and maximizing independence. In achieving this goal, it is important that the care be individualized and multi-faceted planning including the emotional, social and physical aspects of the individual (Dewan et al., 2012).

Evaluation of Physical Condition

Evaluation of the elderly person starts when the patient is first seen (appearance, posture, speech, awareness of his surroundings, ability to move) and continues until he leaves the hospital (dressing on his own, difficulty in finding the way out, etc.). When taking a history, apart from the traditional anamnesis, it is necessary to be asked about specific situations of old age (falling, incontinence, constipation, and the use of situation-specific scales. Questioning many chronic problems such as decreased vision and hearing, depression, deterioration in cognitive functions causes difficulties in taking the history. Especially delirium or cognitive impairment, rapid evaluation and physical examination should be performed, and a comprehensive history should be obtained from the patient and his/her relatives. There is a strong relationship between social relations and functional status and mortality in the elderly. Who the patient lives with should be questioned. The patients should be evaluated especially for neurological, cardiovascular, and mental status. In particular, detailed system diagnosis should be made (Gomes et al., 2013).

Evaluation of Functional Status

Functional status is defined as 'a person's ability to perform their duties and meet the complex social roles required by activities of daily living (ADL)' and is an important element of quality of life. Functional disabilities are common in elderly patients due to many potential causes such as age-related changes, social factors, and diseases. Functional status assessment is done at three levels:

- Basic Daily Living Activities
- Instrumental Daily Living Activities
- Advanced Daily Living Activities

Basic Activities of Daily Living; defines functions that are necessary, but not entirely sufficient, to ensure independent living. Basic functions; feeding,

continence, transfer, using the toilet, dressing and washing. Instrumental Activities of Daily Living include more complex activities necessary to maintain home life. These; These are activities such as paying bills, taking medications, shopping, preparing meals, protecting the home, transport and using the telephone. Advanced Daily Living Activities, on the other hand, are activities such as leisure time, traveling, volunteer activities, creative activities and participation in community services. Various scales used to evaluate patients' ADL are easily used by nurses in the Orthopedics and Traumatology Clinic. Some of those; Katz ADL Scale, Barthel Scale, Lawton-Brody IDA Scale (Savaş & Akçiçek, 2010).

Evaluation of Mobility, Balance and Fall Risk

Falls frequently seen in elderly people; It causes loss of functional capacity, morbidity and mortality. More than 60% of those who die from falls are in the age group of 75 and over. Due to the increased risk of falling, all patients from the age of 65 should be evaluated for mobility, balance and falls (Gomes et al., 2013; Ungar et al., 2013).It has been determined that many factors related to falls pose risks. Physiological changes in age-related sensory functions (sight, color discrimination, decreased adaptability, vestibular disorder, increased ear wax) and changes in the locomotor system (sarcopenia, decreased muscle strength, decreased joint range of motion) are important risk factors for falls. In addition, environmental factors (obstacles, slippery ground, inappropriate shoes, insufficient lighting, etc.) have an impact. In addition, patients may have pathological factors that may pose a risk for falling. These; neurological (dementia, stroke, transient ischemic attack, parkinsonian, delirium, carotid sinus hypersensitivity, vestibular system pathology), cardiovascular (MI, orthostatic hypotension, arrhythmias, valvular diseases), endocrine and internal problems (thyroid diseases, hypoglycemia, hypokalemia, hypohypernatremia) , dehydration, anemia), gastrointestinal (diarrhea, hemorrhage), psychiatric (depression), genitourinary (urinary incontinence), iatrogenic (side effects of drugs such as anxiolytic, neuroleptic, diuretic, antihypertensive and digoxin; limitation of movement-immobilization) and musculoskeletal system (Degenerative joint diseases, muscle diseases, spinal deformities, pathological fractures) (Savaş and Akçiçek, 2010). While the rate of falling was 8% in the last year in those with no risk factors, it was observed that this rate increased to 78% in those with 4 or more risk factors (Naharcı and Doruk, 2009). For this reason, it is important for the nurse to determine the possible risks for the patient and to take the necessary precautions. Objectives for the prevention of falls; To determine the fall risk of the elderly individual, to eliminate the determined fall risk factors, to improve balance, gait, movement and functional

independence with an interdisciplinary approach. In order to determine the risk of falling, forms have been developed to determine the factors that may lead to falls related to the patient and his/her environment. In determining the risk of falling in the elderly; Scales such as Hendrich II Fall Risk Diagnostic Scale, Morse Fall Risk Scale, Fall Behaviors Scale for the Elderly are frequently used (Çeçen & Özbayır, 2011; Uymaz & Nahcivan, 2013). In fact, a simple evaluation of routine movements can also provide information in determining the risk of falling. The elderly individual is observed during movements such as getting up from a chair, turning while walking, lifting his feet from the ground and sitting. Difficulty in any of these activities indicates a high risk of falling. All elderly patients at risk of falling should be evaluated with a history and physical examination. In addition, since the elderly generally do not give spontaneous information about falling, they should be asked at least once a year whether they have fallen and evaluated in terms of balance and walking problems (Erdil & Bayraktar, 2010).

Evaluation of Pain

In the elderly patient who comes to the Orthopedics and Traumatology Clinic, chronic pain such as osteoarthritis or rheumatoid arthritis pain should be dealt with in addition to acute pain due to orthopedic surgical interventions. In addition, clinic-specific applications such as immobilization, tight bandage, plaster, traction, and drain increase the existing pain of the patients. Pain assessment; It starts with observation and requires consideration of physical, psychological and social factors. Various scales used in the assessment determine the severity and quality of pain, eliminating comments among healthcare professionals and turning them into an objective result. In the evaluation of the pain level of elderly patients; Diagnostic tools such as Verbal Category Scale, Numerical Scales, Visual Comparison Scale (VAS), McGill Melzack Pain Questionnaire are used (Güngör et al., 2013).

HIP FRACTURES AND EVIDENCE-BASED NURSING CARE IN ELDERLY PATIENTS

Hip fractures are a serious and common health problem in the elderly, with an incidence of between 20 and 921 per 100,000 people worldwide (Cheng et al., 2011). Geriatric hip fractures are most commonly caused by osteoporosis and are associated with increased mortality and morbidity in the short-term and long-term (Seys et al., 2018). In a study conducted; It was found that there was no significant relationship between the time until surgery and the age of the patient ($p=0.100$, $r=0.03$), and the mortality rate was closely related to age ($p=0.01$, $r=0.87$). Considering the postoperative functions, it was determined

that the relationship with age was moderate (p=0.06, r=0.37) (Uygur et al., 2015). In another study conducted to determine the factors affecting mortality, fracture age (p=0.005), ASA score of 3 and above (p=0.041) and presence of comorbid disease (p=0.033) were found to be effective factors on mortality in the first year. . The preoperative waiting time alone had no effect on mortality in the first year (p=0.143) (Adanır et al., 2017).

In order to plan the post-operative care to be given to the elderly patient, the patient should be evaluated comprehensively in terms of physiological and psychosocial changes due to aging, existing chronic diseases and the risks posed by the surgery. In the postoperative period, the planned care is applied to the patient in the preoperative period. The main goal of care in this period is to contribute to the patient's ability to function independently by preventing complications and providing early mobilization (Savcı and Bilik, 2014).

Prevention of Pressure Wound Development

There are many risk factors in patients for the development of pressure ulcers. In orthopedic surgeries, factors such as staying in the same position for a long time, advanced age, post-surgical immobilization, weight, and nutrition are the most important factors that increase the risk of pressure ulcer development. Studies show that the incidence of pressure ulcers increases as the risk factors related to surgery increase. Aronovitch, in his study with 281 surgical patients, concluded that pressure ulcers may develop when the operation time exceeds 3 hours. Aronovitch also reported that orthopedic and cardiac surgeries performed in the supine position also had an effect on the development of pressure ulcers. There is a relationship between the duration of immobilization and the development of pressure sores (Aronovitch, 2007).

In long-term operations, the risk of pressure ulcer increases when the pre- and post-operative period is added to the immobilization period. In the literature, it is stated that the development of pressure sores is also related to nutrition. Malnutrition is a risk factor that is effective in the development and healing of pressure ulcers. The incidence of pressure ulcers has been reported to be 60% in elderly patients who were operated for hip fractures, whose nutritional support was inadequate, and whose nutritional support was even temporarily discontinued (Avenell and Handoll, 2010).

There are many risk factors that will cause pressure ulcer development in patients undergoing surgical intervention. However, most of these risk factors are preventable with nursing interventions. In order to prevent pressure ulcers in patients who will undergo surgical intervention, a risk assessment for the development of pressure ulcers should be made. Determining the risk status of patients before surgery by surgical nurses will enable them to take initiatives for

preventable risk factors in the preoperative period. By keeping the records of the patients whose risk status is determined before the operation, ensuring the continuity and reporting of the records, they will also shed light on the practice of the operating room nurse to reduce and/or prevent the risk of pressure ulcers during the operation. For patients undergoing surgical intervention, the skin should be evaluated before, during and after the intervention. Under anesthesia, it is recommended to turn the patients with a bed sheet to reduce the risk of pressure ulcers due to friction during position change, to use assistive devices during the transport of the patients, and to place transparent dressing materials on risky areas under pressure such as elbows, heels and sacrum. One of the main responsibilities of the operating room nurses is to make the skin preparation properly so that the humidity caused by the solutions used during the operation does not pose a risk for pressure sores in the patient, and to prevent the solutions from pooling outside the operation area during the operation and to keep the areas under pressure dry (Gül, 2014).

In the study called 'Prospective Prognostic Cohort Study of Pressure Injuries in Elderly Adult Patients', in a study conducted with 467 elderly patients between 2013 and 2014, the patient's skin was evaluated daily by nurses and physiotherapists, and pressure ulcers developed in 27% of the patients. Being over 81 years old, having a limb in a foam splint, and the type of surgery were determined as patient-related risk factors for pressure ulcer development (Forni et al., 2018).

Oxygen Administration

Persistent hypoxia has been reported to be present in all hip fracture patients at the time of presentation up to five days after surgery. Oxygen saturation should be checked at the entrance. Supplemental oxygen should be administered to all patients with hypoxemia. Oxygen saturation should be routinely monitored to reduce the incidence of hypoxemia and should be continued as long as there is a tendency to hypoxemia. Supplemental oxygen is recommended at least six hours after general or spinal/epidural anesthesia, at night, for 48 hours postoperatively, and for as long as hypoxemia persists as determined by pulse oximetry (Evidence Level C) (SIGN, 2009).

Blood transfusion

There is little evidence regarding blood transfusion in patients with hip fractures. A retrospective study of 8,787 hip fracture patients aged ≥60 years found that perioperative transfusion had no effect on mortality in patients with hemoglobin levels ≥80 g/l. However, fewer studies have shown that patients with known cardiac disease may benefit from transfusion at higher hemoglobin levels (SIGN, 2009).

Fluid and electrolyte balance

Electrolyte imbalances, especially hyponatremia and hypokalemia, are common in the postoperative period and reflect the limited renal reserve of these patients. This situation may be exacerbated by the inappropriate combination of diuretics and maintenance intravenous fluids. Fluid management is often poor in the elderly, and elderly women are at risk of developing hyponatremia, especially in the perioperative period. Fluid and electrolyte management should be monitored regularly in the elderly (Evidence Level B). Fluid and electrolyte management should begin in the emergency department (Evidence Level D) (SIGN, 2009).

Delirium

Delirium, or acute confusional state, usually occurs after a hip fracture. Increases in length of stay are associated with nursing home placement rate and mortality. Attention to oxygen saturation, blood pressure, fluid and electrolyte balance, pain control, medication, bowel and bladder function, food intake, early mobilization, and detection and treatment of intercurrent disease will prevent some episodes and minimize the severity of others (SIGN, 2009).

Nutrition

Elderly people with hip fractures are often malnourished at presentation and their nutritional status does not necessarily improve in hospital. Dietary reviews in the postoperative period noted inadequate nutritional intake. Malnutrition can cause mental apathy, muscle wasting and weakness, heart dysfunction, and reduced immunity to infection (SIGN, 2009).

Early mobilization

Early mobilization can prevent complications such as pressure injury and deep vein thrombosis. Mobilization together with pre- and post-operative physiotherapy may be valuable in reducing pulmonary complications. If the patient's general medical condition allows, mobilization and multidisciplinary rehabilitation should begin within 24 hours after surgery. Weight bearing on the injured leg should be allowed unless there are concerns about the quality of the hip fracture repair (eg, poor bone stock or comminuted fracture).

Rehabilitation and discharge

Given the importance of good rehabilitation in the overall quality and cost-effectiveness of hip fracture treatment, the relevant evidence base is disappointing. Factors such as the complexity of the case mix, service context, details of service organization and multidisciplinary input, and even healthcare reimbursement systems can greatly contribute to the problems associated with the organization of large clinical trials that normally involve older patients (SIGN, 2009). Patients with comorbidities, poor functional ability and low

mental test scores should undergo rehabilitation in a geriatric orthopedic rehabilitation unit before admission (Evidence Level B) (SIGN, 2009; Wallace et al., 2018).

HOME CARE

It is not always possible for elderly patients with hip fractures to be rehabilitated in a hospital setting until they are fully independent. In this case, the care opportunities of the patients after discharge come to the fore. Patient care in surgical nursing includes the pre-, intra- and post-operative care of the patient who will be operated on, and the care to be given after discharge is also very important. Patients discharged after surgery are frequently exposed to numerous interventions such as dressings, plasters, mobility aids, drugs (dose, interaction, side effects), diet and exercises, and even limb or organ loss. In addition, because these patients also have other health problems at the same time, their recovery period is complex and they need to be closely monitored. For this reason, the need for care of the patient who is discharged after the surgical intervention and goes home does not end. The need for care may persist for weeks or even months after hospitalization. In order to prevent or reduce the complications that can be seen after discharge, especially in the first month after the operation, when the individuals who underwent surgical intervention are at risk for complications and the recovery process is important, by determining the problems experienced by the individuals, providing patient and family-centered care, and evaluating the effect of environmental conditions on recovery. It is important to plan home care services (Dal et al., 2012).

The surgical nurse should be in contact with the home care nurse to ensure the continuation of patient care after discharge. In the home visits of the home care nurse; It is necessary to remove bad and musty odors by ventilating the house, and the floors such as sofa upholstery can be odorous, so cleaning should be done carefully; Ensuring home, room, bed, clothing and body hygiene, ensuring proper storage and preparation of food, controlling the temperature of the room where the patient's bed is located, the need to be careful about the decrease in the patient's body temperature, the importance of performing non-pharmacological applications as well as maintaining the pharmacological treatment appropriate for the patient's condition, monitoring complications that may occur due to surgical application and poor environmental conditions, providing a suitable hygienic care environment, increasing the patient's sleep quality by minimizing environmental noise, controlling pain, following infection control methods to protect from infections in the post-operative period, getting the patient to exercise, sitting and It should cooperate on issues

such as paying attention to sleeping positions, preventing bedsores, informing the caregiver or people at home on this issue. All these practices performed in the home environment of the patient strengthen the food, medicine, treatment and care program of the patient after discharge, make him feel safe and comfortable in his own home, increase his communication with the family and his environment, and protect him from infections in the home environment, as well as in the home environment with the arrangements in the home environment. (Yilmaz et al., 2017).

As a result, the surgical nurse should be in constant cooperation and communication with the home care nurse in order to meet the needs of the individuals after discharge and to provide effective and comprehensive care. It is very important for the home care nurse to carry out planned home visits, to evaluate the patient together with his/her environment, to warn against the risks that may occur in the home environment, to make the necessary arrangements in the home, to inform the patient's relatives/caregivers about the situations they need to be careful about, to provide health education, and to provide communication between the hospital and the home. is important. In addition, it is thought that the surgical nurse who cares for the patient in the clinic will have an important role in performing interdisciplinary care with the home care nurse through methods such as tele-nursing, home visits, web-based or computer-assisted patient follow-up systems after discharge (Yılmaz et al., 2017).

REFERENCES

1. Adanır, O., Colonel, C., Beytemur, O. (2017). The relationship between preoperative waiting time and mortality in intertrochanteric femur fractures of elderly patients. Okmeydanı Medical Journal, 33(1):23-27.
2. Aronovitch, S.A. (2007). Intraoperatively acquired pressure ulcers: are there common risk factors? Ostomy Wound Manage, 53(2):57–69.
3. Avenell, A., Handoll, H.H. (2010). Nutritional supplementation for hip fracture after care in older people. Cochrane Database Syst Rev., CD001880.
4. Cheng, S.Y., Levy, A.R., Lefaivre, K.A., Guy, P., Kuramoto, L., Sobolev, B. (2011). Geographic trends in incidence of hip fractures: A comprehensive literature review. Osteoporosis Int, 22:2575-2586.
5. Çeçen, D., Özbayır, T. (2011). Determination of fall risk in elderly patients hospitalized in surgical clinics and evaluation of interventions to prevent falls. Journal of Ege University School of Nursing, 27 (1): 11-23.
6. Copuroglu, C., Unver, K.V., Ozcan, M., Ciftdemir, M., Turan, F.N., Copuroglu, E. (2011). Investigation of risk factors identified in hip fracture patients. Bakırköy Medical Journal, 7:136-141.
7. Copuroglu, C., Heybeli, N. (2011). Orthopedic problems in the elderly. Gero Fam., 2(1): 1-10.
8. Dal, Ü., Bulut, H., Demir, S.G. (2012). Problems experienced by patients at home after surgical intervention. Bakırköy Medical Journal, 8(1):34-40.
9. Della Rocca, G.J., Moylan, K.C., Crist, B.D., Volgas, D.A., Stannard, J.P., Mehr, D.R. (2013). Comanagement of geriatric patients with hip fracures: A Retrospective, Controlled, Cohort Study. Geriatric Orthopedic Surgery&Rehabilitation, 4(1): 10-15.
10. Dewan, S.K., Zheng, S.B., Xia, S.J. (2012). Preoperative geriatric assessment: Compherensive, multidisciplinary and proactive. European Journal of Internal Medicine, 23: 487-494.
11. Erdil, F., Bayraktar, N. (2010). Nursing Approaches to Musculoskeletal Problems in Elderly Individuals. Istanbul University Florence Nightingale Nursing Journal, 18 (2): 106-113.
12. Forni, C., D'Alessandro, F., Genco, R., Mini, S., Notarnicola, T., Vitulli, A., Capezzali, D., Morri, M. (2018). Prospective prognostic cohort study of pressure injuries in older adult patients with hip fractures. Adv Skin Wound Care, 31(5): 218-224.

13. Gomes, G.O., Cintra, F.A., Batista, F.S., Neri, A.L., Guariento, M.E., Sousa, M.R., D'elboux, M.J. (2013). Elderly outpatient profile and predictors of falls. Sao Paulo Medicine Journal, 131 (1): 13-8.
14. Gul, S. (2014). Prevention of pressure ulcer development in patients undergoing surgery. Hacettepe University Faculty of Nursing Journal, 1(3): 54-61.
15. Güngör Tavşanlı, N., Özçelik, H., Karadakovan, A. (2013). Investigation of quality of life of elderly individuals with pain. Pain, 25 (3):93-100.
16. Black, H., Bayır, A., Ak, A., Akıncı, M., Tüfekci, N., Değirmenci, S., Azap, M. (2014). Trauma in elderly patients evaluated in a hospital emergency department in Konya, Turkey: A retrospective study. Clinical Interventions in Aging, 9: 17-21.
17. Captain, G. (2013). Geriatric Care Principles. Captain G. (Ed.). Basic Physical Requirements of the Elderly. (1st edition pp. 25-39). Nobel Medicine Bookstores, Istanbul.
18. Naharcı, M.İ., Doruk, H. (2009). Approach to falling in the elderly population. TAF Preventive Medicine Bulletin, 8(5): 437-444.
19. Savaş, S., Akçiçek, F. (2010). Comprehensive geriatric assessment. Aegean Medical Journal, 49 (3): 19-30.
20. Prosecutor, A., Bilik, Ö. (2014). Geriatric evaluation in orthopedics and traumatology clinic. Dokuz Eylül University School of Nursing Electronic Journal, 7 (2), 156-163.
21. Seys, D., Sermon, A., Sermeus, W., Panella, M., Bruynee, L., Boto, P., Vanhaecht, K. (2018). Recommended care received by geriatric hip fracture patients: where are we now and where are we heading? Archives of Orthopedic and Trauma Surgery, 4 (2939): 402-18.
22. Scottish Intercollegiate Guidelines Network (SIGN) (2009) Management of hip fracture in older people. SIGN Guideline no. 111. Access: http://www.sign.ac.uk/guidelines/fulltext/111. Access date: 01.10.2022.
23. Şeliman, D., Kanan, N., Akyolcu, N., Enç, N., Bahçecik, N., Erkal İlhan, S. (2010). Geriatric patient and nursing care. Fındık ÜY, Erol Ö. (Ed.). Elderly and surgery in the elderly patient. (1st ed. pp. 1-8). Yosun Offset Printing, Ankara.
24. Ungar, A., Rafanelli, M., Iacomelli, I., Brunetti, M.A., Ceccofiglio, A., Tesi, F., Marchionni, N. (2013). Fall prevention in the elderly. Clinical Cases in Mineral and Bone Metabolism, 10 (2): 91-95.
25. Uygur, E., Söylemez, S., Aktaş, B., Çarkçı, E. (2015). Postoperative survival outcomes of elderly patients with intertrochanteric femoral

fracture treated with proximal femoral nailing. Medeniyet Medical Journal, 30 (2):63-66.
26. Uymaz, P., Nakhcivan, N. (2013). The validity and reliability of the scale of falling behaviors for the elderly. İ.Ü. Florence Nightingale Journal of Nursing, 21(1): 22-32.
27. Wallace, R., Angus, L.D.G., Munnangi, S., Shukry, S., DiGiacomo1, L.C., Ruotolo, C. (2018). Improved outcomes following implementation of a multidisciplinary care pathway for elderly hip fractures. Aging Clinical and Experimental Research, 952 (17): 405-18.
28. Yılmaz Şenyüz, K., Koçaşlı, S., Topçu, E.T. (2017). The home care role of the surgical nurse in the light of Florence Nightingale. Journal of Education and Research in Nursing, 14 (4): 283-286.

Cytokins And Their Roles in Cancer Mechanism

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Introduction

Cytokines are proteins produced in plant and animal cells, which enable cells to communicate with each other and stimulate their proliferation. Cytokines have important roles in immunity, wound healing, production and activity of blood cells, and in inflammatory and infectious conditions. Cytokines can be produced by a wide variety of cell types (pleiotropic) and have similar effects (redundant), including fibroblasts, endothelialand various stromal cells, as well as B and T lymphocytes, macrophages andmast cells. They can show agonist and antagonist effects among themselves. As with polypeptide hormones, cytokines show their effects by binding to specific receptors on the target cell. The formation, differentiation, activation, and orientation of immune system cells are all influenced by cytokines. The cytokine may act on the cell that synthesizes itself (autocrine effect), may affect a cell around the cell from which it is made (paracrine effect), or rarely interfere with the circulation and act on a distant cell where it reaches (endocrine effect). Lymphokines are cytokines produced by lymphocytes, monokines are cytokines produced by monocytes, chemokines are cytokines with chemotactic properties, and interleukin is a cytokine produced by leukocytes and acting on other leukocytes. Initially, it was called "lymphokine" because it was thought that only lymphocytes were the source of cytokines. Since it was understood that monocytes also produced these factors, it was named "monokine". Today, it has been understood that these mediators are not secreted only by lymphoid cells, so they have been used as "cytokines". Although cytokines cannot be classified precisely because they can be produced by many different cells and affect many different cells, they can be called interleukins (IL), interferons (IFN), transformed growth factor-beta (TGF-β), tumor necrosis factor (TNF) according to their structural and functional properties.

Small peptide proteins called cytokines help cells communicate with one another, promote the growth of antigen-specific effector cells, and control systemic inflammation(1). Although cytokines are secreted from stimulated cells in a very short time, they are not stored. They are produced de novo in response to an immune stimulus (2).Proinflammatory cytokines mediate communication between the immune system and the brain in response to a pathogen encounter and tissue damage (3). Cytokines provide proliferation and differentiation of lymphoid system and some other cells. They activate cells involved in inflammation, attract them to the reaction site and provide wound healing. They participate in hematopoietic regulation by acting on the bone marrow. They provide embryogenesis and the development of the nervous system. In low concentrations, they cause general infection symptoms such as

fever, myalgia, headache, acute phase response, and in high concentrations, they cause shock and death. They cause synthesis and release of some pituitary hormones. They show antiviral activity(2).Cytokines, which are agents that modulate the immune system, are the name given to types of immunotherapy that increase the body's immune response to cancer. Cytokines are produced by white blood cells and havesignificantroles in theimmune system's responses to many different threats, including cancer.

The most important task of cytokines is to receive an extracellular signal and convert it to an intracellular signal, and these receptors are transmembrane proteins that provide signal transmission with their intracellular parts and bind special cytokines with their extracellular parts. Cytokines are found in liver, kidney and lung cells (4).

Cytokines follow two pathways inside the cell.

1. They cause cell repair via microtubule-associated protein(MAP)kinase enzymes or cell death via gene expression by stimulating NF-kB (Nuclear factor-kappaB).
2. They trigger the Janus protein kinase/signaling and activators of transcription (JAK/STAT) pathway, leading to cell proliferation or death through gene expression.

Cytokines are grouped as anti-inflammatory and pro-inflammatory cytokines according to their roles in inflammation reactions.

Essential Proinflammatory Cytokines

- Interleukin-1,6 (IL-1,6)
- Interferons (IFN)
- Tumor necrosis factor (TNF)

Proinflammatory cytokines

- Interferon-α (IFN- α)
- Interferon-β (IFN- β)
- Interferon-γ (IFN-γ)

Essential Anti-Inflammatory Cytokines

- TGF-β
- IL-2
- IL-3
- IL-4
- IL-5
- IL-10
- IL-12
- IL-13

Pro-inflammatory cytokines like IL-6 and IL-1β play a role in the creation of an immediate immune response as well as the development of inflammatory alterations.. Anti-inflammatory cytokines such as IL-13 and IL-10 can suppress the synthesis of some cytokines and immune response. An imbalance between pro-inflammatory and anti-inflammatory cytokines inhibits resolution of inflammation and instead leads to disease progression and tissue destruction (5).It was shown that excessively increased pro-inflammatory cytokines, encouraged cancer cell proliferation and survival, angiogenesis, stimulated DNA damage, and altered the extracellular matrix, allowing cancer cells to invade and migrate more easily (6).

Interleukins (ILs)

Interleukin is known as a group of cytokines, which are latent signaling molecules expressed by lymphocytes of white blood cells where they are first seen(7).A large part of the immune system depends on interleukins. The interleukin family is released from macrophages and T-lymphocytes. They stimulate B-lymphocytes to mature and differentiate. They regulate the activity of B and T lymphocytes. They play a significant part in the immune system's response to viruses, defense against microbes, and triggering joint inflammation (8).

IL-1

IL-1 is a very crucial cytokine in systemic and local inflammation. It is one of the most produced cytokines during injury, immunological threat or infection (4). IL-1 is a pluripotent cytokine that has been linked to aggressive tumor biology in various cancers and has been shown to increase angiogenesis, tumor development, and metastasis in experimental animals(9). IL-1, one of the most important proinflammatory cytokines in the pathogenesis of periodontal disease, has many biological activities. Regulates many genes expressed in inflammation(10). IL-1 is synthesized from activated mononuclear phagocytes by monocytes, macrophages, lymphocytes, neutrophils and fibroblasts in the tissue. Members of the IL-1 family are linked to both acute and chronic inflammation and are crucial for the immune response. Members of the IL-1 family often have pro-inflammatory biological traits. The IL-1 family consists of 11 individuals.

Dendritic cells, T cells, B cells, Natural killer (NK) cells, vascular endothelium, fibroblasts, and keratinocytes all produce IL-1, despite macrophages being its primary source. T, B, NK, neutrophils, eosinophils,

dendritic cells, fibroblasts, endothelial cells, and hepatocytes are among the cells that IL-1 affects(12).

IL-1Ra

IL-1Ra is a receptor antagonist of IL-1, which consists of a protein with two main subunits, IL-1α and IL-1β. Periodontitis, vaginitis, non-Hodgkin lymphoma, gastric cancer, osteoarthritis, precancerous lesions, and inflammatory bowel illnesses are all linked to IL-1Ra, a powerful anti-inflammatory cytokine(13).

IL-1α

It is a pro-inflammatory cytokine that plays an important role in sterile inflammation (14). Malignant cells that express membrane-bound IL-1 trigger immunological responses that are anti-tumor, whereas intracellular IL-1 precursors regulate homeostatic processes like gene expression, cell differentiation, and proliferation (15).

IL-1β

IL-1β is induced in many immune cells via inflammatory signaling (16). IL-1β is a pro-inflammatory cytokine transported in the blood and produced by inflamed brain cells (17). As a byproduct of cells such monocytes, macrophages, dendritic cells, B-lymphocytes, and NK, IL-1 is a highly inflammatory cytokine(4). IL-1 has a significant impact on stress, developmental disorders like schizophrenia, and neurodegenerative diseases like Alzheimer's(18).While high concentrations of IL-1 promote inflammation-related tissue damage and tumor invasiveness, low concentrations of IL-1 downregulate inflammatory responses and immune mechanisms(15).An important pro-inflammatory cytokine called IL-1 has been linked to many illnesses, including lung cancer(19).IL-1 is regarded as a cytokine that promotes tumorigenesis. According to studies, IL-1 promotes tumor growth, metastasis, and angiogenesis (9).

IL-2

IL-2 is a cytokine produced by activated helper T cells. In the treatment of IL-2 cancer, Bindon et al. (1983) its use was reported (20). Native IL-2 was derived from the lymphocytes of two patients with melanoma and was approved for treatment by the Food and Drug Administration (FDA) after further studies. Then, figures such as IL-2, IL-7, IL-11, IL-13, IL-15, which were approved by the International Congress of Immunology, were given to interleukins. The

therapeutic effects of interleukins are directly related to the patient's immune system. IL-2 activates lymphocytes and macrophages and stimulates lymphokine release (21).

IL-6

IL-6 is a pleiotropic cytokine produced by lymphoid and non-lymphoid cells, it also helps regulate inflammation, hematopoiesis, immune reactivity and ontogenesis.IL-6 has both growth factor and is produced at the site of inflammation. The regulation of the neurological and immunological systems, liver regeneration, and body metabolism are all impacted by IL-6. At the site of inflammation, IL-6 is generated. IL-6 is released from T lymphocytes and stimulates differentiation of B lymphocytes, but B lymphocytes, monocytes, fibroblasts, endothelial cells, astrocytes, mesangial cells can also secrete IL-6, it has also been shown that cardiac myxoma, myeloma and hypernephroma cells can also produce and release IL-6. This interleukin stimulates differentiation and antibody secretion in B lymphocytes without stimulating proliferation. IL-6 release is triggered by bacterial endotoxins, IL-1 and TNF-α.

Studies on IL-6 in cancer cases were mostly encountered in ovarian cancers. In a study on ovarian cancer cells, stated that IL-6 may promote tumor growth by affecting cell attachment and migration(22).There was a significant positive correlation between serum IL-6 levels and the depth of tumor invasion, according to a study, and serum IL-6 levels were found to be significantly higher in gastric cancer patients than in healthy controls(23).In another study, it was reported that IL-6 has important tumor-supportive properties such as increasing cell proliferation and anti-apoptotic effects in tumor cells, and that it causes inflammation due to the increase in the level of Caspase-1, an inflammatory caspase, in HepG2 cells (24).

IL-9

IL-9 is a stem cell growth factor secreted only by Th2 cells and promotes the development of T helper cells and mast cells (12).

IL-10

IL-10, a cytokine that plays an active role in regulating the systemic response in conditions such as cell growth, healing, inflammation and injury, increases the secretion of CD8+ T cells (25). Human cytokine synthesis inhibitory factor (CSIF), another name for IL-10, is a cytokine that reduces inflammation. Utilizing IL-10 helps to reduce the burden of metastatic disease and regulate tumor growth(26). IL-10 has anti-inflammatory properties at low

doses, but increases the activation and proliferation of lethaÏ CD8+ T cells, especially at higher doses(27).An immunoregulatory cytokine called IL-10 suppresses both innate and adaptive immune reactions. Six members of the IL-10 family are encoded in two groups on various chromosomes. On chromosome 1, IL-10, IL-19, IL-20, and IL-24 are syntenic; on chromosome 12, IL-22 and IL-26 are syntenic.

IL-16

Inflammatory cytokine IL-16 has chemotactic properties for spleen lymphocytes (28).

IL-17

IL-17, a family of cytokines involved in the regulation of the immune response, is released by T memory cells. The IL-17 family increases cytokine synthesis by fibroblasts, keratinocytes, epithelial and endothelial cells. In addition to these issues, IL-17 stimulates proliferation of T cells and cells of myeloid origin (12).

IL-18

Like IL-1, IL-18 is produced as an inactive precursor that must be processed by caspase-1 in order to become biologically active. The IL-18 precursor, in contrast to IL-1, is structurally found in almost all healthy human and animal cells. IL-18 binding protein (IL-18BP), a naturally occurring protein with high affinity, counteracts the function of IL-18. The generation of interferon- from T cells and NK cells is significantly influenced by IL-18. An increase in the amount of free IL-18 in the blood may result in a worsening of the condition in humans.Numerous autoimmune illnesses, myocardial function, emphysema, metabolic syndrome, psoriasis, inflammatory bowel disease, hemophagocytic syndrome, macrophage activation syndrome, sepsis, and acute kidney injury have been shown to be affected by IL-18. This protective effect of IL-18 (29).

IL-19,

B cells and active monocytes release IL-19, which acts on monocytes to boost their production of IL-19, IL-6, and TNF-, leading to monocyte death. Th1 cells secrete IL-26, which promotes the growth of T cells and keratinocytes (12).

IL-21

It belongs to the cytokine family of IL-2. Other members of this family include IL-2, IL-4, IL-7, IL-9, and IL-15. Studies conducted in vivo and in vitro reveal that IL-21 aids in the development of anti-tumor immune responses. It has been shown that IL-21 released from tumor cells reduces tumor growth and causes tumor rejection in mouse colorectal cancer model created in mouse using cell line transfected with IL-21 gene.IL-2 is a cytokine from the same family as IL-21 and is a cytokine used clinically in malignant melanoma and renal carcinoma, but the side effects of IL-2 limit its clinical use.

IL-32

IL-32 is a multifunctional proinflammatory cytokine released from epithelial cells (colon, stomach, lung), endothelial cells such as breast, brain, pancreas, IL-2, IL-18, IFN-γ stimulated monocytes, activated NK cells and T lymphocytes. . The signaling pathways associated with IL-32 are closely related to immune responses. IL-1, TNF-, IL-6, and IL-8 are among the proinflammatory cytokines and chemokines that are stimulated, and the NF-B and p38 MAPK pathways are also activated. IL-32 stimulates NK cells, causing the expression of DR3 ligand (APO3 ligand) and caspase-3 in colon cancer and prostate cancer cells. In this way, it is known that by inhibiting cancer cell growth, it provides an increase in the effector functions of NK cells. For this reason, IL-32 is thought to be beneficial in cancer treatment with its antitumor effect as well as being a pro-inflammatory cytokine.

IL-33

IL-33, believed to act as a distress alert to the immune system, is present in the nucleus under constant conditions and requires signaling for extracellular binding to ST2.Only during active infection and after influenza infection does IL-33 begin to quickly leak passively from damaged cells in response to stress circumstances such infection, injury, and inflammation.

IL-36Ra

IL-36Ra is a cytokine (IL-36, IL-36R, IL-36Ra) antagonist. It is linked to several inflammatory illnesses and stimulates the T-helper cell response, playing a significant role in immune system adaptability. Inhibiting IL-36 through treatment with both IL-36Ra and IL-36R may be the best course of action for treating inflammation in human skin(13).IL-36Ra shows similar homology to IL-1Ra, but cannot bind to the IL-1R1 receptor since it has structurally important differences from IL-1Ra.

IL-36

In addition to their importance in the etiology of rheumatoid arthritis, inflammatory lung conditions, obesity, gallbladder occlusion disorders, and chronic glomerulonephritis, IL-36 cytokines are proinflammatory cytokines. Keratinocytes, bronchial epithelium, brain tissue, monocytes, and macrophages are the key tissues where IL-36 cytokines are expressed. The cytokines IL-36, IL-36, IL-36, and IL-36 receptor antagonist have been given new names.

IL-37

IL-37 has transcription regulatory factor and anti-inflammatory effects. The synthesis of pro-inflammatory cytokines is nearly entirely inhibited by the expression of IL-37 in macrophages and other epithelial cells, but the presence of these cytokines strengthens the inhibition of endogenous IL-37 in human blood cells. Thus, IL-37 seems as a natural inhibitor of the immunological and inflammatory response.

Interferons (IFN)

Leukocytes and virus-infected somatic cells create interferons as a defense against viral infections, immunological activation, inflammatory stimulation, and chemical stimuli. There are primarily two classes of interferons. Type I interferons produced by virus-infected mononuclear cells and fibroblasts, as well as Type II interferons produced after stimulation by T lymphocytes and natural killer cells. IFN-alpha, IFN-beta, and IFN-lamda have been identified as three subsets of virus-induced Type I IFN. IFN- plays a key function in the modulation of the Th1 cell response, which is essential for the management of intracellular pathogen infections.

IFN-α

IFN-α is a functionally pleiotropic cytokine and can regulate the expression of the protein. IFN-a is also an FDA-approved cytokine and is in the first class of immunotherapy to induce specific antigen presentation and co-stimulatory factors to induce APC maturation, trigger T cell activation and increase their cytotoxicity.

IFN- β

IFN-β has been shown to reduce the number and severity of relapses in patients with fibroblast, Nκ cell activation, innate immunity increase, experimental autoimmune encephalomyelitis and relapsing-remitting multiple sclerosis.

IFN-γ

IFN-γ and its targets play an important role in the destruction of tumor cells by directly inducing cytotoxic activities and enhancing Th1-related immune response (30).IFN-γ provides immune response regulation, phagocytic cell activation and suppresses humoral immunity with increased Th1 activity, increased cellular immunity, Th2 and B lymphocyte inhibition.

Tumor necrosis factor (TNF)

Along with being a critical cytokine contributing to physiological and pathological processes, it is a cytokine that has attracted the attention of scientists because of its role in normal physiology, acute inflammation, chronic inflammation, autoimmune diseases and cancer-related inflammation. The majority of tissues that may create TNF are macrophages, however other tissues such as lymphoid cells, mast cells, endothelial cells, fibroblasts, and neural tissue can also do so.It helps heal tissue damage, and helps the body to organize attacks against invading bacteria and viruses. TNF might stand for one of the molecular connections between persistent inflammation and the emergence of cancer. The tumor microenvironment's dysregulated TNF expression encourages the invasion, migration, and eventual metastasis of cancerous cell tissue.TNF, which causes necrosis in tumors, is also a cytokine that plays a role in apoptosis and inflammation processes. In recent studies, it has been emphasized that TNF causes necrosis in tumors, such as cervical cancer cells, as well as growth in tumors. It has been reported that low secretion of TNF increases the growth of cancer cells, and excessive secretion causes apoptosis or necrosis.*In vitro* studies have shown that low-dose TNF triggers tumor development by causing proliferation, dissemination and metastasis of tumor cells in some cancer cell cultures. It has been proposed that TNF increases the formation of NO and free oxygen radicals in macrophages that are phagocytosing cancer cells or tumors, causing DNA damage and impeding DNA repair pathways. It is also believed that when NF-B activation prevents apoptosis in cells, this promotes the growth of tumors by ensuring cell survival. Studies using mice have demonstrated that this action causes tumors to recede. TNF also helps dendritic cells become macrophages, which then transmit antigens to T cells, assisting in the development of the cellular immune response.

TNF is of two types, α and β. It is mostly secreted by active macrophages and monocytes. It can also be synthesized from activated B and T cells, NK

cells, mast cells, fibroblasts, keratinocytes, Kuppfer cells, smooth muscle cells, basophils, and tumor cells. TNF-β is mainly released from T lymphocytes. Its effects on the host cell are similar to TNF-α, but it is known to have a weaker effect. The amino acid similarity between the two biomolecules is very low. In contrast, their receptors and mechanisms of action are the same.

TNF-α

TNF-α has important roles in innate and adaptive immunity, cell regulation, differentiation and apoptosis processes; IL-6 is cytokines with many functions, preventing proliferation in innate and adaptive immunity, carcinoma and B-cell leukemia, regulating the growth and differentiation of different tissues. TNF-α is also called cachectin. From many normal and tumor cells; It is produced by various stimuli, mainly viruses, bacteria, parasites, cytokines and mitogens. TNF-α is a pleiotropic cytokine with important roles in innate and adaptive immunity, cell regulation, differentiation and apoptosis processes. It is known that TNF-α is responsible for some tumor-related local and systemic effects, including cachexia and neoplastic tissue destruction, tumor cells are killed by TNF, and macrophages are directly stimulated by tumor cells. The effector effect of TNF-α is synergistic with IL-6. It is thought that the necrosis of tumors with the effect of TNF-α does not affect the tumor cells directly, but probably by damaging the vasculature of the tumor tissues .

TNF Super-Family(TNF-SF)

Both innate and adaptive immunity, including inflammation, apoptosis, cell proliferation, and immune system stimulation, depend heavily on TNF-SF. The homotrimeric structure of TNF-SF proteins. Many TNF-SF members are considered to be stimulating substances as opposed to cytokines. Members of the TNF-SF that can be regarded as cytokines include TNF, lymphotoxin (LT), LT-, and B cell activating factor (BAFF)(27). TNF-α is produced by macrophages, mast cells, T cells, endothelial, Band fibroblasts cells. TNF-α can trigger the killing of some tumor cells and virus-infected cells (12). Mammals' large tissue compatibility complex class III (MHC class III) area is where TNF-, LT-, and LT- congregate. The development of lymph nodes and other secondary lymphoid organs depends heavily on LT genes (29).

Transformed Growth Factor-β (TGF-β)

Three of the five families of glycoproteins that make up TGF-—TGF-1, TGF-2, and TGF-3are present in mammals. TGF-family members are produced by platelets, neutrophils, activated macrophages, B cells, and T cells. They also

act on dendritic cells, fibroblasts, T cells, and B cells. TGF- suppresses T and B cell growth and promotes T and B cell death(31). TNF-β kills tumor cells and activates neutrophils, macrophages, endothelial and B cells (12).TGF-β1 suppresses the anti-tumor activities of T cells, NK cells, neutrophils, monocytes and macrophages, which have important roles in tumor progression, and supports tumor progression, indicating that TGF-β1 is an effective cytokine in the tumor microenvironment.

Growth/differentiation factor-15 (GDF-15)

GDF15/MIC-1 (macrophage inhibitor cytokine-1) was discovered as a member of the TGF-β superfamily. It is known that GDF15 is associated with the development of cancers such as cervical cancer, multiple myeloma and colon cancer, and also supports metastasis in many cancer types such as lung, colon and prostatecancer (32). GDF-15 has reportedly been linked to breast cancer cells spreading through metastasis in recent years(33).While GDF-15 functions as a tumor suppressor by arresting the cell cycle and causing apoptosis, it also has a pro-tumorogenic function (34,35). Studies have reported that both TGF-β1 and GDF-15 have roles in promoting tumor progression (36).

Nuclear factor-κB (NF-κB)

NF-B is a transcription factor that regulates the expression of several genes that code for proteins, governs the transcription of numerous cellular genes that control the inflammatory response, and is significant in immunological and inflammatory processes(37).Apoptosis is crucial for the proliferation and migration stages of cancer genesis and progression(38). NF-kB is a transcription factor that can bind to DNA directly and controls the transcription of several inflammatory cytokines that are involved in the human immune response, including IL-6 and TNF.

Chemokines

Chemokines are proteins that promote differentiation of immune cells, including chemotaxis, induce tissue extravasation, and have roles in the anti-tumor immune response in the cancer setting (39). Chemokines are low molecular weight peptide groups. It contains various receptors and their associated ligands. Chemokiler, whose main task is to recruit leukocytes to the areas of inflammation, are involved in tumor growth, angiogenesis and metastasis (40). Proteins are of great interest for their role in the anti-tumor immune response in the cancer setting, as they play an important role in inducing immune cell differentiation, chemotaxis, and tissue extravasation (39).

Chemokines also enhance the host response to infections, tumors and vaccines. The combination of chemokines with other cytokines is more effective and provides the formation of antitumor therapy (41).According to a study, cancer cells alter the normal chemokine system, making these molecules and their receptors crucial parts of the tumor microenvironment that assist the growth of the tumor in several ways (42).

Cytokines are mediators whose 50 different members take part in normal biological and pathological processes in a regulatory type. They are named by classifying them according to the position of the amino acid cysteine (C) in the molecule. Alpha-chemokines are defined as CXC chemokines because there is an amino acid between the two cysteines at the amino terminal end. Beta-chemokines are called CC-chemokines because the terminal cysteines are side by side. About 20 chemokine receptors to which the chemokine family binds on cells have been identified. Chemokine receptors are G-protein-dependent types of intracellular signal-transmitting structures. Cells stimulated by signal transduction as a result of binding of chemokines to the appropriate receptor pass through tissue injury, inflammation or chemotaxis to the required site. During the inflammation process, cell traffic from intravascular to endothelial cell wall, transendothelial migration and inflammation area takes place under the influence of different types and numbers of chemokines and chemokine receptors. In particular, CXC chemokines affect the process in coordination in both inflammation and angiogenesis. Disturbances in the regulation of this process can lead to chronic inflammation and even to the neoplastic transformation of cells by affecting immune system cells (41).

The pro-inflammatory chemokines CXCL9, CXCL10 and CXCL11 mainly lead to tumor suppression by regulating immune cell migration, differentiation and activation (39).One study hypothesized that CXCR3, along with its ligands CXCL10, CXCL9, and CXCL11, may contribute to the development and spread of tumors by overexpressing CXCR3 in tumor cells as opposed to immune-competent cells that are infiltrating the area. Apoptosis induction, chemotaxis, cell growth control, and mediating angiostatic effects are all mediated by CXCL10. It is linked to conditions like immune system failure, persistent inflammation, infectious illnesses, tumor growth, metastasis, and spread. According to definitions, CXCL10 is a significant biomarker that influences how severe a disease is and can be used to predict the prognosis of numerous illnesses(43). CXCL11 is one of the small secretory proteins and members of the chemokine family involved in inflammatory and immune responses (44). CXCL11 release contributes to induction of immunity as a systemic tumor-protective.

The CC chemokine CCL2 activates CCR2 to control the migration of macrophages, monocytes, and other inflammatory cells to areas of inflammation(45). CCL2 is expressed by immune cells, various malignant cells and stromal cells, and has a role in tumor progression and metastasis (46). CCL2 stimulation is effective in the growth, survival, invasion and migration of tumor cells (47). CCL2 is a promising drug target for cardio-metabolic, inflammatory and some malignant diseases (48). One study shows that targeting CCL2-CCR2 signaling can lead to unexpected adverse effects in breast cancer, furthermore, interruption or interruption of CCL2 inhibition in four mouse models has been shown to increase metastases and accelerate death (49).

When T cells reach the site of injury and are triggered by particular antigens, CCL5, another member of the CC chemokine family, starts to be generated in high amounts within 3 or 5 days, protecting and boosting the immune response (50). A study shows that CCL2 and CCL5 play an important role in tumor cell invasion and lead to metastasis formation at distant sites (51).

Chemotactic factor CCL7 is extensively expressed in a variety of cell types and has the ability to influence the recruitment of immune cells to help reduce inflammation. CCL7 causes cancer progression and increased metastasis. A study shows that overexpression of CCL7 is associated with tumor metastasis and acts as a prognostic factor in patients with gastric cancer (52).

The Role of Cytokines in the Mechanism of Cancer

Cancers, in which intercellular communication is impaired; They are defined as diseases in which cellular growth and proliferation go beyond the normal line. Cytokines are effective in the pathogenesis and treatment of cancer. The important role of cytokines in cancer biology was understood in the 1893s when William Coley showed that some malignant tumors regressed after certain bacterial infections. Again in the 1970s, the discovery of tumor inhibitory factors such as IFNs and TNFs and the demonstration of their release by bacterial endotoxin supported this view. Cytokines may be associated with the malignant process in different ways. Cytokines are often produced as part of control mechanisms between cells or as inducers of other molecules responsible for these mechanisms. For example; some proto-oncogenes or oncogenes are codes for cytokine receptors or normal or abnormal components of cytokine signaling pathways (53,54).Cytokines can cause tumor regression by directly inhibiting growth on cancer cells. Cytokines also play a role in increasing antitumor effects. Conversely, some cytokines can be growth factors for malignant cells, and by inhibiting the effects of these cytokines, treatment can occur. In addition, cytokines mediate paraneoplastic syndromes.

In the early stages of tumor development, immune cells that have invaded the tumor release inflammatory mediators such as cytokines, reactive oxygen species, and reactive nitrogen species that cause epigenetic alterations in premalignant lesions and mute tumor suppressor genes(55). Numerous epidemiological studies have found a link between obesity and a higher risk of cancer in a number of organs, including the breast, prostate gland, endometrial, colorectum, and stomach. These results imply that adipocytokines play a role in the development of tumors and the induction of carcinogenesis(56).

It has been claimed that cytokines such as TGF-ß, TNF-α, IL1, IL-6, IL-10 and interferons play an important role in the immune system's anti-tumor response in innate and adaptive immunity. It has been determined that IL-6 inhibits proliferation in cancer cells through the induction of apoptosis, and DNA fragmentation, which is the characteristic feature of apoptosis, occurs in these cells.In studies of melanoma, renal cancer, and colorectal cancer treated with IL-2, bCRP and IL-6 levels have been shown to be associated with response to therapy (57).In patients who respond to IL-2 treatment, CRP and IL-6 levels are lower before treatment and increase greatly during treatment. Soluble IL-2 receptor (sIL-2R) concentrations are increased in a group of cancers, especially leukemias and lymphoid system cancers (58). High sIL-2R levels in children with acute lymphoid leukemia are an indicator of disease recurrence and correlate with survival. In the management of hematological malignancies, sIL-2R measurements may also have an important place. Solid tumors (lung cancer) require special attention; because elevated sIL-2R levels may be associated with post-treatment disease status and survival. High sIL-2R levels found in small cell lung cancer (59). Most cancer cell lines and biopsies show TNF-a expression and TNF protein production. For example; 50-70% of ovarian and breast cancers have TNF mRNA or TNF protein (53).

Measurement of high TNF and sTNFR levels in a wide group of malignancies may be useful in follow-up studies. The majority of patients with hematologic cancer have elevated Macrophage-CSF levels, and most studies show that M-CSF levels are associated with disease type, stage, and response to therapy.

Serum concentrations of TGF-α are elevated in various cancers. TGF-α levels are almost always high in breast cancer, regardless of stage (60). It is known that fructose has a carcinogenic effect by stimulating tumor angiogenesis, increasing proliferation and inhibiting immune tracking agents that fight against malignant cells. Some of these effects are mediated by cytokines, which can trigger the development of cancer in damaged tissues, as in inflammatory bowel disease. In addition, fructose increases the secretion of

IFN-g, IL-1b, IL-6, TNF-α, IL-1β and IL-2(61,62).In another study with lung cancer cells, it was observed that it increased the production of cytokines such as IL-6, IL-8 and MCP-1 in Mesenchymal stem cells (MSC) cells cultured with exosomes originating from cancer cells (63).MSCs show that it can increase the growth, division and metastasis of cancerous cells through the cytokines it secretes. The fact that some cytokines secreted by MSCs stimulate cancer cells and increase the growth and metastatic properties of cancer cells has limited their use as a treatment tool. Many cytokines released from MSC and adversely affecting cancer cells have been identified. Some of these cytokines are IL-6, SDF-1, CCl-5, TGFβ1, EGF, FGF, VEGF and CXCL7 (64). In the study, it was stated that the communication between stem cells and cancer cells is provided by cytokines and IL-8 cytokine comes to the fore (65). In a study with mice, it was shown that with the cytokine TREG secreted by MSCs, it suppressed CD8 T lymphocytes and natural killer cells, and cancer cells got rid of the cellular immune response and the cancer spread (66). Increase the expression of TGF-βI and periostin cytokines secreted by MSCs and many metastasis proteins that they use in attachment to connective tissue in prostate cancer cells.

Another important group of cytokines that enable cancer to grow and spread are pro-angiogenic cytokines. These cytokines include fibroblast growth factor-2, VEGF, angiogenin, TGF beta, and platelet-derived growth factor-BB (67). It has been shown that MSC increases the release of IL-8 cytokine against colon cancer cells in-vitro and in vivo, and this cytokine increases the formation of new vessels in the colon cancer stroma and enlarges the cancer mass (68). In the study, genetically modified stem cells that cannot secrete cytokines known to cause carcinogenic effects can be produced in order to benefit from the cancer-curing properties of MSCs (69).

Conclusion

Cancer, which is one of the diseases with the highest mortality rate of recent times, is not an easy disease because it is not a single system disease. For this reason, it is an important study to examine the disease by considering different components of the immune system in order to better control cancer. It is known that the risk of developing cancer is higher in patients whose immune system is suppressed. Cells with innate immunity create cytokines in response to pathogens and tumor antigens. These membrane-bound or secreted proteins mediate intercellular signaling to control immune system homeostasis. The role of inflammation in tumor development and other diseases is largely dependent on the secretion of cytokines and their interaction with other cells in the microenvironment.

REFERENCES

1. Neuman MG. Immune dysfunction in inflammatory bowel disease. Transl Res. 2007;149(4):173-86.
2. Akdoğan M, Yöntem M. Sitokinler. OTSBD. 2018;3(1):36-45.
3. Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. Progress in Neuro-psychopharmacology Biological Psychiatry, 2005;29(2):201-17.
4. Dinarello CA. A clinical perspective of IL-1β as the gatekeeper of inflammation. European Journal of Immunology 2011;41(5):1203-17.
5. Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol. 2014;14(5):329-42.
6. Jager A, Sleijfer S, Van Der Rijt CCD. The pathogenesis of cancer related fatigue: could increased activity of pro-inflammatory cytokines be the common denominator? Eur JCancer. 2008;44(2):175-81.
7. Sahoo A, Im SH. Interleukin and interleukin receptor diversity: role of alternative splicing. Int Rev Immunol. 2010;*29*(1):77-109.
8. Bocci V. Interleukins. ClinPharmacokinet. 1991;21(4):274-84.
9. Elaraj DM, Weinreich DM, Varghese S, et al. The role of interleukin 1 in growth and metastasis of human cancer xenografts. Am Clin Cancer Res. 2006;12(4):1088-96.
10. Dinarello C. Biologic basis for interleukin-1 in disease. Blood. 1996;87:2095-147.
11. Yi-June L, Cheing Meii L, Man-Ying W, Lein-Tuan H, Weikuei C. Interleukin-1 secreting cells in inflamed gingival tissue of adult periodontitis patients. Cytokine. 1999;11:626-33.
12. Tizard IR. Cytokines and the Immune System. I. R. Tizard içinde, Veterinary Immunology (7th ed b). W.B. Saunders Co. s. 2004;133-143.
13. Yuan X, Peng X, Li Y, Li M. Role of IL-38 and its related cytokines in inflammation. Mediators Inflamm .2015; 2015:807976.
14. Rider P, Carmi Y, Guttman O, et al. IL-1α and IL-1β recruit different myeloid cells and promote different stages of sterile inflammation. J Immunol. 2011;187(9):4835-43.
15. Apte RN, Krelin Y, Song X, et al. Effects of micro environment and malignant cell derived interleukin-1 in carcinogenesis, tumour invasiveness and tumourhost interactions. Eur J Cancer. 2006;42:751-9.
16. Bent R, Moll L, Grabbe S,Bros M.Interleukin-1 beta-a friend or foe in malignancies? Int J Mol Sci.2018;19(8):2155
17. Liu T, McDonnell PC, Young, PR, et al. Interleukin-1 beta mRNA expression in ischemic rat cortex.Stroke. 1993;24(11):1746-50.

18. Meyer U. Anti-inflammatory signaling in schizophrenia. Brain, Behavior, and Immunity 2011;25(8):1507-18.
19. Bhat IA, Naykoo NA, Qasim I, et al. Association of interleukin 1 beta (IL-1beta) polymorphism with mRNA expression and risk of non-small cell lung cancer. Meta gene. 2014;2:123-33.
20. Bindon C, Czerniecki M, Ruell P, et al. Clearance rates and systemic effects of intravenously administered interleukin 2 (IL-2) containing preparations in human subjects. Br JCancer. 1983;47(1);123-33.
21. Widmaier EP, Raff H, Strang KT. Çeviren: Özgünen, T. Vander İnsan Fizyolojisi, Vücud Foksiyon Mekanizmaları, 13. Baskı, Güneş Tıp Kitapevi, Ankara, 2014.
22. Obata NH, Tamakoshi K, Shibata K, et al. Effects of interleukin-6 on in vitro cell attachment, migration and invasion of human carcinoma. Anticancer Res.1997;17:337-42.
23. Ikeguchi M, Hatada T, Yamamoto M, et al. Serum interleukin-6 and -10 levels in patients with gastric cancer. Gastr Cancer. 2009;12:95-100.
24. Balkan BM, Kısmalı G, Turan D, et al. IL-6 ilavesi HEPG hücrelerinde kaspaz aktivitelerini nasıl etkiler?MAKÜ Sag Bil Enst Derg.2017;5(2):85-92.
25. Chan IH, Wu V, Bilardello M, et al. The potentiation of IFN-γ and induction of cytotoxic proteins by pegylated IL-10 in human CD8 T cells. J Interferon Cytokine Res. 2015;35(12):948-55
26. Fujii S,Shimizu K,Shimizu T, LotzeMT. Interleukin-10 promotes the maintenance of antitumor CD8(+) T-cell effector function in situ. Blood.2001;98(7):2143-51.
27. NaingA, Papadopoulos KP, Autio KA, et al. Safety, antitumor activity and immune activation of pegylated recombinant human interleukin-10 (AM0010) in patients with advanced solid tumors. J Clin Oncol. 2016;34(29):3562-69.
28. KaiserP, Staheli P. Avian Cytokines and Chemokines. K. A. Schat, B. Kaspars, & P.Kaiser (Dü) içinde, Avian Immunology (second ed b., s.). USA: Elsevier Ltd. 2014;189-204.
29. Dinarello CA, Novick D, Kim S,Kaplanski G. Interleukin-18and IL-18 binding protein. Front Immunol. 2013;4:289.
30. Derynck R, Turley SJ, Akhurst RJ. TGFβ biology in cancer progression and immunotherapy. Nat Rev Clin Oncol. 2021;1:9-34
31. Tizard IR. Regulation of Adaptive Immunity. IR Tizard, Vet Immunology (9th ed. b.). Saunders. 2012;209-224.

32. Mehta RS, Song M, Bezawada N, et al. A prospective study of macrophage inhibitory cytokine-1 and risk of colorectal cancer. J Natl Cancer Inst. 2014;106 (4)
33. Wang T, Mao B, Cheng C, et al. YAP promotes breast cancer metastasis by repressing growth differentiation factor-15. Biochim Biophys Acta Mol Basis Dis. 2018;1864(5):1744-53.
34. Bauskin AR, Brown DA, Kuffner T, et al. Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer. Cancer Res. 2006;66 (10):4983-6.
35. Unsicker K, Spittau B, Krieglstein K. The multiple facets of the TGF-beta family cytokine growth/differentiation factor-15/macrophage inhibitory cytokine-1. Cytokine Growth Factor Rev. 2013;24(4):373-84.
36. Dilmaç S, Ertosun MG, AçıkgözE, Tanrıöver G. Kök hücreler kanser hücrelerinin mikroçevresindeki sitokin yanıtlarını etkiler: Meme kanseri ve dental pulpa kök hücreleri arasındaki etkileşim. Ege Tıp Derg. 2021;60(4): 314-23.
37. O'Neill LA, Kaltschmidt C. NF-kB: a crucial transcription factor for glial and neuronal cell function. TrendsNeurosci. 1997;20(6):252-8.
38. Dolcet X, Llobet D, Pallares J, Matias-Guiu X. NF-kB in development and progression of human cancer.Virchows Archiv. 2005;446(5):475-82.
39. Nennig SE, SchankJ. The role of NFkB in drug addiction: beyond inflammation. Alcohol Alcohol. 2017;*52*(2),172-9.
40. Ding Q, Lu P, Xia Y, et al. CXCL9: evidence and contradictions for its role in tumor progression.Cancer Med. 2016;5(11):3246-59.
41. Çağlar M, Kansu E. Kemokinler, kemokin reseptörleri ve inflamasyon. ANKEM Derg. 2004;18(2):164-8.
42. AldinucciD,Colombatti A. The inflammatory chemokine CCL5 and cancer progression.Mediators Inflamm, 2014;292376
43. LiuM, Guo S,Stiles JK.The emerging role of CXCL10 in cancer.Oncol Let. 2011;2(4),583-9.
44. Booth V, Clark-Lewis I, Sykes BD.NMR structure of CXCR3 binding chemokine CXCL11 (ITAC).Protein Sci. 2004;13(8):2022-8.
45. Hensbergen PJ, Wijnands PGB, Schreurs MW, et al. The CXCR3 targeting chemokine CXCL11 has potent antitumor activity in vivo involving attraction of CD8+ T lymphocytes but not inhibition of angiogenesis.JImmunother. 2005;28(4):343-51.
46. Charo IF, TaubmanMB.Chemokines in the pathogenesis of vascular disease.Circ Res. 2004;95(9):858-66.

47. ZhangJ, Patel L, Pienta KJ.Targeting chemokine (CC motif) ligand 2 (CCL2) as an example of translation of cancer molecular biology to the clinic.Prog Mol Biol Transl Sci. 2010;95:31-53.
48. DommelS, BlüherM.Does CC motif chemokine ligand 2 (CCL2) link obesity to a pro-inflammatory state? Int J Mol Sci.2021;22(3):1500.
49. Lim SY, Yuzhalin AE, Gordon-Weeks AN, Muschel RJ.Targeting the CCL2-CCR2 signaling axis in cancer metastasis. Oncotarget. 2016;7(19):28697-710.
50. Kawka E, Witowski J, Fouqet N, et al. Regulation of chemokine CCL5 synthesis in human peritoneal fibroblasts: a key role of IFN-γ. MediatorsInflamm, 2014;590654.
51. Soria G, Ben-Baruch A.The inflammatory chemokines CCL2 and CCL5 in breast cancer.Cancer Let. 2008;267(2);271-85.
52. Hwang TL, Lee LY, Wang CC, et al. CCL7 and CCL21 overexpression in gastric cancer is associated with lymph node metastasis and poor prognosis.World J Gastroenterol. 2012;18(11):1249-56.
53. Heikkila K, Ebrahim S, Debbie, Lawlor A. A systematic review of the association between circulating concentrations of C reactive protein and cancer. JEpidemiol Community Health. 2007;61:824-32.
54. Bilen E, Balcerska A. Serum soluble interleukin 2 receptor α in human cancer of adults and children: a review. J Biomarkers. 2008;13(1):1-26
55. Grivennikov SI, KarinM. Inflammation and oncogenesis: avicious connection. Curr Opin GenetDev. 2010;20:65-71.
56. Calle EE, Thun MJ. Obesity and cancer. Oncogene. 2004;23:6365-78
57. Behne T, Copur MS. Biomarkers for Hepatocellular Carcinoma. Int J Hepatol 2012;7.
58. Söderberg-Naucler C, Fish KN,Nelson JA. Interferon-gamma and tumor necrosis factor-alpha specifically induce formation of cytomegalovirus-permissive monocyte-derived macrophages that are refractory to the antiviral activity of these cytokines. JClin Invest. 1997;100(12):3154-63.
59. Olson TS, Ley K. Chemokines and chemokine receptors in leukocyte trafficking. Am JPhysiol. 2002;283:R7–R28.
60. Snyder DS, Unanue ER. Corticosteroids inhibit murine macrophage Ia expression and interleukin 1 production.JImmunol. 1982;129(5):1803-05.
61. TogashiN, UraN, HigashiuraK, et al.The contribution of skeletal muscle tumor necrosis factor- to insulin resistance and hypertension in fructose-fed rats. J Hypertens.2000;18:1605-10.

62. WangL, Higashiura K, TogashiN, et al. Effects of the Chinese medicine Jiang-Tang-Ke-Li on insulin resistance in fructose-fed rats. Hypertens Res. 2001;24:303-9.
63. Li X, Wang S, Zhu R, et al. Lung tumor exosomes induce a pro-inflammatory phenotype in mesenchymal stem cells via NFκB-TLR signaling pathway. JHematol Oncol. 2016;18:9(1).
64. TorsvikA, BjerkvigR. Mesenchymal stem cell signaling in cancer progression. Cancer Treat Rev. 2013;39(2):180-8.
65. Li W, Zhou Y, Yang J, et al. Gastric cancer-derived mesenchymal stem cells prompt gastric cancer progression through secretion of interleukin-8. J Exp Clin Cancer Res.2015;34(1):1-15.
66. Djouad F, Plence P, Bony C, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood.2003;*102*(10):3837-44.
67. LinG, Yang R, Banie L,et al. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor.Prostate.2010;70(10):1066-73.
68. Wang J, Wang Y, Wang S, et al. Bone marrow-derived mesenchymal stem cell-secreted IL-8 promotes the angiogenesis and growth of colorectal cancer. Oncotarget.2015.
69. Şahin E, Bayçu C, Koparal A, et al. The adverse effects of mesenchymal stem cell cytokines on the cancer cells. Osmangazi JMed. 2016;37(3):7-12.

Trending Neuromotor Assessment Methods in the Diagnosis Of Cerebral Palsy: A Review

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INTRODUCTION:

Cerebral palsy (CP) is a group of permanent disorders of movement and posture that occur in the developing fetal or infant brain, which are non-progressive but cause activity limitation (Rosenbaum et al., 2007). The diagnosis of CP is usually made between 12-24 months as a result of clinical findings and neurological evaluation (Hubermann et al., 2016). CP has 4 motor types. These are spastic, dyskinetic, ataxic and mixed types(Rosenbaum et al., 2007). Comorbidities such as pain, cognitive problems, epilepsy, secondary musculoskeletal problems, behavioral disorders, vision problems, and hearing problems may accompany of CP (Novak et al., 2012).

While the prevalence of CP is 2.1 per 1000 live births in European countries, this rate is even higher in middle and less developed countries (Oskoui et al., 2013). Recently, many interventions (cooling therapy, steroid treatments, etc.) to reduce the rate of CP are applied in the prenatal and perinatal period (Novak et al., 2020). Despite current technological developments, CP continues to be common especially in middle and less developed countries (Novak et al., 2020).

While many approaches to the prevention of CP continue to develop, there are also studies for the early diagnosis of CP. Novak et al. states in their study that CP can be predicted with neuroimaging and neuromotor assessment tools performed before the 5th month (Novak et al., 2017). In this study, it is stated that Hammersmith Infant Neurological Examination (HINE) and General Movements (GMs) evaluation have an important place in the diagnosis of CP (Novak et al., 2017). The aim of this review study is to define the HINE and GMs evaluation methods and to synthesize the predictive values for the diagnosis of CP.

Hammersmith Infant Neurological Examination (HINE)

HINE is a neurological assessment battery applied to infants aged 2-24 months to determine the developmental status. HINE has three subcategories. One of them is neurological assessment section.The neurologic assessment section, which is a scoreable part, includes 26 items that evaluate different aspects of functions such as cranial nerves, posture, movements, tone and reflexes. Second subcategories is motor milestones. The last subcategories is behaviour section. HINE performed by health professionels easily and can be completed in 10 minutes. All items in neurological assessment section is individually scored from zero to three. The HINE minimum score can be 0, while the maximum score can be 78. Optimization scores for infants aged 2 to 24 months are based on the frequency distribution of neurological signs in the population of that age group. A substance is considered optimal when it is present in at least 90% of infants. The use of the HINE test's total and cut-off

scores provide prognostic information regarding the level of motor development (Maitre, et al., 2016; Morgan et al., 2019; Romeo et al., 2016).

HINE is an assessment tool with predictive values in diagnosing CP. The predictive power of the HINE test battery performed at 3 months was 88% sensitive and 62% specific(Morgan et al., 2019). Similarly, sensitivity and specificity rates of the HINE test battery performed at 3-6 months in children with hypoxic ischemic encephalopathy (HIE) who received cooling therapy were reported as 83.3% and 87.8%, respectively (Apaydın et al., 2021). In another study, infants with HINE scores of ≤56 at 3 months and ≤65 at 12 months showed highsensitivity and specificity (~90%) for the diagnosis of CP (Romeo et al., 2016). Based on the results of the current literature, the use of the HINE test battery is important for the early identification of CP.This test battery has been translated into Turkish and made available for use in order to make its use widespread in our country (Adıgüzel et al., 2022). It is thought that with the widespread use of the Turkish HINE test battery, it will be useful in terms of early identification of CP and initiating early rehabilitation in our country.

Prechtl's General Movements Assessment (GMs)

Heinz Prechtl, an Austrian pediatric neurologist; suggested the use of spontaneous movements in the functional assessment of the young nervous system (Heinz F Prechtl, 1990). GMs are movement patterns in which the whole body participates, with a variable sequence of arm, leg, neck and trunk movements. GMs whose beginning and end are gradual; density, power and speed are of increasing and decreasing character. Moreover; GMs are fluid, elegant, complex and variable in character, with a rotation component and minor changes in the direction of motion (Einspieler, et al., 1997). GMs are examined under three periods: fetal/preterm GMs, writhing movements (WMs) and fidgety movements (FMs). Considering different criteria according to the periods, GMs can be considered as normal or abnormal. If a GMs is variable and complex in all periods this means the infant has normal GMs. Preterm GMs; It begins in the fetal period and continues until the term. WMs; Starting at term and continuing until the end of the postterm 2nd month, FMs; It occurs gradually between the 6th and 9th weeks of the postterm and continues until the 20th week,(Hadders-Algra, 2004; Morgan et al., 2019).

Abnormal GMs in the writing period are defined as:

a) Poor repertoire (PR) In this abnormal movement pattern, the sequence of movements of different body parts is monotonous. PR; does not have the variable and complex characters seen in normal GMs;
b) Cramped synchronized (CS) In this abnormal pattern, in which all limb and trunk muscles contract and relax almost simultaneously, movements

are blocky and rigid. It doesn't have the fluid and smooth characters seen in normal GMs;

c) Chaotic (Ch) GMs, means all four extremities' movements are of large amplitude, occur irregularly and lack fluency or smoothness (Einspieler & Prechtl, 2005).

Normal fidgety movements are defined as short-amplitude, circular movements that occur in all extremities. Abnormal GMs in the fidgety period are classified as:

a) Absent fidgety movements; The absence of fidgety movements between 9 and 20 weeks postterm is called absent fidgety.;

b) Abnormal fidgety; This pattern, which is seen rarely and has a low predictive value; looks like normal fidgety movements but has exaggerated speed and amplitude (Morgan et al., 2019).

GMs have been widely used in the world for the early identification of CP. In a study, it was stated that the sensitivity and specificity ratio of GMs assessment performed during the fidgety period to predict CP was 95% and 97%, respectively (Morgan et al., 2019). According to the study of Prechtl et al., (1997) the specificity was 96% and sensitivity was 95% in predicting CP (Prechtl et al., 1997). In a study conducted in infants with HIE who received cooling therapy, the rate of prediction of CP by GMs was reported as 83.3% sensitivity and 100% specificity (Apaydın et al., 2021). In another study examining the predictive power of GMs in infants with a history of HIE, the sensitivity of the assessment was 80% and the specificity was 100% during the fidgety period (Soleimani et al., 2015). Considering the results of the literature, it is necessary to use the GMs evaluation method in terms of its early predictive power for the diagnosis of CP. GMs is a method based on visual perception and requires a course. It is not widely used in Turkey. However, the rate of predicting the diagnosis of CP is quite high. Therefore, using of this method should be expanded in our country. It is thought that early diagnosis and initiation of early rehabilitation will ensure that the functional disabilities of children with CP will be minimized in the early period, especially with the physicians and health professionals working in the field of pediatrics learning and using this evaluation method.

Conclusion: Predicting the diagnosis of CP in the early period is important in order to benefit from neural plasticity. The first 2 years of life, when neural plasticity is the fastest, are critical for cognitive, motor and sensory development. In this regard, it is important to be able to diagnose CP, especially in the first 5 months. In addition to neuroimaging methods for the diagnosis of CP, the use of HINE and GMs evaluations together if possible, or separately if not, has high sensitivity and specificity rates for estimating the diagnosis of CP.

References

1. Adıgüzel, H., Sarıkabadayı, Y. Ü., Apaydın, U., Kırmacı, Z. İ. K., Gücüyener, K., Karadeniz, P. G., & Elbasan, B. (2022). Turkish Validity and Reliability of the Hammersmith Infant Neurological Examination (HINE) with High-Risk Infant Group: A Preliminary Study. *Turkish Archives of Pediatrics,* 57(2), 151.
2. Apaydın, U., Erol, E., Yıldız, A., Yıldız, R., Acar, Ş. S., Gücüyener, K., & Elbasan, B. (2021). The use of neuroimaging, Prechtl's general movement assessment and the Hammersmith infant neurological examination in determining the prognosis in 2-year-old infants with hypoxic ischemic encephalopathy who were treated with hypothermia. *Early human development,* 163, 105487.
3. Einspieler, C., & Prechtl, H. F. (2005). Prechtl's assessment of general movements: a diagnostic tool for the functional assessment of the young nervous system. *Mental retardation and developmental disabilities research reviews,* 11(1), 61-67.
4. Einspieler, C., Prechtl, H. F., Ferrari, F., Cioni, G., & Bos, A. F. (1997). The qualitative assessment of general movements in preterm, term and young infants—review of the methodology. *Early human development,* 50(1), 47-60.
5. Hadders-Algra, M. (2004). General movements: a window for early identification of children at high risk for developmental disorders. *The Journal of pediatrics,* 145(2), S12-S18.
6. Hubermann, L., Boychuck, Z., Shevell, M., & Majnemer, A. (2016). Age at referral of children for initial diagnosis of cerebral palsy and rehabilitation: current practices. *Journal of Child Neurology,* 31(3), 364-369.
7. Maitre, N. L., Chorna, O., Romeo, D. M., & Guzzetta, A. (2016). Implementation of the Hammersmith Infant Neurological Examination in a high-risk infant follow-up program. *Pediatric neurology,* 65, 31-38.
8. Morgan, C., Romeo, D. M., Chorna, O., Novak, I., Galea, C., Del Secco, S., & Guzzetta, A. (2019). The pooled diagnostic accuracy of neuroimaging, general movements, and neurological examination for diagnosing cerebral palsy early in high-risk infants: a case control study. *Journal of clinical medicine,* 8(11), 1879.
9. Novak, I., Hines, M., Goldsmith, S., & Barclay, R. (2012). Clinical prognostic messages from a systematic review on cerebral palsy. *Pediatrics,* 130(5), e1285-e1312.

10. Novak, I., Morgan, C., Adde, L., Blackman, J., Boyd, R. N., Brunstrom-Hernandez, J., Eliasson, A.-C. (2017). Early, accurate diagnosis and early intervention in cerebral palsy: advances in diagnosis and treatment. *JAMA pediatrics,* 171(9), 897-907.
11. Novak, I., Morgan, C., Fahey, M., Finch-Edmondson, M., Galea, C., Hines, A., . . . Popat, H. (2020). State of the evidence traffic lights 2019: systematic review of interventions for preventing and treating children with cerebral palsy. *Current neurology and neuroscience reports,* 20(2), 1-21.
12. Oskoui, M., Coutinho, F., Dykeman, J., Jette, N., & Pringsheim, T. (2013). An update on the prevalence of cerebral palsy: a systematic review and meta-analysis. *Developmental Medicine & Child Neurology,* 55(6), 509-519.
13. Prechtl, H. F. (1990). Qualitative changes of spontaneous movements in fetus and preterm infant are a marker of neurological dysfunction. *Early human development*. 23, 151-158.
14. Prechtl, H. F., Einspieler, C., Cioni, G., Bos, A. F., Ferrari, F., & Sontheimer, D. (1997). An early marker for neurological deficits after perinatal brain lesions. *The Lancet,* 349(9062), 1361-1363.
15. Romeo, D. M., Ricci, D., Brogna, C., & Mercuri, E. (2016). Use of the Hammersmith Infant Neurological Examination in infants with cerebral palsy: a critical review of the literature. *Developmental Medicine & Child Neurology,* 58(3), 240-245.
16. Rosenbaum, P., Paneth, N., Leviton, A., Goldstein, M., Bax, M., Damiano, D., . . . Jacobsson, B. (2007). A report: the definition and classification of cerebral palsy April 2006. *Dev Med Child Neurol Suppl,* 109, 8-14.
17. Soleimani, F., Badv, R. S., Momayezi, A., Biglarian, A., & Marzban, A. (2015). General movements as a predictive tool of the neurological outcome in term born infants with hypoxic ischemic encephalopathy. *Early human development,* 91(8), 479-482.

Intraoperative Monitoring

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Monitoring during anesthesia and surgery; is to record the measured value at regular intervals. Devices that perform this function, monitor and warn are also called monitors.It ranges from simple and inexpensive methods to complex, expensive and time-consuming methods, depending on the patient's condition and the type of surgical intervention.The purpose of monitoring; examining the variables, recognizing the problems, determining the severity of the problems, and evaluating the response to treatment.

The most important monitor in the operating room is the careful anesthetist who collects objective and subjective information.While monitoring based on the senses (visual, auditory, tactile) and experiences of the anesthesiologist creates subjective monitoring, sensitivity to negative developments in cases under anesthesia is increased with monitors that provide objective data.An ideal monitor should be non-invasive, should not cause physiological or psychological changes in the patient, should be reliable, easy to understand, easy to use, easy to calibrate, portable, inexpensive, easy to maintain, and should not complicate the procedures to be performed on the patient.

There are various classifications for monitoring methods, but according to the procedure applied on the patient; While noninvasive (ECG, pulse oximetry, NIBP, capnography, measurement of gases in respiratory air) do not require any intervention on the patient, invasive methods (such as arterial and central catheter, urinary catheter, nasopharyngeal temperature measurement) require patient-specific catheter placement procedures.Monitoring standards in anesthesia applications are linked to the following standards by the American Society of Anesthesiologists (ASA):

Standard 1: A staff experienced in regional or general anesthesia should be at the patient's side

Standard 2: During the entire anesthesia, the patient's oxygenation, ventilation (pulse oximetry, capnography, oxygen analyzer, disconnection alarm), circulation: ECG and blood pressure (5 min intervals) and body temperature should be monitored.

In routine anesthesia applications, the following systems are monitored:

1- Cardiovascular system
2- Respiratory system
3- Renal system
4- Neuromuscular system
5- Heat monitoring
6- Central nervous system

1- MONITORIZATION OF THE CARDİOVASCULAR SYSTEM

a-) Arterial Blood Pressure (ABP) : Monitoring blood pressure is an indispensable intervention in determining the effects of anesthesia on the cardiovascular system.Rhythmic contraction of the left ventricle pumps blood into the vascular system, resulting in pulsatile arterial pressure.Peak pressure during systolic contraction is considered systolic arterial pressure (SAP), and pressure during diastolic relaxation is considered diastolic arterial pressure (DAP).Pulse pressure is the difference between these two pressures. The time-weighted average of arterial blood pressure forms the mean arterial pressure (MAP) and is calculated with the following formula: MAP= (SAP + 2 DAP)/ 3

Non-invasive blood pressure: Blood pressure is usually measured using either manual palpation, doppler, auscultation, or the automatic oscillation method.

i. Palpation method; It is one of the easiest methods of measuring blood pressure. To localize a pulse is to inflate the cuff until that pulse disappears and then deflate the cuff until the pulse is palpated again.

ii. Doppler principle; It can be used to detect arterial wall movements compressed with an inflated cuff. It has been reported that Doppler measurement shows a close relationship with intra-arterial measurements in pediatric cases, but gives slightly lower blood pressure values. The advantage of this technique is that it is suitable for children and adults with low blood flow. Its disadvantage is that mean and diastolic arterial pressures cannot be easily obtained; movement, electrocautery, and displacement of the Doppler probe.

iii. Auscultation method: The cuff wrapped around the extremity is inflated with air until it reaches a suprasystolic pressure, and then, as it is slowly deflated, Korotkoff sounds are heard. The systolic pressure value when the sounds are heard and the pressure value at which the sounds disappear are accepted as the diastolic blood pressure.Certain conditions must be met for an accurate ABP measurement:

- Cuff width should be 20% greater than the diameter of the extremity
- The pouch must be connected to a calibrated aneroid or mercury manometer
- Wrapping the cuff too tightly or loosely can also prevent accurate measurement.

iiii. Oscillometric method: A pressure cuff is inflated to the extent that it blocks arterial blood flow. Pulsation of the artery as the cuff is deflated causes a pressure change within the cuff and these changes are evaluated by a computer. Measurement errors; Caused by conditions that cause changes in intracuff pressure, such as improper cuff size and patient tremors.

Measurement of arterial pressure by the direct method (invasive): Continuous recording of blood pressure with a catheter placed in the peripheral artery allows blood pressure monitoring. Arterial pressure is ideally measured in the ascending aorta. Pressures measured from the periphery very often differ from the central aortic pressure as they become more and more deformed as they are conducted through the arterial system. This effect is most common in the dorsalis pedis artery. In this artery, SAP is 10-20 mmHg higher and DAP 20 mmHg lower than in the central aorta. Despite such changes, MAP is normally equal to central aortic pressure.Although peripheral arteries (radial, ulnar, axillary, femoral, dorsalis pedis, tibialis posterior) are used, it is the most commonly used radial artery. Before cannulation, the "Allen" test is performed to evaluate the adequate collateral circulation.To apply the Allen test, compression is applied to the radial and ulnar arteries, while the hand is exercised until it becomes pale. The ulnar artery is then released and the time taken for the hand to reach its normal color is recorded. In the presence of a normal collateral circulation, this time is around 5-7 seconds (sec). On the other hand, if this time exceeds 15 seconds, it is controversial whether the radial artery can be cannulated.If the hand is hyperextended or the fingers are tautly spread apart, the hand may still remain pale despite the presence of normal collateral circulation, resulting in a false test result. The Allen test can be modified using a Doppler device or pulse oximeter.

Cannulation of the radial artery is performed by dorsiflexing the wrist. Generally, a 20 G Teflon catheter is chosen for the adult.The catheter is placed at a 15-30 degree angle. After the catheter is placed, it should be flushed continuously with heparinized saline (1-2 U/mL) at a rate of 1-3 mL/h.

Indications:

- When there are rapid changes in blood pressure
- Elective hypotension
- Conditions where minor blood pressure changes can cause organ damage
- Frequent monitoring of arterial blood gases
- Conditions where non-invasive blood pressure measurements are unreliable
-

b-) Electrocardiography (ECG): Continuous visual monitoring of the ECG is standardmonitoring in all anaesthetized patients. Thus, information about cardiac dysrhythmias, myocardial ischemia (ST segment depression) and electrolyte changes (especially potassium) can be obtained.Usually, the heart rate is calculated from the EKG trace. Different derivations are obtained with the evaluation made according to the insertion of the ECG electrodes.

- Standard leads (D I , D II, D III)
- Unipolar limb leads (avR , avL, aVF)
- Precordial leads (V1-6)

What disorders can be diagnosed by EKG during anesthesia?

- Rhythm disorders
- Conduction disorders (AV block, premature atrial contractions, premature ventricular contractions)
- Myocardial ischemia
- Ventricular and atrial hypertrophy
- Pace maker function
- Preexcitation rhythms
- Toxic effects of drugs (digital, antiarrhythmics, tricyclic antidepressants)
- Electrolyte level disorders (Ca, K,)
- Different medical problems (pericarditis, hypothermia, pulmonary embolism, corpulmonale, cerebrovascular events or increased intracranial pressure)

Generally, 5 or 3prong cable configurations are used. A standard 3lead ECG is recorded from the right arm (RA), left arm (LA), and left leg (LL). The standard limb leads are known as DI (LA-RA), DII (LL-RA), and DIII (LL-LA). During monitoring, lead DII is mostly used for the diagnosis of rhythm disorders. 75% of ischemic events were found when V5 lead was followed, it was possible to detect 85% ischemic events when V4-V5 leads were followed. It is possible to obtain the most information by monitoring the **DII** and **V5** leads on the monitor.

2- MONITORING RESPIRATORY FUNCTIONS

a-) Pulse Oximeter: Pulse oximetry is mandatory intraoperative monitör.It is a continuous and non-invasive measurement of peripheral arterial Hb oxygen saturation, which is a reflection of arterial oxygen saturation. It provides early detection of arterial hypoxemia.Measurement can be made by placing well-perfused tissues such as fingertips, earlobes, etc. between the sensor, which consists of a light source and a light detector. Under normal room air conditions, the SpO_2 value is between 97-99%. When SpO_2 is 90%, the PaO_2 value is less than 65 mmHg.

b-) End-expiratory carbon dioxide pressure: End-tidal carbon dioxide ($PEtCO_2$) shows a close relationship with $PACO_2$ (alveolar partial pressure of CO_2) and thus with $PaCO_2$. Normally the difference between $PEtCO_2$ and $PaCO_2$ is 3-5 mmHg. Although this relationship is impaired in patients with lung disease, it is an appropriate monitoring method for monitoring the

adequacy of ventilation.On the other hand, sudden changes in $PEtCO_2$ may be caused not only by the respiratory system but also by the cardiovascular system. For example, a sudden decrease in cardiac output due to cardiac or pulmonary embolism will also cause a sudden decrease in $PEtCO_2$ as it will increase the physiological dead space ratio.

Continuous $PEtCO_2$ measurement; It can be used to detect hypoventilation, hyperventilation and apnea conditions in spontaneously breathing cases, to adjust ventilator settings to achieve the desired $PaCO_2$ level, to detect disconnection, leakage or obstruction in the breathing circuit, or ventilator dysfunction.

c-) Transcutaneous Oxygen And CO_2 Monitoring: Transcutaneous oxygen sensors measure oxygen diffused from the dermal capillaries under the electrode to the skin surface, and this measurement is capillary blood flow dependent.

d-)Anesthetic Gas Analyzes: Gas used in procedures using inhalation anesthetics can be analyzed.

3- MONİTORİNG RENAL FUNCTIONS

The primary purpose of monitoring renal function is to evaluate the extracellular fluid volume and cardiac output (and thus renal blood flow). The bladder is usually catheterized with a Foley catheter, with urine collected in a sterile, closed system and recorded hourly. As one of the most common causes of oliguria and anuria is catheter occlusion, the catheter should be checked at regular intervals under aseptic conditions. Hourly urine monitoring with a urinary catheter is usually an adequate monitoring method for controlling renal perfusion in patients with adequate blood volume and without kidney problems.

Decreased urine output in resuscitation of an acute injury; It will mean that renal perfusion is impaired or that acute renal failure has started. However, urine output may not always be an adequate indicator as it may be sufficient even in shock situations.Plasma and urine osmolality, osmolar and free fluid clearances are the most sensitive and most important functions of the kidneys' ability to concentrate urine.

4- NEUROMUSCULAR MONITORING

In some cases, it is necessary to monitor the effects of neuromuscular blocking agents, which are frequently used during anesthesia. A peripheral nerve stimulator is used for this purpose. The stimulating electrodes of the stimulator are fixed on pallets placed on the ulnar nerve trace. The responses of the thumb to electrical stimuli applied at the supramaximal level are evaluated

visually, by tactile (tactile) methods, mechanomyographic recordings or by acceleration method. With such monitoring, it is possible to evaluate the level of muscle relaxation, the type of block (depolarizing, nondepolarizing), the time of the maintenance dose, the time and adequacy of muscle relaxation at the end of the operation.Some drugs used in anesthesia, especially muscle relaxants and some antibiotics can change the duration of action of muscle relaxants. In the absence of a nerve stimulator, clinical findings can be used for evaluation: Maintaining respiratory functions, making a fist and raising the head for a while are the clinical criteria used for this purpose.

5- HEAT MONITORING

Body temperature is a parameter that is routinely measured together with blood pressure, pulse and respiratory rate. Central body temperature; It is better evaluated in measurements made from the tympanic membrane or esophagus. Since the pulmonary artery temperature will reflect the central body temperature, pulmonary artery thermodilution catheters can also be used for this purpose. A large difference between rectal temperature and thumb temperature indicates a decrease in peripheral flow, and a decrease in this difference indicates an increase in peripheral flow.

During the application of anesthesia, hypothermia often occurs and the reasons that may cause this are:

- Operating room temperature < 21°C
- Infusion of intravenous fluids at room temperature
- Cold irrigation fluids
- Heat loss by breathing gases
- Decreased basal metabolic rate
- Vasodilation with anesthesia
- Changes in the hypothalamic thermoregulatory mechanism with anesthesia

6- CENTRAL NERVOUS SYSTEM (CNS) MONİTORİNG

EEG: Electroencephalography is a method that is frequently used for the diagnosis of critical cases with CNS deficits, especially when they are semicomatous or comatose. It is also used in the evaluation of brain death. Less frequently, serial EEGs are taken to monitor changes in electrical activity during the worsening of comatose conditions. It is also not used very frequently during continuous EEG monitoring, anesthesia administration and carotid artery surgery.

Indications for Monitoring of Central Nervous System Functions:

- Controlled hypotension
- Hypothermia
- Deep sedation
- Cardiopulmonary surgery
- Surgeries in which focal brain ischemia is expected
- Surgery where spinal cord, cranial nerve traction or ischemia is expected
- Peripheral nerve and plexus reconstructions
- Cases in which sedative and anticonvulsant drugs are used in the intensive care unit.

RESOURCES:

1. Morgan GE, Mikhael MS, Murray MJ. Clinical Anesthesiology 7. Edition. McGraw Hill LLC, 2022, p:132-221.
2. Esener Z: Klinik Anestezi. Logos Yayıncılık, 3. baskı, 2004, p:37-64
3. Hasta monitörizasyonu ve girişimler: In James Duke (Çev: Yalım Dikmen) Anestezinin sırları. ikinci baskı Nobel kitap evi. Ankara, 2006, p:115-148

BASAL GANGLIA

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Basal ganglia are five pairs of deep nuclei in the cerebral hemispheres(Figure 1).These are caudate nucleus, putamen, globus pallidus, substantia nigra, and amygdala (Van-derah and Gould, 2021).

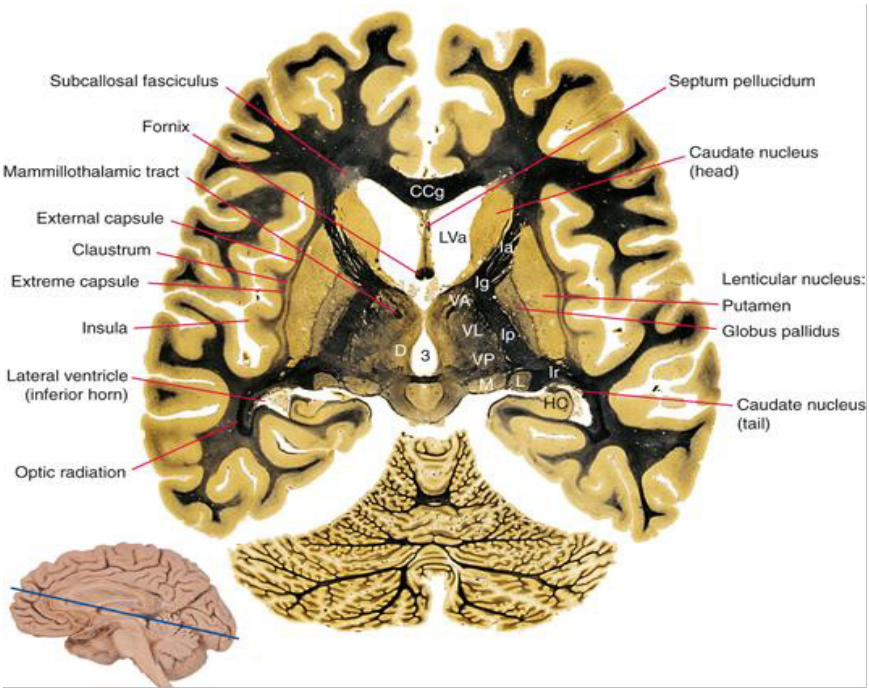

Figure 1. Basal nuclei and surrounding structures, as seen in an axial section (Vanderah and Gould, 2021:19).

(3, Third ventricle; CCg, genu ofthe corpus callosum; D, dorsomedial nucleus; HC, hippocampus; Ia, Ig, Ip, and Ir, internal capsule—anterior limb, genu, posterior limb, and retrolenticular part; L, lateral geniculate nucleus; LVa, anterior horn ofthe lateral ventricle; M, medial geniculate nucleus; VA, VL, and VP, ventral anterior, ventral lateral, and ventral posteriornuclei.)

The basal ganglia (BG) are distributed from the mesencephalon to thetelencephalon (Fig. 2). The striatum is in the ventral partof thetelencephalon.The structures that assume different roles in motor, emotional and cognitive behavior by receiving other inputs from the prefrontal cortex includethe caudate nucleus, putamen, and nucleus accumbens (Burbaud et al.,2022).

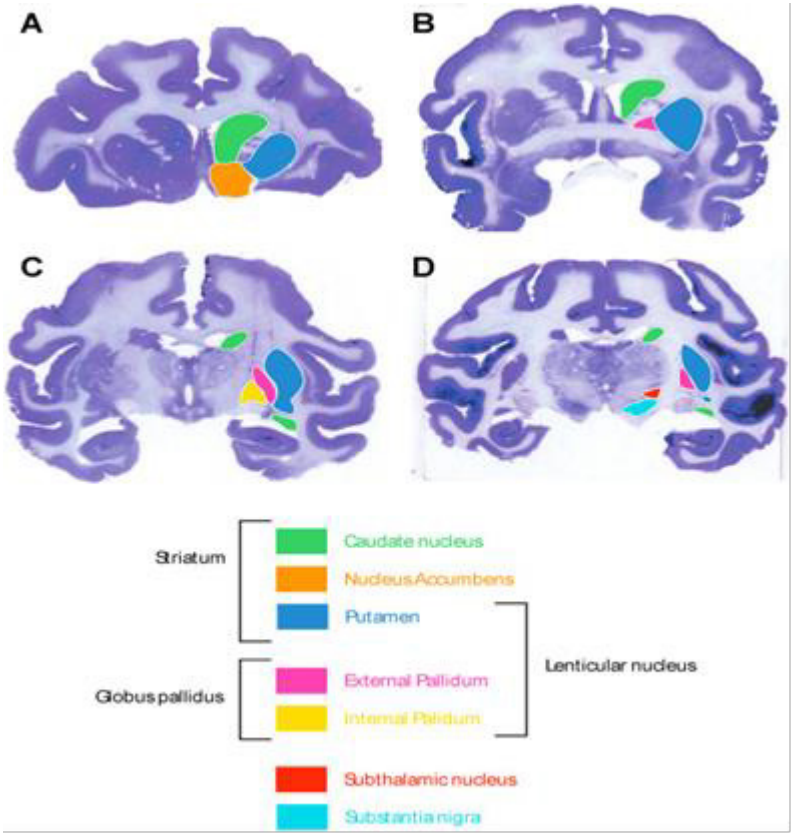

Figure 2. Coronal view of basal ganglia from different angles (Burbaud et al., 2022). A. Ön striatum. B. Posterior striatum. C. Lentiküler çekirdek. D. Mesensefalon

It contains two areas: the globus palli-dus globus pallidus externalis(GPe) and the globus pallidus interna-lis (GPi), located medial and posterior to the putamen.The substantia nigra is found in the red ruler.It is dividedinto two areas, dorsal and ventral. Pars compacta (SNc) contains dopamine nerve neurons, while pars reticulata (SNr) contains GABA nerve neurons. In theupper lateral part of thesubstantia nigra, there are subthalamic nuclei containing glutamatergic neurons. Dopaminergic neurons are in the ventraltegmental area.As a result, the primary circuits of the basal ganglia(Figure 3) contain information about the cerebral cortex, striatum andsubthalamic nucleus extents (Gal-van and Yoland, (2023).

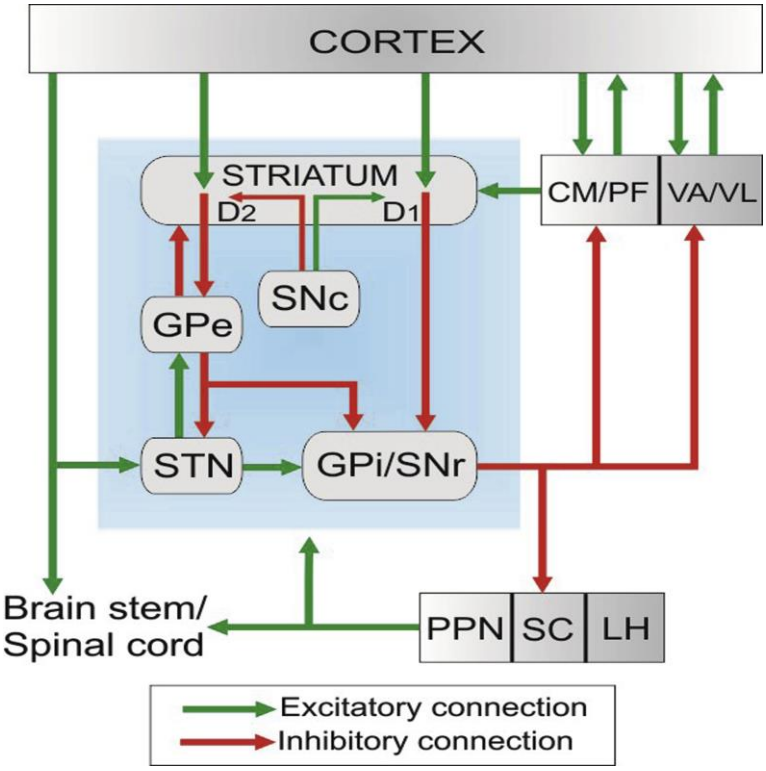

Figure 3. Connections of basal ganglia (Galvan and Yoland, 2023).

There are three neurocycles in the basal ganglia that function as senseriomotor, cognitive, and limbic (Figure 4). It also plays aactive role in the thalamostrial system between the cerebellum and basal ganglia(Macpherson and Hikida, 2019, Galvan and Yoland, 2023).

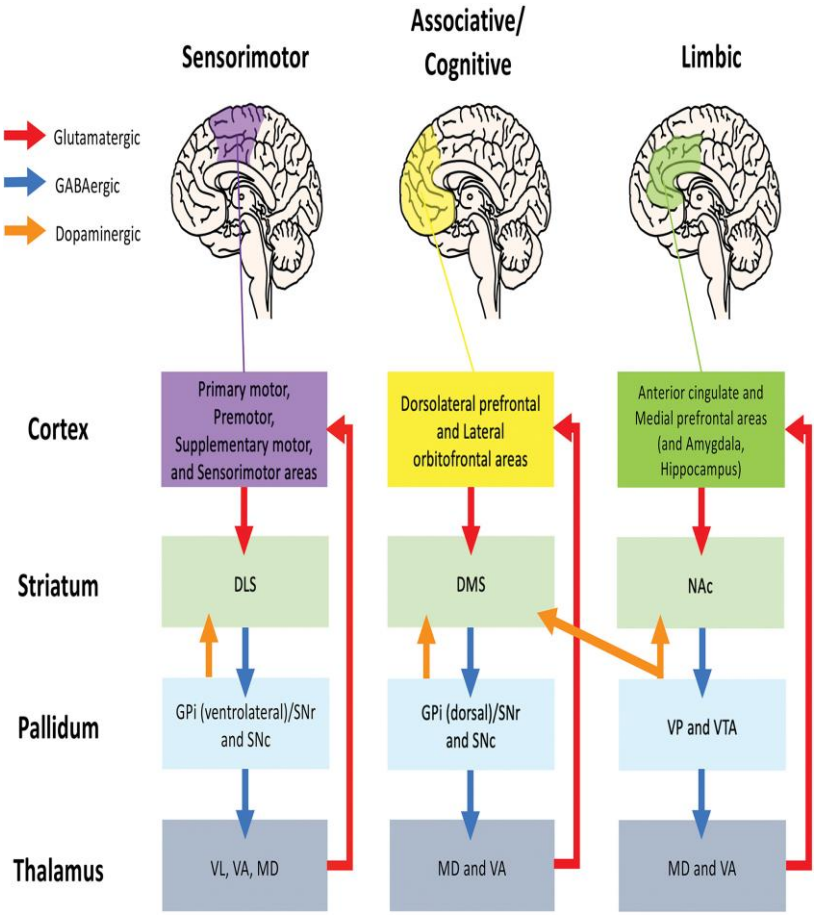

Figure 4. Neuron circuits of basal ganglia (Macpherson and Hikida, 2019).

There are two ways basal ganglia related to motor activity, defined as direct (direct) and indirect (indirect) (Figure 5) (Roshan et al., 2016)

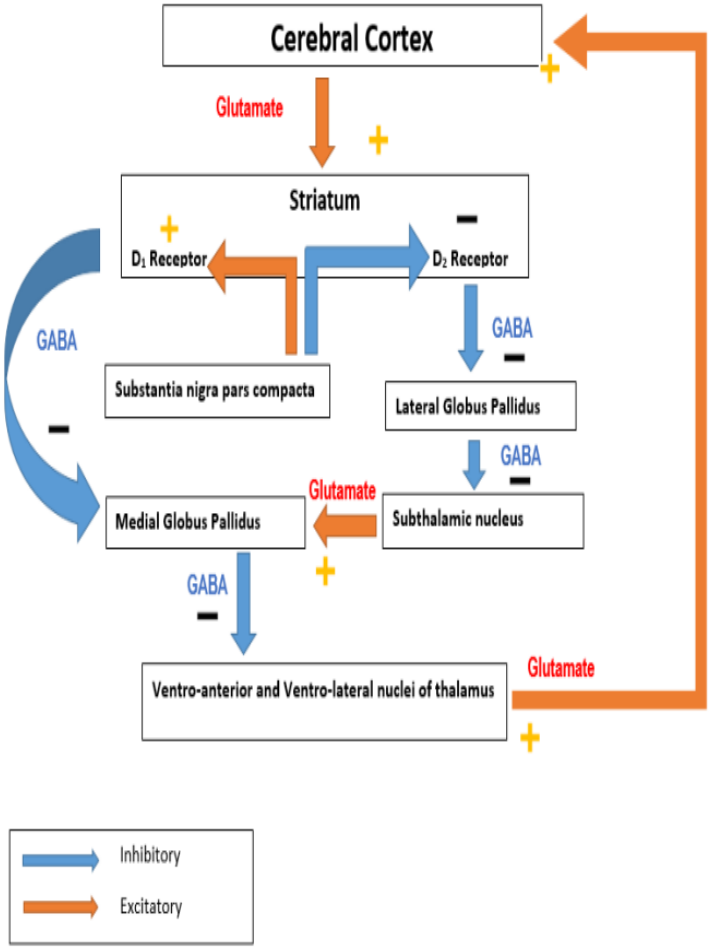

Figure 5. Direct and indirect pathways of Basal Ganglia in initiating motor activity(Roshan et al., 2016).

The globus pallidus internal [GPi], ventral anterior nuclei of thethalamus [VA],ventral lateral nuclei of the thalamus [VL],globus pallidus external [GPe],subthalamic nucleus [STN].

An increased inhibitory output pathway in the globus is the direct pathway, causing cerebral cortical input to the mar-gins of inhibitory neurons inthe striatum. As a result,this pathway participates in the expectation of tonic excitation in the premotor cortex about the planning of movement.The indirect pathway, on the other hand,is prohibitive.

The indirect pathway inhibits the creation of movement when the excitatory projections from the cerebral cortex are facilitated by inhibitory projecting

neurons in theglobus palli-dus external. This inhibits tonic inhibitory output neurons, which reduces tonic inhibition of thesubthalamic nucleus. As a result,the excitatory feedback in thecerebral cortex,which leads to theinhibition of motor activity, is reduced (Roshan et al., 2016).The direct pathway transmits signals from the motor cortex, activating motor responses, while the indirect pathway reduces motor activity. In addition, support information is also obtained from thecognitive functions of the limbic system, where non-motor responses (Figure 6) are produced in these interactions. These, in turn,support the formation of responses such as attention, choice of actions (Miller, E.K, 2000, Swick et al., 2011, Floresco, S.B, 2015, Jahanshahi et al., 2015, Criaud et al., 2017 ; Criaud et al., 2021).

It shows that the cerebral cortex and basal gangliaare distributed in most places where they enter and exit. It reveals the cortico-striato-thalamo-cortical loop due to the modulus of attention, working memory, conditioned fear memory, and cognition with the basal ganglia, especially about the frontal lobe (Leisman et al., 2014).

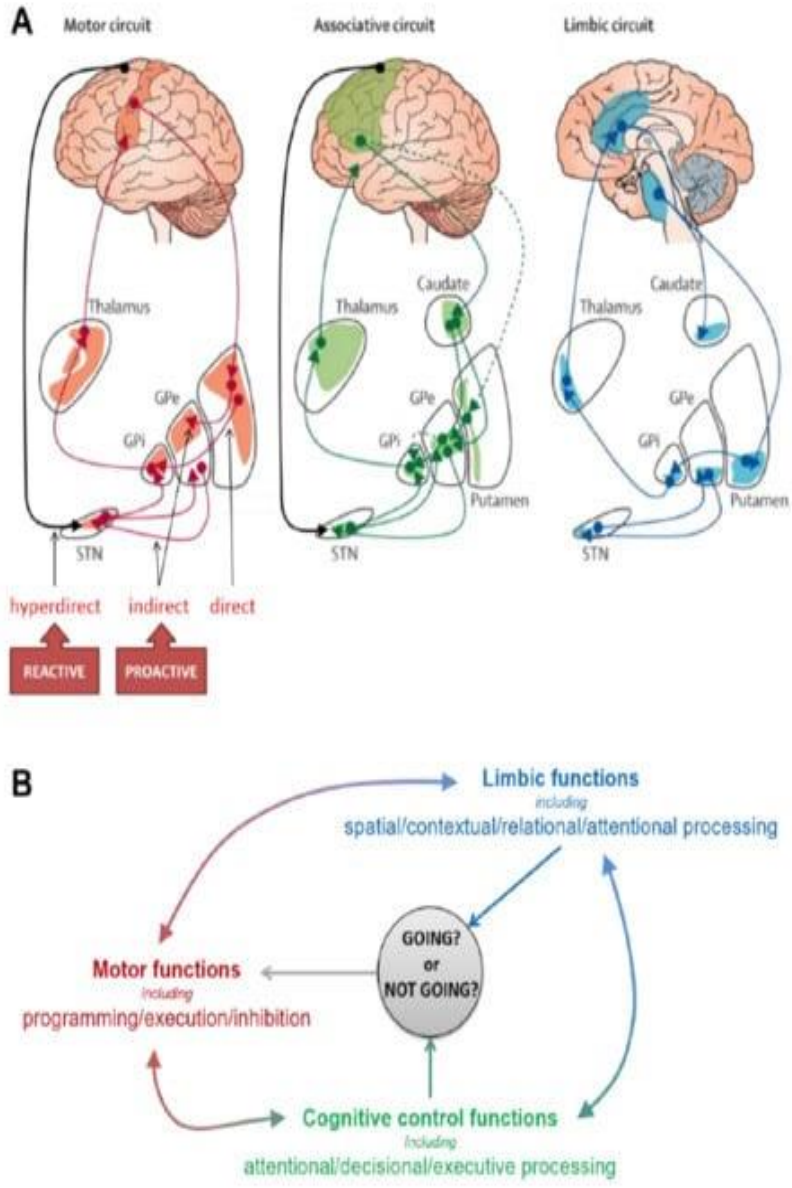

Figure 6. Cortico-striatal circuits (A) and related elements (B) (Criaud et al.,2021)

Movement disorder includes various motor problems such as rigidity, akinesia/bradykinesia, Parkinson's, dystonia, and chorea-gunning that does not spread with complete dysfunction of the motor pathways of the basal ganglia (Lancego et al., 2012). Huntington's disease also occurs due to damage to the

basal ganglia. There is degeneration in some of the dopamine-secreting nerve fibers of the substantia nigra(pars compacta) in patients with Parkinson's disease whohave pupils with standard features such as involuntary tapping, akinesia, dysphagia, speech disorders, walking weights, and rigidity. In Huntington's, which shows an autosomal dominant hereditary feature, progressive severe distortional movements and demands exist. The problems in this disease are recovery from the action of GABA-secreting neurons in the caudate nuclei and putamen (John and Hall, 2021).

As a result,there is a need for amore detailed investigation of our current general knowledge that the learning pathways with reinforcement in motor movements receive and process, and transmit multiple inputs through loops with both motor, cognitive and limbic structures in the realization of new activities, with the newly discovered microneuroanatomical relationships.

REFERENCES

1. Aron, A.R. From Reactive to Proactive and Selective Control: Developing a Richer Model for Stopping Inappropriate Responses. Biol. Psychiatry 2011, 69, e55–e68.
2. Burbaud P., Courtin E., Ribot B., Guehl D., (2022). Basal ganglia: From the bench to the bed, European Journal of Paediatric Neurology,36, 99-106.
3. Criaud, M., Longcamp, M., Anton, J.-L., Nazarian, B., Roth, M., Sescousse, G., Strafella, A.P., Ballanger, B., Boulinguez, P., (2017). Testing the Physiological Plausibility of Conflicting Psychological Models of Response Inhibition: A Forward Inference FMRI Study. Behav. Brain Res. 333, 192–202.
4. Criaud M, Anton J-L, Nazarian B, Longcamp M, Metereau E, Boulinguez P, Ballanger B., (2021). The Human Basal Ganglia Mediate the Interplay between Reactive and Proactive Control of Response through Both Motor Inhibition and Sensory Modulation. Brain Sciences,11(5):560.
5. Floresco, S.B., (2015). The Nucleus Accumbens: An Interface between Cognition, Emotion, and Action. Annu. Rev. Psychol. 2015, 66, 25–52.
6. Galvan A., Yoland S., (2023). Anatomy and Synaptic Connectivity of the Basal Ganglia. Youmans and Winn Neurological Surgery, Eighth Edition; 102, 671-671.e22.
7. Jahanshahi, M., Obeso, I., Rothwell, J.C., Obeso, J.A., (2015). A Fronto-Striato-Subthalamic-Pallidal Network for Goal-Directed and Habitual Inhibition. Nat. Rev. Neurosci. 2015, 16, 719–732.
8. John E.H, Hall M.E., (2021). Cerebellum and Basal Ganglia Contributions to Overall Motor Control. Guyton and Hall Textbook of Medical Physiology, Chapter 57, 711-726.
9. Lanciego JL, Luquin N, Obeso JA., (2012). Functional neuroanatomy of the basal ganglia. Cold Spring Harb Perspect Med;2(12).
10. Macpherson, T., Hikida, T. (2019). Role of basal ganglia neurocircuitry in the pathology of psychiatric disorders. Psychiatry and clinical neurosciences, 73(6), 289-301.
11. Miller, E.K., (2000). The Prefrontal Cortex and Cognitive Control. Nat. Rev. Neurosci. 2000, 1, 59–65.
12. Roshan, M. H., Tambo, A., Pace, N. P. (2016). Potential role of caffeine in the treatment of Parkinson's disease. The open neurology journal, 10, 42.
13. Swick, D., Ashley, V. Turken, U., (2011) Are the Neural Correlates of Stopping and Not Going Identical? Quantitative Meta-Analysis of Two Response Inhibition Tasks. NeuroImage, 56, 1655–1665.

EFFECT OF VARIOUS PROBIOTIC CHARACTERS ON CHOLESTEROL ASSIMİLATION OF STRAINS

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It is widely acknowledged that having high serum cholesterol levels increases the risk of developing cardiovascular diseases (CVD), overweight, heart disease and other metabolic disturbances (Park et al., 2018). The main causes of death in the globe and in our nation are cardiovascular illnesses. One of the most prevalent and fatal diseases in developed nations, this ailment is directly linked to blood levels of excessive cholesterol. According to the World Health Organization (WHO), coronary heart disease will impact 23.6 million people globally by 2030 and be a leading cause of death (WHO 2002).

It is well recognized that having too much blood cholesterol puts people at risk for developing coronary heart disease (Ahn et al., 2003). Intake of meals high in cholesterol and saturated fatty acids contributes to the rise in cardiac illnesses (Belviso et al., 2009). Dietary changes, behavioral changes, regular exercise, and pharmaceutical methods can all help lower high serum levels of cholesterol. Although there are effective cholesterol-lowering medications, they are known to have negative side effects that include liver damage and muscle soreness (Park et al., 2018). As a result, non-pharmacological methods, particularly the administration of probiotics, have drawn interest for controlling steadily rising cholesterol levels and so preventing cardiovascular illnesses (Kumar et al. 2012; Saikia et al. 2018).

To treat hyperlipidemic patients, a significant variety of medications have been created, with statins serving as the greatest description (Atorvastatin, Simvastatin , Rosuvastatin and Lovastatin). However, these substances have been shown to have adverse effects, which raises questions regarding how long they may be used therapeutically. As a result, various non-pharmacological strategies (such as nutrition) to lower blood cholesterol levels have been investigated. Probiotics have been utilized as one strategy among many to address this issue. According to its definition, a probiotic is "a living microbial supplementation that benefits the person by enhancing the gut microbial balance" (Fuller, 1992). Probiotics are non-pathogenic bacteria that are beneficial to the host when taken in sufficient quantities. The principal probiotics are certain lactic acid bacteria (LAB), which are responsible for the biological found in the gastrointestinal system of both animals and humans. Examples of these LABs include *Lactobacillus* and *Bifidobacterium*. Over time, lactobacilli have been linked to reductions in blood cholesterol, an increase in natural resistance to infectious infections in the gastrointestinal tract, improvements in lactose intolerance, and suppression of cancer (Gibson et al., 1995). A naturally occurring component of the microbial community called LAB is the subject of intense scientific investigation due to its potential health benefits, including the reduction of serum

cholesterol, induction of immune function, protection against cancer, and relief from diahorrea.

Hypercholesterolemia due to high serum low-density lipoprotein (LDL) cholesterol levels is an important risk factor for CVD. Meta-analysis showed that a 1% reduction in serum cholesterol can reduce CVD risk by 2 to 3% (Manson et al., 1992). The liver is a critical organ for clearance of LDL cholesterol (LDL-C) from the circulating blood, and LDL receptor (LDLR)-mediated endocytosis in the liver is responsible for removing 70 to 80% of serum LDL-C. Therefore, a complementary relationship between hepatic LDLR expression and serum LDL-C level is well established, suggesting that LDLR is a promising target for preventing the development and progression of CVD (Song et al., 2015).

Cholesterol absorption capacity (Kumar et al. 2011), bile acids and cholesterol (Anandharaj et al. 2015), cholesterol reducing potential of probiotic strains, bile salt deconjugation ability and bile salt hydrolase (BSH) activity, and cholesterol (Choi and Chang, 2015), reported probiotic adhesion to cell walls, while Tsai et al. (2014) reported micelle-mediated cholesterol cleavage, Park et al. (2018) reported cholesterol conversion to the insoluble compound coprostanol, and Choi and Chang (2015) reported short-chain fatty acid-mediated cholesterol reduction (Kumar et al. 2012).

BSH activity can be examined as an useful probiotic biomarkers for the selection of hypocholesterolemic strains because it is directly related to the ability of probiotics to decrease cholesterol (Kumar et al. 2012a, b). BSH action has been documented for numerous probiotic species and strains, including Lactobacillus, *Bifidobacterium*, Enterococcus, and *Saccharomyces boulardii* (Saikia et al. 2018). Probiotic management may be a potent non-pharmacological strategy to lower cardiovascular disease risk factors by reducing increased blood cholesterol levels, according to analysis of recent research data (Wang et al., 2018).

In vivo and in vitro cholesterol assimilation studies

Various *Lactobacillus* spp. isolated from human breast milk samples. In the study in which the probiotic properties of the species were investigated, *Lactobacillus paracasei* M3, *L. casei* M5 and *L. paracasei* M7 strains with hypocholesterolemic potential were found to have commercial potential. According to the study's findings, LAB probiotics reduce cholesterol via a variety of mechanisms, including bile salt hydrolase activity, bile salt deconjugation, cholesterol absorption, cholesterol biosorption on cell walls, inhibition of micelle formation, and retention of cholesterol by micelles (Bhat and Bajaj, 2020).

Identified strains of *Lactobacillus* from raw goat milk. The strains of *L. plantarum* GM-12 and GM-15 were discovered to have the highest levels of

cholesterol assimilation *(Tulumolu et al., 2022).* In the study conducted for the selection of potential probiotic microorganisms with cholesterol-lowering properties for the production of probiotic yogurt, three strains with good cholesterol-lowering properties were identified from 61 LAB isolates. The strain with the highest cholesterol assimilation of these was *Limosilactobacillus fermentum* KUB-D18 (68.75%) (51 g/10^9 CFU), while the strains with the highest bile salt hydrolase (BSH) activities were *Lactiplantibacillus pentosus* HM04-25 and *L. pentosus* HM04-3 (22.60 and 21.45 U/mL, respectively) (Wongrattanapipat et al., 2022).

In a study investigating the effects of a proven probiotic strain of *Enterococcus faecalis* AG5 on cholesterol assimilation, the cholesterol assimilation percentage of the strain inoculated with a mixture of 0.3% sodium glycolate (glycine conjugated) and bile salts in different time periods varied between 14% and 33.1% in the bile salt mixture, while sodium thioglycolate alone However, this rate was found to be slightly higher between 16.55 and 53.1%. As a result, it has been proven that the AG5 strain is a strain with strong probiotic properties, promising to be beneficial to human health due to the deconjugation of bile salt and its short-chain fatty acid production capacity (Mishra et al., 2019).

In the study investigating the cholesterol-lowering effect of 79 LAB isolated from various fermented foods (pickles, yoghurt, fermented sausage, etc.) in rats fed a high-cholesterol diet, it was determined that the best cholesterol-assimilating strain was *L. plantarum* LP96 among 79 strains. It was applied to test its effects on rats given a diet high in cholesterol. In comparison to the model group, the groups receiving the LP96 strain solution had significantly lower levels of serum triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol, liver total cholesterol, and serum triglycerides, while serum levels of high-density lipoprotein cholesterol were not significantly different. It has been found to increase without (Liu et al., 2017).

Cholesterol assimilation efficiency was assessed using in vitro and in vivo experiments in the study that looked at probiotic qualities including acid resistance, bile tolerance, adhesion to HT-29 cells, and cholesterol absorption ability of *Lactobacillus acidophilus* NS1 identified from newborn stool. Male C57BL/6 mice aged 7 weeks were given either a standard diet, a high-fat diet (HFD), or an HFD including *L. acidophilus* NS1 (around 1.010(8) cfu/mL). For 10 weeks, mice fed HFD with *L. acidophilus* NS1 had considerably lower levels of total cholesterol and minimal lipoprotein (LDL) cholesterol than mice fed HFD only. We measured the mRNA levels of genes involved in cholesterol homeostasis in the liver in order to comprehend the mechanisms of the cholesterol-lowering impact of *L. acidophilus* NS1 on the HFD-mediated raise in

plasma cholesterol levels. When compared to mice on a regular diet, mice fed the HFD had considerably lower levels of the liver enzymes sterol regulatory element-binding protein 2 (Srebp2) and LDL receptor (Ldlr). The findings demonstrate that oral delivery of *L. acidophilus* NS1 to mice on an HFD enhanced the synthesis of Srebp2 and Ldlr in the liver, which was otherwise suppressed by a high fat diet. This resulted in a drop in blood cholesterol levels (Song et al., 2015)

It has also been reported that *L. oris* isolated from infant feces can reduce cholesterol up to 90% depending on the cholesterol concentration (Afrin et al., 2021). *Bifidobacterium animalis* subsp. In a study investigating in vitro Cholesterol Assimilation by lactis (BPL1), it was reported that up to 44.4% cholesterol assimilation was observed under intestinal and anaerobic conditions (Fernández-Calderón et al., 2022). Several techniques were used to test the capacity of the *Lactobacillus paracasei* M3, *L. casei* M5, and *L. paracasei* M7 strains isolated from breast milk to decrease cholesterol. The M6 isolate demonstrated a greater assimilation rate of 82.15%, whereas the *L. casei* M5 strain displayed highest bile salt hydrolase (BSH) activity and showed the capability to assimilate cholesterol at a rate of 76.51% (Bhat and Bajaj 2020).

In the study investigating the probiotic properties of various LAB species isolated from turnip juice and pickle juice, *L. plantarum* T4 strain observed cholesterol assimilation of 49.6% while *L. platarum* T8 and *L. brevis* S6 strains were found cholesterol assimilation 42% and 36.3%, respectively (Erdem et al., 2021). *Lactobacillus* spp. isolated from the vaginal flora of healthy women. Cholesterol assimilation rate of *L. gasseri* strain was found to be quite high compared to other strains with a rate of 63% (Kiray et al., 2020).

Possible cholesterol assimilation mechanisms of probiotics

Although various mechanisms have been proposed for the inducing resistance of *Lactobacillus* strains to diet-induced cholesterol increase and increased plasma cholesterol and triglyceride levels, how lactobacilli reduce serum cholesterol levels has not been fully determined. The absorption of cholesterol by *Lactobacillus acidophilus* is one of the suggested processes. Some lactobacilli, including bile acid, *Bifidobacterium longum*, *Clostridium perfringens*, and *Bacteroides fragilis* ssp., generate bile salt hydrolase, which makes it easier for bile acid to be deconjugated (Liong and Shah 2005; Pereira and Gibson, 2002). Short-chain fatty acid production by probiotics upon fermentation may be another mechanism contributing to cholesterol reduction. Short-chain fatty acid can inhibit hepatic cholesterol synthesis.

Some reports suggest that a single probiotic or a mixture of probiotics may regulate cholesterol synthesis in the liver (Park et al., 2008; Ooi and Liong, 2010). This inhibition in cholesterol synthesis was caused by a decrease in the activity of hydroxymethyl glutarate (HMG) CoA reductase, the rate-limiting enzyme of the mevalonate pathway. In other reports, some strains of LAB lowered circulating cholesterol through the induction of hepatic LDLR expression, which removes LDL-C from the circulation, leading to cholesterol catabolism in the liver (Park et al., 2008b). Reducing total plasma and LDL-C are major strategies for reducing cardiovascular disease risk (Liong and Shah, 2005).

Conclusion

In recent years, studies on various LAB species with probiotic character isolated from different origins have shown that the cholesterol assimilation abilities of these microorganisms are quite high. This ability has also been proven in many species of LAB with the genus *Lactobacillus*, *Enterococcus*, *Pediococcus* and *Bifidobacterium*. These data were also supported by in vitro studies. Since each probiotic strain has its own characteristics, strains with strong probiotic properties should be investigated in this area and human experiments should be done. It is hoped that probiotic strains with strong cholesterol assimilation properties can be effective with various foods or by taking them directly orally, and can replace cholesterol drugs with high side effects.

Reference

1. Ahn YT., Kim GB., Lim KS., Baek YJ., Kim HU. (2003). Deconjugation of bile salts by *Lactobacillus acidophilus* isolates. Int Dairy J. https://doi.org/10.1128/aem.33.1.15-18.1977.
2. Afrin S., Akter S., Begum S., Hossain MN. (2021). The Prospects of *Lactobacillus oris* as a Potential Probiotic With Cholesterol-Reducing Property From Mother's Milk. Front Nutr: 8: 619506. doi: 10.3389/fnut.2021.619506.
3. Belviso S., Giordano M., Zeppa PDG. (2009). In vitro cholesterol-lowering activity of *Lactobacillus plantarum* and *Lactobacillus paracasei* strains isolated from the italian castelmagno pdo cheese. Dairy Sci Technol. https://doi.org/10.1051/dst/2009004.
4. Bhat B., Bajaj BK. (2020). Multifarious cholesterol lowering potential of lactic acid bacteria equipped with desired probiotic functional attributes. Biotech. 10(5):200. doi: 10.1007/s13205-020-02183-8.
5. De Vrese M., Schrezenmeir J. (2008). Probiotics, prebiotics, and synbiotics advances. Adv Biochem Eng Biotechnol. https://doi.org/10.1007/10_2008_097.
6. Erdem B., Kıray E., Kariptaş E., Tulumoğlu Ş., Akıllı A. (2021). Characterızatıon of probıotıc abılıtıes of lactıc acıd bacterıa from tradıtıonal pıckle juıce and shalgam. Gece kitaplığı Research & Reviews in Science and Mathematics, 31-50.
7. Fernández-Calderón MC., Sánchez-Moro MDH. (2022). In vitro Cholesterol Assimilation by *Bifidobacterium animalis* subsp. *lactis* (BPL1) Probiotic Bacteria Under Intestinal Conditions. Endocr Metab Immune Disord Drug Targets. 22(4):433-439. doi: 10.2174/1871530321666210908124848.
8. Fuller R. Probiotics: the scientific basis Chapman and Hall, London, UK (1992).
9. Gibson GR. (1995). Roberfroid M.B. Dietary modulation of the human colonic microbiota: introducing the concept of probiotics J. Nutr., 125, 1401-1449.
10. Kiray E., Kariptas E., Dagli SS. (2020). Characterisation Of Probiotic Properties Of Lactic Acid Bacteria Isolated From Vaginal Microflora Of Healthy Turkish Women. Fresenius Environmental Bulletin. 29(6).
11. Kumar M., Nagpal R., Kumar R., Hemalatha R et al. (2012). Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp Diabetes Res. https://doi.org/10.1155/2012/902917.
12. Liong MT., Shah NP. (2005). Acid and bile tolerance and cholesterol removal ability of lactobacilli strains J. Dairy Sci, 88 55-66

13. Liu Y., ZhaoF., Liu J., Wang H., Han X., Zhang Y., Yang Z. (2017). Selection of Cholesterol-Lowering Lactic Acid Bacteria and its Effects on Rats Fed with High-Cholesterol DietCurrent Microbiology 74, 623–631.
14. Manson JE., Tosteson H., Ridker PM., Satterfield S., Hebert P., O'Connor GT., Buring JE., Hennekens CH. (1992). The primary prevention of myocardial infarction N. Engl. J. Med., 326, 1406-1416.
15. Mishra AK., Kumar SS., Ghosh AR. (2019). Probiotic *Enterococcus faecalis* AG5 effectively assimilates cholesterol and produces fatty acids including propionate. FEMS Microbiol Lett. 1;366(4).
16. Ooi L.G., Liong M.T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo and in vitro findings Int. J. Mol. Sci., 11 2499-2522.
17. Park YH., Kim JG., Shin YW., Kim HS., Kim YJ., Chun T., Kim SH., Whang KY. (2008). Effects of *Lactobacillus acidophilus* 43121 and a mixture of *Lactobacillus casei* and *Bifidobacterium longum* on the serum cholesterol level and fecal sterol excretion in hypercholesterolemia-induced pigs Biosci. Biotechnol. Biochem., 72, 595-600.
18. Park MY, Kim J, Kim S, Whang KY (2018). *Lactobacillus curvatus* KFP419 and *Leuconostoc mesenteroides* subsp. mesenteroides KDK411 isolated from kimchi ameliorate hypercholesterolemia in rats. J Med Food 21:647–653.
19. Pereira DIA., Gibson GR. (2002). Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut Appl. Environ. Microbiol., 68, 4689-4693.
20. Saikia D, Manhar AK, Deka B, Roy R, Gupta K, Namsa ND, Chattopadhyay P, Doley R, Mandal M (2018). Hypocholesterolemic activity of indigenous probiotic isolate Saccharomyces cerevisiae ARDMC1 in a rat model. J Food Drug Anal 26:154–162.
21. Song M, Park S, Lee H, Min B, Jung S, Park S, Kim E, Oh S. (2015). Effect of *Lactobacillus acidophilus* NS1 on plasma cholesterol levels in diet-induced obese mice. J Dairy Sci, 98(3):1492-501. doi: 10.3168/jds.2014-8586.
22. Tulumoğlu Ş., Kariptaş E., Erdem B. (2022). *Lactobacillus* spp. isolated from prebiotic-derived raw goat milk: probiotic characteristics, cholesterol assimilation and folate production. Biotechnology Letters.
23. Wang L, Guo MJ, Gao Q, Yang JF, Yang L, Pang XL, Jiang XJ (2018). The effects of probiotics on total cholesterol: a meta-analysis of randomized controlled trials. Medicine. https://doi.org/10.1097/MD.0000000000009679.
24. World Health Organization (WHO) (2002) Joint working group report on drafting guidelines for the evaluation of probiotics in food. World Health Organization, Geneva, Switzerland.

25. Wongrattanapipat S., Chiracharoenchitta A., Choowongwitthaya B., Komsathorn P., La-Ongkham O., NitisinprasertS., Tunsagool P., Nakphaichit M. (2022). Selection of potential probiotics with cholesterol-lowering properties for probiotic yoghurt production. Food Sci Technol Int., 28(4):353-365.

Delivery Strategies Used in the Generation of Induced Pluripotent Stem Cells

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Introduction

Stem cells have the potential to renew themselves and differentiate into other cells. The embryonic stem cells, which have the highest differentiate potency can differentiate into 220 different tissue cells. However, there are ethical and clinical restrictions on the use of these valuable cells. Because isolation of these cells results in the death of the embryo. On the other hand, the immune rejection problem is encountered in the use of these cells in patients. Therefore, scientists have begun to investigate the generation of cells with embryonic stem cell characteristics. At the line of these studies, Yamanaka et al. in 2006 succeeded in generating cells like mouse embryonic stem cells by reprogramming from mouse skin fibroblasts. One year later, the same group obtained Induced Pluripotent Stem Cells (iPSCs) from human fibroblasts by using this method. iPSCs generation are based on the principle of reprogramming somatic cells into pluripotent cells with different techniques.

iPSCs have the potential to self-renew and differentiate into other cells like embryonic stem cells. Recently, iPSCs generated from somatic cells have been used in regenerative medicine. However, the low efficiency of reprogramming and the use of viruses, tumorigenic factors, mutation, unstable proliferation, etc., in generation iPSCs are affected to use these cells in clinical applications. Recently many studies designed for the development of safe and new techniques to generate iPSCs. The aim of this chapter is to evaluate the generation of cells with the potential for clinical replication by newly developed reprogramming methods. The purpose of this chapter is to evaluate and comparing developed and under development reprogramming methods to use in the clinical application.

History

Ethel Browne Harvey, who conducted research on cell division on the embryo of sea urchins in 1909, was the first to discover that cell transplantation could create a secondary axis of polarity in the host. Harvey's work on embryonic cell division formed the basis of Spemann's discovery (Lenhoff, 1991)

In 1928, Hans Spemann and Hilde Mangold conducted various experiments on salamander embryos to discover the factors responsible to embryonic differentiation. They demonstrated that the cells have different induction that determined the fate of cells that differentiate at the embryonic stage. (De Robertis & M, 2006)

Inducing factors responsible for embryonic differentiation and cell fate are called Spemann promoters. Many studies are designed with the Spemann promoters leading to find the factors that are responsible to determine the cells fate. He designed experiments to understand whether cells undergoing

differentiation could be brought back to the embryonic state. Spemann was awarded the Nobel Prize in 1935 for his work that describing the organizing centers of embryonic cells.

Until 1952, it was thought that when cells differentiate, they lose parts of their genome. In 1952 Briggs and King, it has been shown that the nucleus of the adult frog cell can form a dividing blastula by transferring it to an enucleated egg. Later, the same technique was used in the cloning of sheep (Dolly). These experiments showed that no genomic loss occurred during the embryonic stages (Briggs & King, 1952).

John Gurdon was the first person to simply describe the cell reprogramming technique in 1962. John Gurdon produced normal tadpoles by transferring the nuclei of fully differentiated adult intestinal epithelial cells to eggs: he proved that the nucleus contains the genetic information necessary to enable differentiation to all cells in the organism. It has been shown that when cells differentiate, their genetic potential does not decrease (Gurdon, 1962).

The cloning of "Dolly" in 1996 marked a milestone in the history of cell reprogramming research. The first successful mammalian cloning experiment by transplanting the nuclei of adult sheep mammary cells into unfertilized sheep oocyte was performed by Ian Wilmut and Keith Campbell et al. (K. H. Campbell, McWhir, Ritchie, & Wilmut, 1996)(K. H. S. Campbell & Wilmut, 1997)

In 2006 and 2007 Yamanaka et al. identified genes required to reprogram somatic cells into induced pluripotent stem cells (iPSCs). iPSC was obtained by transferring transcription factors (Oct4, Sox2, Klf4 and c-Myc) to mouse and human fibroblasts via retroviruses. Yamanaka received the Nobel Prize in 2012 for iPSC technology(Takahashi et al., 2007; Takahashi & Yamanaka, 2006).

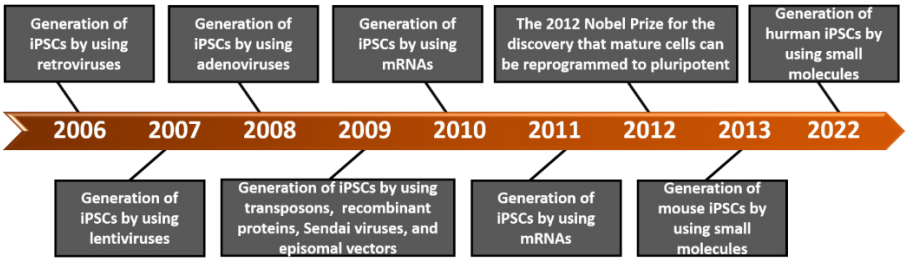

Figure 1: Historic of reprograming techniques

Different techniques are being developed in the field of cell reprogramming. In 2007, Takahashi et al. succeeded in producing iPSCs cells by using lentivirus (Takahashi et al., 2007). In 2008, Stadtfeld et al. had used adenoviruses in iPSC technology (Stadtfeld, Nagaya, Utikal, Weir, & Hochedlinger, 2008). In 2009,

Zhou et al. using recombinant proteins, Yu et al. using episomal vectors, Woltjen et al. using transposons, and Fusaki et al. using Sendai viruses to succeed to cell reprogramming (H. Zhou et al., 2009) (Yu et al., 2009)(Woltjen et al., 2009)(Fusaki, Ban, Nishiyama, Saeki, & Hasegawa, 2009). In 2010, Warren et al. performed the first somatic cell reprogramming based on mRNAs (Warren et al., 2010). In 2011, two separate research groups, Anokye-Danso et al. and Miyoshi et al. performed reprogramming using only miRNA (Anokye-Danso et al., 2011)(Miyoshi et al., 2011). Although iPSCs was obtained from mice by using only small molecules by Hou et al. in 2013 (Hou et al., 2013). But the generation of human iPSCs using the same method was carried out by Guan et al. in 2022 (Guan et al., 2022).

DELIVERY STRATEGIES TO CELL REPROGRAMMING

1. Integrative Methods

One of the first and most widely used methods in reprogramming researches is the integrative method. In this method, the transferred genetic material is integrated into the genome of the cell and enables to be reprogrammed. In this techniques, genetic materials are transferred to cells by viral and non-viral methods to reprogramming:

1.1. Viral techniques

1.1.1. Using Retrovirus

Retroviruses were used in reprogramming studies by Yamanaka et al. (Takahashi & Yamanaka, 2006). In this method, transcription factors called Yamanaka factors (OCT4, KLF4, SOX2, c-MYC) are transferred to cells through retroviruses. Because of these factors are integrated into the cell genome, this method is called integrative methods. The genetic material of retroviruses is RNA. When this material is transferred to the cell, it is translated into DNA and integrated into the cell genome (Nisole & Saïb, 2004). When these transcription factors are expressed, they activate the same endogenous genes and pluripotency pathways to reprogram cell (O'Malley, Woltjen, & Kaji, 2009). However, since the reprogramming efficiency of retroviruses is low, different methods have been developed.

1.1.2. Using Lentivirus

The genetic material of lentiviruses, like retroviruses, is RNA. Takahashi et al. first time used these viruses in reprogramming in 2007 (Takahashi et al., 2007). Reprogramming with lentiviruses is one of the methods with high efficiency (Al Abbar, Ngai, Nograles, Alhaji, & Abdullah, 2020). However, the

genes transferred by this method continue to have some disadvantages such as not silencing and creating mutations in the use of the same retrovirus. To avoid these disadvantages, excisable Cre/LoxP and Doxcycline-inducible lentiviruses have been developed (Bailly, Milhavet, & Lemaitre, 2022). In this method, we can silence transferred genes however, the efficiency was low and other disadvantages is remaining loxP in the genome. LoxP remaining may lead to mutagenesis in generated iPSCs (Zhang, De Los Angeles, & Zhang, 2010).

1.2. Non-viral techniques

1.2.1. Using linear DNA and transposons

To generate iPSCs without using viruses, linear DNAs can be transferred to cells via electroporation or liposomes. The transferred DNA is integrated into the genome and reprogramming is happen. However, the efficiency of this method is very low (Bailly et al., 2022).

Transposons or jumping genes have the capacity to move from one region to another in the genome (Ravindran, 2012). It is known that Yamanaka factors can be transfer to cells via the transposon and reprogramming is starting. Nowadays, iPSCs has been obtained by using the piggyBac and Sleep Beauty. (Woltjen et al., 2009)(Davis et al., 2013). On the other hand, the use of transposons in cell reprogramming has some disadvantages. The most important problem of this method is the use of transposase enzyme.

When this enzyme is used in this method, endogen transposons in reprogramming cells genome become active and cause unexpectable mutations (Al Abbar et al., 2020).

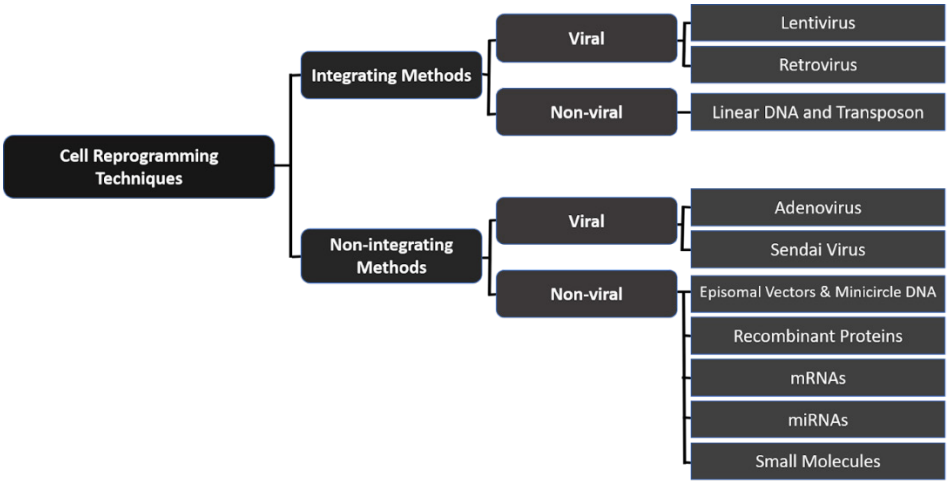

Figure 2: Delivery strategies of reprograming

2. Non-Integrative Methods

In these methods, Yamanaka factors are transferred without integrating into to reprogramming cells genome:

2.1. Viral techniques

2.1.1. Using Adenovirus

Adenoviruses for reprogramming were used by Stadtfeld et al. in 2008 for the first time (Stadtfeld et al., 2008). Adenoviruses DNA can replicate without integrating into the reprogramming cell genome (W. Zhou & Freed, 2009). Therefore, it is one of the important non-integrative methods of cell reprogramming. However, the low transduction capacity and rapid elimination from reprogramming cells is important limitations of this method (Bailly et al., 2022).

2.1.2. Using Sendai Virus

Sendai virus, like adenovirus, has the capacity to reproduce without integrating into the genome of reprogramming cell. This virus is widely used for reprogramming. The virus used in this method has been modified according to the virus used by Fusaki et al.. This virus has been sensitized to temperature, so that virus particles can be eliminated from the cell with an increase in temperature (Fusaki et al., 2009) (Ban et al., 2011). This method is among the non-integrative methods. Although it is accepted as the standard reprogramming method, any unexpectable mutation that may occur in the virus genetic material can cause to become pathogen or not completely eliminated from host cells. In this case, the clinical use of iPSCs obtained by this method is limited.

Reliable and efficient methods are required to use İPSCs in the clinic application. Although the Adenovirus and Sendai virus methods are non-integrative reprogramming methods, the lack of complete elimination from cells is one of the weak points of these methods. For this reason, scientists aim to develop non-viral and non-integrative methods.

2.2. Non-Viral techniques

2.2.1. Using episomal Vectors and minicircle DNA

Cell reprogramming is carried out with episomal vectors without using viruses. Plasmid or minicircle DNAs transferred to the cell divide autonomously within the cell and ensure the expression of reprogramming factors (Yu et al., 2009).

In episomal vectors and minicircle DNA-based reprogramming methods, permanent exogenic genetic material is transferred to the cell. Therefore, its use seems unsafe in the clinic application (Bailly et al., 2022).

2.2.2. Using Recombinant Proteins

Zhou et al. generate iPSCs by transferring the proteins of Yamanaka factors into the cell in 2009 (H. Zhou et al., 2009). In order to facilitate the transfection of Yamanaka factors proteins into the cell, these proteins carried a special amino acid sequence (Hitsuda et al., 2012). iPSCs were obtained through recombinant proteins was used in clinical studies of AMD (Age-related macular degeneration) disease (Cyranoski, 2013). However, the low efficiency of cell reprogramming with this method makes a limitation to widespread use of this method.

2.2.3. Using mRNAs

mRNA transfer to cell is easier than gene and protein transfer to reprogramming cell. iPSCs generation with use mRNA, was first performed by Warren et al. (Warren et al., 2010). mRNAs of Yamanaka factors are transferring into the cell through the pores with different method such as microinjection, electroporation, or chemicals compounds (Lipofectamine). After the transferred mRNA is translated into protein in the cell, the cell reprogramming is initiated. The problem encountered in this method is that exogenously transferred mRNAs activate inflammation pathways in the cell. As a result of this activation, it causes cell-cycle arrest and leading to apoptosis. As a solution to this problem, scientists reprogram cells by silencing the p53 gene or suppressing the immune response (Watanabe et al., 2019) (Poleganov et al., 2015) In recent studies, miRNAs have been used in addition to mRNAs.

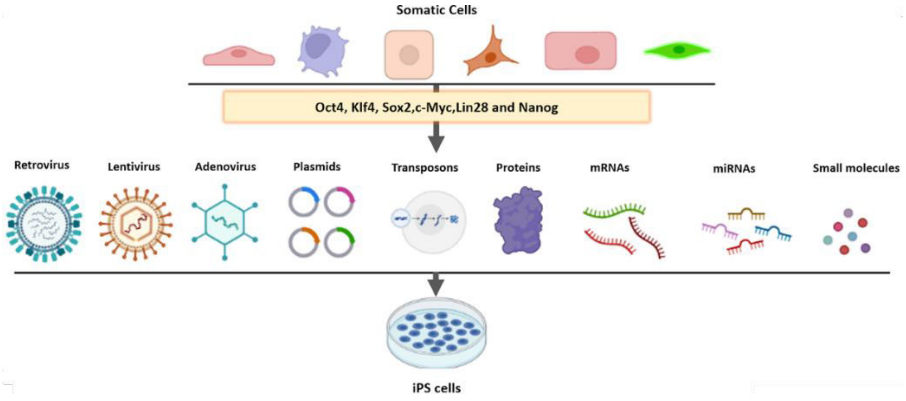

Figure 3: Methods used in iPSCs generation

2.2.4. Using miRNAs

miRNAs are small RNAs that regulate mRNAs. miRNAs, which affect mRNAs quickly and effectively, play an important role in cell functions. In a study conducted by Houbaviy et al. in 2003, it was shown that embryonic stem cells specifically express some miRNAs (Houbaviy, Murray, & Sharp, 2003). According to this study, it has been shown that the reprogramming efficiency is higher when selected miRNAs used beside Yamanaka factors (Judson, Babiarz, Venere, & Blelloch, 2009). Using miRNA for cell reprogramming was performed by two separate research groups (Anokye-Danso et al., 2011)(Miyoshi et al., 2011). While the efficiency of iPSCs formation was about 1% in other methods, the efficiency reached 10% when miRNAs were transferred into the cell with retroviruses. This study was performed by Anokye-Danso et al. (Anokye-Danso et al., 2011). Miyoshi et al. generate iPSCs by transferring miRNAs to the cell via Lipofectamine without using viruses. Although the efficiency is lower, the iPSCs obtained are completely virus-free, DNA-free and integration-free. This method has a high potential for clinical application. With the technical optimizations of this method and the testing of different miRNAs combinations, it will be possible to obtain iPSCs with high efficiency and completely suitable for clinical application.

2.2.5. Using Small Molecules

Small molecules are smaller than 500 Da that can easily pass through the cell membrane (Sidal, Colakoglu Erkan, Uslu, & Kocabas, 2020). These molecules have function on different cell mechanisms. These small molecules influence on epigenetic actors, cell cycle and metabolism enabling cell reprogramming. Small molecules have been shown to increase reprogramming efficiency when used with Yamanaka factors (Lin & Wu, 2015). The first study was carried out by Huangfu et al. in 2008 in this field. It has been shown that valproic acid, a histone deacetylase inhibitor, increases the reprogramming efficiency 100 times more than common reprogramming methods (Huangfu et al., 2008). In subsequent studies, it has been shown that some small molecules can replace the Yamanaka factors. For example, oxysterol induce cell reprogramming without the need for Sox2, Klf4 and c-Myc factors (Moon et al., 2011). Hou et al., obtained iPSCs from mouse somatic cells by using only small molecules in 2013 (Hou et al., 2013). Recently scientists were tried to be obtained iPSCs from human somatic cells by using small molecules. Guan et al. obtained iPSCs from fetal and parental human cells by using only small molecules. This find is very important in the field of regenerative medicine (Guan et al., 2022).

Conclusion

Since the studies initiated by Yamanaka et al. in 2006, iPSCs cells have been obtained by using different methods and techniques for reprogramming. İPSCs technology was started with gene integration and viral transduction methods and developed with non-integrated and non-viral methods during recent years. Beside of this improvement methods, the potential for clinical use of iPSCs cells has increased. iPSCs cells with high efficiency, footprint-free, DNA-free and safe for clinical application obtain by using miRNA and small molecules is the last point reached in this field. The iPSCs cells produced with the latest technology have high potential for experiments in in vitro conditions as well as for use in the field of regenerative medicine.

References

1. Al Abbar, A., Ngai, S. C., Nograles, N., Alhaji, S. Y., & Abdullah, S. (2020). Induced Pluripotent Stem Cells: Reprogramming Platforms and Applications in Cell Replacement Therapy. *BioResearch Open Access*, *9*(1), 121–136. https://doi.org/10.1089/biores.2019.0046
2. Anokye-Danso, F., Trivedi, C. M., Juhr, D., Gupta, M., Cui, Z., Tian, Y., … Morrisey, E. E. (2011). Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell Stem Cell*, *8*(4), 376–388. https://doi.org/10.1016/j.stem.2011.03.001
3. Bailly, A., Milhavet, O., & Lemaitre, J.-M. (2022). RNA-Based Strategies for Cell Reprogramming toward Pluripotency. *Pharmaceutics*, *14*(2), 317. https://doi.org/10.3390/pharmaceutics14020317
4. Ban, H., Nishishita, N., Fusaki, N., Tabata, T., Saeki, K., Shikamura, M., … Nishikawa, S.-I. (2011). Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. *Proceedings of the National Academy of Sciences*, *108*(34), 14234–14239. https://doi.org/10.1073/pnas.1103509108
5. Briggs, R., & King, T. J. (1952). Transplantation of living nuclei from blastula cells into enucleated frogs' eggs *. *Proceedings of the National Academy of Sciences*, *38*(5), 455–463. https://doi.org/10.1073/pnas.38.5.455
6. Campbell, K. H., McWhir, J., Ritchie, W. A., & Wilmut, I. (1996). Sheep cloned by nuclear transfer from a cultured cell line. *Nature*, *380*(6569), 64–66. https://doi.org/10.1038/380064a0
7. Campbell, K. H. S., & Wilmut, Ian. (1997). Totipotency or multipotentiality of cultured cells: Applications and progress. *Theriogenology*, *47*(1), 63–72. https://doi.org/10.1016/S0093-691X(96)00340-8
8. Cyranoski, D. (2013). Stem cells cruise to clinic. *Nature*, *494*(7438), 413–413. https://doi.org/10.1038/494413a
9. Davis, R. P., Nemes, C., Varga, E., Freund, C., Kosmidis, G., Gkatzis, K., … Mummery, C. L. (2013). Generation of induced pluripotent stem cells from human foetal fibroblasts using the Sleeping Beauty transposon gene delivery system. *Differentiation; Research in Biological Diversity*, *86*(1–2), 30–37. https://doi.org/10.1016/j.diff.2013.06.002
10. De Robertis, & M, E. (2006). Spemann's organizer and self-regulation in amphibian embryos. *Nature Reviews Molecular Cell Biology*, *7*(4), 296–302. https://doi.org/10.1038/nrm1855

11. Fusaki, N., Ban, H., Nishiyama, A., Saeki, K., & Hasegawa, M. (2009). Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences*, *85*(8), 348–362. https://doi.org/10.2183/pjab.85.348
12. Guan, J., Wang, G., Wang, J., Zhang, Z., Fu, Y., Cheng, L., … Deng, H. (2022). Chemical reprogramming of human somatic cells to pluripotent stem cells. *Nature*. https://doi.org/10.1038/s41586-022-04593-5
13. Gurdon, J. B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *Journal of Embryology and Experimental Morphology*, *10*, 622–640.
14. Hitsuda, T., Michiue, H., Kitamatsu, M., Fujimura, A., Wang, F., Yamamoto, T., … Matsui, H. (2012). A protein transduction method using oligo-arginine (3R) for the delivery of transcription factors into cell nuclei. *Biomaterials*, *33*(18), 4665–4672. https://doi.org/10.1016/j.biomaterials.2012.02.049
15. Hou, P., Li, Y., Zhang, X., Liu, C., Guan, J., Li, H., … Deng, H. (2013). Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science (New York, N.Y.)*, *341*(6146), 651–654. https://doi.org/10.1126/science.1239278
16. Houbaviy, H. B., Murray, M. F., & Sharp, P. A. (2003). Embryonic stem cell-specific MicroRNAs. *Developmental Cell*, *5*(2), 351–358. https://doi.org/10.1016/s1534-5807(03)00227-2
17. Huangfu, D., Maehr, R., Guo, W., Eijkelenboom, A., Snitow, M., Chen, A. E., & Melton, D. A. (2008). Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nature Biotechnology*, *26*(7), 795–797. https://doi.org/10.1038/nbt1418
18. Judson, R. L., Babiarz, J. E., Venere, M., & Blelloch, R. (2009). Embryonic stem cell-specific microRNAs promote induced pluripotency. *Nature Biotechnology*, *27*(5), 459–461. https://doi.org/10.1038/nbt.1535
19. Lenhoff, H. M. (1991). Ethel Browne, Hans Spemann, and the Discovery of the Organizer Phenomenon. *The Biological Bulletin*, *181*(1), 72–80. https://doi.org/10.2307/1542490
20. Lin, T., & Wu, S. (2015). Reprogramming with Small Molecules instead of Exogenous Transcription Factors. *Stem Cells International*, *2015*, 794632. https://doi.org/10.1155/2015/794632
21. Miyoshi, N., Ishii, H., Nagano, H., Haraguchi, N., Dewi, D. L., Kano, Y., … Mori, M. (2011). Reprogramming of mouse and human cells to

pluripotency using mature microRNAs. *Cell Stem Cell*, *8*(6), 633–638. https://doi.org/10.1016/j.stem.2011.05.001

22. Moon, J.-H., Heo, J. S., Kim, J. S., Jun, E. K., Lee, J. H., Kim, A., … You, S. (2011). Reprogramming fibroblasts into induced pluripotent stem cells with Bmi1. *Cell Research*, *21*(9), 1305–1315. https://doi.org/10.1038/cr.2011.107
23. Nisole, S., & Saïb, A. (2004). Early steps of retrovirus replicative cycle. *Retrovirology*, *1*, 9. https://doi.org/10.1186/1742-4690-1-9
24. O'Malley, J., Woltjen, K., & Kaji, K. (2009). New strategies to generate induced pluripotent stem cells. *Current Opinion in Biotechnology*, *20*(5), 516–521. https://doi.org/10.1016/j.copbio.2009.09.005
25. Poleganov, M. A., Eminli, S., Beissert, T., Herz, S., Moon, J.-I., Goldmann, J., … Sahin, U. (2015). Efficient Reprogramming of Human Fibroblasts and Blood-Derived Endothelial Progenitor Cells Using Nonmodified RNA for Reprogramming and Immune Evasion. *Human Gene Therapy*, *26*(11), 751–766. https://doi.org/10.1089/hum.2015.045
26. Ravindran, S. (2012). Barbara McClintock and the discovery of jumping genes. *Proceedings of the National Academy of Sciences*, *109*(50), 20198–20199. https://doi.org/10.1073/pnas.1219372109
27. Sidal, H., Colakoglu Erkan, P., Uslu, M., & Kocabas, F. (2020). Development of small-molecule-induced fibroblast expansion technologies. *Journal of Tissue Engineering and Regenerative Medicine*, *14*(10), 1476–1487. https://doi.org/10.1002/term.3112
28. Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G., & Hochedlinger, K. (2008). Induced pluripotent stem cells generated without viral integration. *Science (New York, N.Y.)*, *322*(5903), 945–949. https://doi.org/10.1126/science.1162494
29. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, *131*(5), 861–872. https://doi.org/10.1016/j.cell.2007.11.019
30. Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, *126*(4), 663–676. https://doi.org/10.1016/j.cell.2006.07.024
31. Warren, L., Manos, P. D., Ahfeldt, T., Loh, Y.-H., Li, H., Lau, F., … Rossi, D. J. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells using synthetic modified mRNA. *Cell Stem Cell*, *7*(5), 618–630. https://doi.org/10.1016/j.stem.2010.08.012

32. Watanabe, T., Yamazaki, S., Yoneda, N., Shinohara, H., Tomioka, I., Higuchi, Y., … Sasaki, E. (2019). Highly efficient induction of primate iPS cells by combining RNA transfection and chemical compounds. *Genes to Cells: Devoted to Molecular & Cellular Mechanisms*, *24*(7), 473–484. https://doi.org/10.1111/gtc.12702
33. Woltjen, K., Michael, I. P., Mohseni, P., Desai, R., Mileikovsky, M., Hämäläinen, R., … Nagy, A. (2009). PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature*, *458*(7239), 766–770. https://doi.org/10.1038/nature07863
34. Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, I. I., & Thomson, J. A. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. *Science (New York, N.Y.)*, *324*(5928), 797–801. https://doi.org/10.1126/science.1172482
35. Zhang, X., De Los Angeles, A., & Zhang, J. (2010). The Art of Human Induced Pluripotent Stem Cells: The Past, the Present and the Future. *The Open Stem Cell Journal*, *2*(1). Retrieved from https://benthamopen.com/ABSTRACT/TOSCJ-2-2
36. Zhou, H., Wu, S., Joo, J. Y., Zhu, S., Han, D. W., Lin, T., … Ding, S. (2009). Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell*, *4*(5), 381–384. https://doi.org/10.1016/j.stem.2009.04.005
37. Zhou, W., & Freed, C. R. (2009). Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. *Stem Cells (Dayton, Ohio)*, *27*(11), 2667–2674. https://doi.org/10.1002/stem.201

The Use of Molecular Biomarkers in Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a clonal malignant proliferation of immature myeloid progenitors caused by a lack of differentiation (El Achi & Kanagal-Shamanna, 2021). Recurrent cytogenetic abnormalities are known diagnostic and prognostic markers in AML, showing that acquired genetic abnormalities play an important role in leukemogenesis (Dohner et al., 2015) The biology of AML determines the prognosis for patients. At diagnosis, about 45% of patients have a normal karyotype as defined by conventional cytogenetics, but a somatic mutation can be found in 97.3% of patients (Patel et al., 2012) (Kayser & Levis, 2019). Six groups of AML are identified by the World Health Organization (WHO) in its 2016 updated criteria: (1) AML with recurring genetic abnormalities, (2) AML with changes caused by myelodysplasia, (3) myeloid neoplasms associated to therapy, (4) AML Not Otherwise Specified, (5) myeloid sarcoma, and (6) myeloid proliferations linked to Down syndrome. The diagnosis is determined if there are 20% or more blasts in the bone marrow or peripheral blood, or if there are specific genetic abnormalities (t(8;21), inv(16), or t(15;17)) present in the bone marrow independently of blast count. Three prognostic risk categories are also used to categorize AML: “favorable”, “intermediate”, and “poor” (Kayser & Levis, 2019) (Pelcovits & Niroula, 2020). These are based on cytogenetics as well as the relatively recent identification of subsets of molecular disorders that are independent of the influence of cytogenetic risk. These recently discovered molecular subgroups respond to conventional treatments differently (Pelcovits & Niroula, 2020).

In a significant retrospective analysis of patients under the age of 55, the prognostic groups were able to predict the survival and response to standard therapy. The overall survival (OS) rate at 5 years was 44%, but when the survival rates were broken down by risk profile, they were 64%, 41%, and 11% for favorable, intermediate, and adverse risk, respectively. Even if the addition of elderly adults diminishes total survival, the stratification of survival does not change (Pelcovits & Niroula, 2020).

The group with an intermediate prognosis was the most striking. This group is heterogeneous, and optimal treatment approaches for patients in this category are mostly unknown. Patients with a favorable prognosis are treated with normal chemotherapy, whereas those with a poor prognosis should get allogeneic hematopoietic stem cell transplantation. However, 40-50% of adult patients with AML have an intermediate prognosis, and the majority have normal karyotypes. FLT3, NPM1, and CEBPA mutations are the first discovered in patients with an intermediate prognosis and a cytogenetically normal karyotype (Yohe, 2015).

This section summarizes the most important molecular biomarkers linked with AML and discusses their clinical significance in relation to disease prognosis and diagnosis.

FLT3 (FMS-Like Tyrosine Kinase 3)

One of the most frequent genetic alterations in AML, activating FLT3 mutations are found in 30% of newly diagnosed patients (Kayser & Levis, 2019). An early hematopoietic progenitor's ability to differentiate and proliferate is regulated by the membrane-bound receptor FLT3, which also has tyrosine kinase activity, also known as STK1 (stem cell kinase I) or FLK2 (fetal liver kinase 2) (Prada-Arismendy et al., 2017), (Damiani & Tiribelli, 2019). FLT3 receptor consists of an immunoglobulin-like extracellular ligand-dependent domain, a transmembrane domain, a juxtamembrane domain (JM), and a highly conserved intracellular kinase domain (Prada-Arismendy et al., 2017).When the FLT ligand (FL) binds to the FLT3 receptor, the receptor undergoes homodimer formation. This receptor dimerization activates the tyrosine kinase domain and leads to phosphorylation of various regions of the intracellular domain and causes activation of some proteins. These events result in the activation of the cascade MAP kinase, STAT, AKT/PI3K signaling pathways ultimately regulates hematopoietic homeostasis. (Prada-Arismendy et al., 2017).

Two categories of FLT3 mutations are analyzed: 1. Internal tandem duplications in the receptor's juxtamembrane domain (FLT3-ITD mutations)

2. Point mutations in the tyrosine kinase domain of the FLT3 receptor (FLT3-TKD mutations)

1. FLT3-ITD;

FLT3-ITD are in-frame mutations that result in receptors with longer juxta-membrane domains by duplicating short sequences, ranging in size from 3 to more than 400 base pairs. Internal tandem duplication is the most significant mutation identified in the FLT3 gene (FLT3-ITD) (El Achi & Kanagal-Shamanna, 2021). The most prevalent mutation in AML is FLT3-ITD, which is also usually linked to the prognosis of the disease. This mutation indicates continuous FLT3 kinase activity (Prada-Arismendy et al., 2017). AML has a 20–50% prevalence of FLT3-ITD mutations (El Achi & Kanagal-Shamanna, 2021). The prevalence of FLT3-ITD is age-related; While it is extremely rare in children, young adults up to the age of 60 have the highest frequency and the elderly have the lowest incidence (Kayser & Levis, 2019). Proliferative AML with a high WBC (white blood cell) count is linked to FLT3-ITD. The allelic burden affects the prognostic significance of the mutation. One of the two parameters, allele

ratio (AR), which is calculated as the ratio of the area under the curve of the mutant to the wild type or allele frequency (AF), which is calculated as the ratio of the area under the curve of the mutant to the total (mutant + wild type), can be used to determine the allele burden of FLT3-ITD (El Achi & Kanagal-Shamanna, 2021), (Damiani & Tiribelli, 2019). For the 2017 ELN (European LeukemiaNet), a cut-off of 0.5 is used to distinguish between low and high AR. Only high FLT3-ITD AR patients (0.5) responded from allogeneic stem cell transplantation. (Boddu et al., 2019). Patients with high AR FLT3-ITD in AML exhibited lower complete remission (CR) rates, poor survival, and higher rates of relapse. Allogeneic stem cell transplantation was beneficial only in high FLT3-ITD AR patients (≥0.5). According to ELN, a stem cell transplant is not advised for NPM1 mutant AML with FLT3-ITD and low allelic burden because this subgroup is considered to have a favorable prognosis. Loss of heterozygosity could partially account for the difference in allelic ratio. (El Achi & Kanagal-Shamanna, 2021).

2. FLT3-TKD;

FLT3-TKD mutations are a less common type of mutation, although they are seen in approximately 7% of patients, they are common in AML patients in the cytogenetically favorable prognosis risk group. (Levis, 2013). Less frequently than FLT3-ITD, FLT3-TKD alterations have included missense point mutations, deletions, or insertions within the TK domain. A point mutation that involves a nucleotide substitution at codon 835 is the most common modification (El Achi & Kanagal-Shamanna, 2021). Although the presence of FLT3 mutations is a key marker in AML patients included in the poor prognosis risk group, the largest study has been on the identification of FLT3 therapeutic inhibitors (Prada-Arismendy et al., 2017). Tyrosine kinase inhibitor (TKI) treatments in combination with conventional chemotherapy can target FLT3 mutations. Three FLT3 subgroups: FLT3-TKD, FLT3-ITD low, and FLT3-ITD high AR saw significantly higher overall survival (OS) and event-free survival (EFS) after adding the TKI, **Midostaurin**, to the usual chemotherapy treatment for AML. A non-selective first generation TKI, Midostaurin was the first targeted therapy for AML to receive FDA approval. **Gilteritinib**, a "second-generation" FLT3 inhibitor with greater selectivity and fewer side effects, was approved by the FDA in 2018 for the treatment of AML with FLT3-ITD or FLT3-TKD mutation positive that has relapsed or become resistant (El Achi & Kanagal-Shamanna, 2021). Several other FLT3 first- and second-generation inhibitors, including **Sorafenib** (Levis, 2013) and **Quizartinib** (El Achi & Kanagal-Shamanna, 2021) (in newly diagnosed AML), are currently in the last stages of clinical testing. Through the activation of alternate intracellular pathways, resistance mutations

to FLT3 inhibitors can develop over the course of therapy. As a result, the appearance of a FLT3-TKD mutation during treatment, especially the FLT3 D835 mutation, would confer resistance to TKI (Perl et al., 2019). Some type II (second generation) FLT3 inhibitors do not have effectiveness against TKD mutations. **Crenolanib**, a second-generation inhibitor that has effect against ITD and TKD mutations, can overcome treatment resistance caused on by FLT3-TKD changes (Daver et al., 2019).

NPM1 (Nucleophosmin)

A molecular chaperone known as NPM1, which is necessary for ribosome synthesis and transport, apoptosis in response to stressors, maintaining genomic stability and DNA repair, is involved in the advancement of the cell cycle. The most frequent genetic change in AML, occurring in 25% to 41% of patients, is an NPM1 mutation (El Achi & Kanagal-Shamanna, 2021). NPM mutations are called "NPM1c mutations" and cause changes in the C-terminal region of the protein. This alters the nuclear localization of the protein, preventing correct folding (Prada-Arismendy et al., 2017), (Yohe, 2015). At positions 956–959, the most frequent mutation is the addition of 4 base pairs of TCTG. There are several NPM1 gene variations that all have the same biological outcome. They are mutually exclusive with other known recurring genetic disorders like RUNX1 and CEBPA and are typically linked with normal karyotype (El Achi & Kanagal-Shamanna, 2021). AML with NPM1 mutations is typically seen as having a favourable prognosis and responding well to induction treatment (Courtney D DiNardo, 2016). Recurrent mutations in other genes, particularly the FLT3 allele ratio, have an impact on the prognosis. According to the 2017 ELN, AML patients with NPM1 mutations and low or absent FLT3-ITD AR (0.5) have similar OS and are categorized as having favorable risk, whereas AML patients with mutated NPM1 and high FLT3 ITD AR are categorized as having intermediate risk besides the patients with wild-type NPM1 and absent or low FLT3 ITD AR. CR eliminates NPM1 mutations in AML. Patients over 65 years old with NPM1 mutant AML treated with the BCL2 inhibitor **Venetoclax** in combination with hypomethylating drugs saw a significant improvement in their overall survival (Lachowiez et al., 2020). When combined with **Azacitidine** or **Decitabine**, Venetoclax was given the FDA's approval to treat newly diagnosed AML in patients over the age of 75 or with significant comorbidities (El Achi & Kanagal-Shamanna, 2021).

CEBPA (CCAAT/Enhancer Binding Protein)

A transcription factor for granulocyte differentiation is encoded by the CEBPA gene, which is for CCAAT/enhancer binding protein alpha. Mutations in CEBPA inhibit DNA from binding, which prevents granulocyte differentiation. 10% of AML patients had CEBPA variants described, and half of them are biallelic mutations that often affect both the N- and C-terminal domains (Kayser & Levis, 2019). Independent of other biomarkers, AML with biallelic CEBPA mutations in a heterozygous or homozygous form is strongly related with better overall prognosis and outcomes (Li et al., 2015; Walker & Marcucci, 2012). Biallelic CEBPA mutations appear to be age-dependent and become less common as individuals get older (Kayser & Levis, 2019). NT5E, which encodes CD73, was found to be elevated in bi-allelic CEBPA mutant leukemia by Jakobsen et al (Jakobsen et al., 2019). In bi-allelic CEBPA mutant AML, the effectiveness of CD73 inhibitors, especially their combination with immune checkpoint inhibitors like PD-1/PD-L1, is being examined.

RUNX1 (Runt-related transcription factor)

The RUNX1 gene, which is located on chromosome 21q22.12, encodes for a transcription factor that interacts with numerous cofactors and enhancers and is therefore crucial for both hematopoiesis and embryogenesis (Damiani & Tiribelli, 2019). Up to 15% of AML have somatic mutations, while secondary AML resulting from MDS (Myelodysplastic syndrome) has a higher frequency of somatic mutations (Dicker et al., 2010). 2017 ELN recommendations indicate that mutant RUNX1 is a poor prognostic indicator. According to research done on 219 AML patients by You et al., patients with RUNX1 mutations showed shorter relapse-free survival than those with wild-type RUNX1 (You et al., 2017). Although there are currently no direct targeted therapeutics, pre-clinical investigations have shown that enhancer suppression with a bromodomain and extra-terminal motif (BET) inhibitor inhibits abnormal RUNX1 (Antony et al., 2020). By inhibiting RUNX1 via CRISPR/Cas9, the BET protein antagonist increased the rate of apoptosis in leukemic cells that express mutant RUNX1 compared to wild-type cells, improving mouse survival (Mill et al., 2019).

KIT

The 145 kDa stem cell factor receptor (c-kit, CD117), also known as "KIT proto-oncogene receptor tyrosine kinase," is encoded by a single copy gene with twenty-one exons on chromosome 4(4q12). All lineages undergo downregulation during maturation, and mature cells only detect c-kit in activated platelets, mast cells, and CD56+ natural killer cells (132-136). SCF binding triggers KIT

dimerization, phosphorylation, and activation of numerous downstream pathways, including PI3K, MEK, RAS, and RAF, involved in controlling the survival, proliferation, and migration of hematopoietic cells (Damiani & Tiribelli, 2019).

In "core binding factor" (CBF) leukemias, which include AML with t(8;21) and AML with inv(16), activating mutations of KIT (encoding a transmembrane glycoprotein) that cause constitutional activation of the receptor tyrosine kinase pathway are most frequently seen (Damiani & Tiribelli, 2019), (El Achi & Kanagal-Shamanna, 2021). Despite the better overall prognosis of CBF-AML, the majority of studies indicate that the co-occurrence of KIT mutations results in a poor prognosis for AML with both inv(16) and t (8;21), as well as a greater relapse rate for AML with inv (16) (Yohe, 2015) (El Achi & Kanagal-Shamanna, 2021). The National Comprehensive Cancer Network has categorized CBF-AML with KIT mutations into the intermediate-risk group as a result (Kuykendall et al., 2018). Although other investigators claimed that KIT mutations only had a poor prognostic impact when present at an allelic burden more than 25% or 35%, the findings are still unclear (Kuykendall et al., 2018), (Allen et al., 2013), (Duployez et al., 2016). Given the prognostic significance of gain-of-function KIT mutations and the overexpression of KIT found in the majority of CBF-AML, including those with KIT mutations studies have investigated the adding of KIT inhibitors, such as Dasatinib and Avapritinib to priority target therapy to enhance outcomes. The potential to lower relapse rates of KIT-mutated CBF-AML to levels equivalent to non-KIT-mutated CBF-AML is demonstrated by the results (Luck et al., 2010), (Borthakur & Kantarjian, 2021).

TP53 (Tumor protein P53)

A DNA-binding protein that functions as a tumor suppressor is encoded by the TP53 gene. It induces cell cycle arrest, apoptosis, and DNA repair in response to biological stress. TP53 mutations are more frequent in solid than hematologic malignancies and are present in 8–14% of all cases of AML. Patients with complex karyotypes had a much higher incidence, ranging from 69% to 73% (Damiani & Tiribelli, 2019). The mutations result in phenotypes such as loss-of-function, dominant negative, and gain-of-function (Oren & Rotter, 2010). An independent marker of poor survival, a high risk of recurrence, and treatment resistance is the presence of a TP53 mutation. The occurrence of a TP53 mutation is classified in the 2017 ELN classification as a poor risk category (Dohner et al., 2015). In phase I and phase II trials, the effects of Idasanutlin, an MDM2 inhibitor, and Cobimetinib, a MEK inhibitor, both of which influence TP53 expression, are being compared (El Achi & Kanagal-Shamanna, 2021).

DNA methyltransferase 3a (DNMT3A)

DNA methylation leads to addition of methyl group (5-methylcytosine) to cytosine bases in the context of cytosine-guanine sequences expressed as CpG islands. CpG islands are localized within or near promoter regions in DNA. The methylation of these islands is a crucial epigenetic mechanism in the regulation of gene expressions and inherited methylation patterns underlying genomic imprinting. In addition, abnormal DNA methylation contributes to the pathogenesis of cancers including myeloid tumors. Various factors regulate the DNA methylation process. Recurrent mutations have been found in some of these factors in myeloid tumors (Matynia et al., 2015).

The DNA methyltransferase family catalyzes the methylation of cytosine in CpG dinucleotides to generate 5-methylcitosine. The downstream genes are transcriptionally silenced as a result of the increased methylation of CpG islands. Adult AML patients with normal cytogenetics have a greater frequency of DNMT3A mutations (12–22%). Compared to the wild type analogue, DNMT3A mutations are associated with older age and a higher WBC count. They cause a missense mutation that, in the majority of cases, results in an arginine to histidine substitution at codon R882, resulting in the generation of a shortened protein with diminished methyltransferase function (Damiani & Tiribelli, 2019). It is generally recognized that DNMT3A mutations constitute an early occurrence in leukemic transformation, may be present in pre-leukemic stem cells, and can remain even after CR success, even if the exact mechanism by which they contribute to leukemogenesis is not well understood (Shlush et al., 2014), (Corces-Zimmerman et al., 2014). DNMT3A changes are the most frequently repeated changes in AML after NPM1 and FLT3 mutations (Prada-Arismendy et al., 2017).

Decitabine and guadecitabine are DNA methyltransferase inhibitors undergoing ongoing clinical trials to determine their effectiveness (183-185). AML patients carrying the DNMT3A mutation develop a response to the Decitabine inhibitor with a high percentage of clinical remission and high survival rates (Prada-Arismendy et al., 2017).

IDH1 and IDH2 (Isocitrate Dehydrogenase)

Metabolism-related isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) genes have epigenetic roles in histone and DNA methylation. (Yohe, 2015). These genes encode NADP-dependent isocitrate dehydrogenase found in the cytosol and mitochondria. They catalyze the decarboxylation of isocitrate to alpha-ketoglutarate in the citric acid cycle, and this alpha-ketoglutarate is used by TET proteins during histone demethylation (Prada-Arismendy et al., 2017). IDH

mutations cause epigenetic methylation, particularly the TET family of methylators, to be dysregulated. The pathways used to differentiate hematopoietic progenitors will eventually become suppressed as a result of these abnormalities, causing maturation arrest. Additionally, IDH mutations impair DNA repair and cause a buildup of secondary mutations. AML is linked to IDH1 and IDH2 mutations in 4-9% and 8-19% of patients, respectively. Codon 132 in exon 4 of the IDH1 gene and codons 140 or 172 in exon 4 of the IDH2 gene are the amino-acid substitution hotspots. IDH2-R172 is classified as a distinct sub-category by genomic study and is mutually exclusive with NPM1 mutation, but it is not yet recognized by the WHO. IDH1/2 mutations are a typical example of targeted therapy in AML, similar to FLT3 mutations (El Achi & Kanagal-Shamanna, 2021).

Enasidenib, an oral selective inhibitor of mutant R140Q, R172S, and R172K IDH2 enzyme variations, received FDA approval for the treatment of relapsed/refractory AML patients in 2017 (Stein et al., 2019). **Ivosidenib** targets mutant IDH1 enzyme, which results in normal malignant cell development and maturation. It was FDA-approved for the treatment of relapsed/refractory AML cases in 2019 (El Achi & Kanagal-Shamanna, 2021).

TET 2 (Ten-Eleven translocation 2)

A key player in the epigenetic regulation of cellular processes is the TET2 protein, which is encoded on chromosome 4q24. It converts 5-methyl- to 5-hydroxymethylcitosine with α-ketoglutarate as a cofactor. Most frequently in exons 3–12, TET2 mutations have been identified in 8–28% of AML patients and are linked to decreased catalytic performance or reduced DNA targeting. Pre-leukemic stem cells in humans have TET2 inactivation, which is linked to clonal growth (Jan et al., 2012). By specifically affecting enhancer areas that control tumor suppressor genes, the TET2 mutations-induced hypermethylation has been shown to facilitate leukemogenesis in experimental mice (Rasmussen et al., 2015). TET2 mutations' prognostic significance is still up for discussion. TET2 mutations were found to be associated with an adverse result in such studies (Gaidzik et al., 2012; Nibourel et al., 2010), but not in others, which either looked at the entire population or only certain subgroups (such as CN-AML, those under the age of 65, and people at favorable risk for ELN) (Metzeler et al., 2011; Patel et al., 2012; Weissmann et al., 2012). TET2 mutations were found in 395 AML patients, and Liu and colleagues conducted a meta-analysis to reach the conclusion that it could be a poor prognostic factor in patients with a normal karyotype (Liu et al., 2014).

References

1. Allen, C., Hills, R. K., Lamb, K., Evans, C., Tinsley, S., Sellar, R., O'Brien, M., Yin, J. L., Burnett, A. K., Linch, D. C., & Gale, R. E. (2013). The importance of relative mutant level for evaluating impact on outcome of KIT, FLT3 and CBL mutations in core-binding factor acute myeloid leukemia. *Leukemia*, *27*(9), 1891-1901. https://doi.org/10.1038/leu.2013.186
2. Antony, J., Gimenez, G., Taylor, T., Khatoon, U., Day, R., Morison, I. M., & Horsfield, J. A. (2020). BET inhibition prevents aberrant RUNX1 and ERG transcription in STAG2 mutant leukaemia cells. *J Mol Cell Biol*, *12*(5), 397-399. https://doi.org/10.1093/jmcb/mjz114
3. Boddu, P. C., Kadia, T. M., Garcia-Manero, G., Cortes, J., Alfayez, M., Borthakur, G., Konopleva, M., Jabbour, E. J., Daver, N. G., DiNardo, C. D., Naqvi, K., Yilmaz, M., Short, N. J., Pierce, S., Kantarjian, H. M., & Ravandi, F. (2019). Validation of the 2017 European LeukemiaNet classification for acute myeloid leukemia with NPM1 and FLT3-internal tandem duplication genotypes. *Cancer*, *125*(7), 1091-1100. https://doi.org/10.1002/cncr.31885
4. Borthakur, G., & Kantarjian, H. (2021). Core binding factor acute myelogenous leukemia-2021 treatment algorithm. *Blood Cancer J*, *11*(6), 114. https://doi.org/10.1038/s41408-021-00503-6
5. Corces-Zimmerman, M. R., Hong, W. J., Weissman, I. L., Medeiros, B. C., & Majeti, R. (2014). Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. *Proc Natl Acad Sci U S A*, *111*(7), 2548-2553. https://doi.org/10.1073/pnas.1324297111
6. Courtney D DiNardo, J. E. C. (2016). <Mutations in AML prognostic and therapeutic implications.pdf>. *Hematology Am Soc Hematol Educ Program*
7. Damiani, D., & Tiribelli, M. (2019). Molecular landscape in adult acute myeloid leukemia: where we are where we going? *Journal of Laboratory and Precision Medicine*, *4*, 17-17. https://doi.org/10.21037/jlpm.2018.09.08
8. Daver, N., Schlenk, R. F., Russell, N. H., & Levis, M. J. (2019). Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia*, *33*(2), 299-312. https://doi.org/10.1038/s41375-018-0357-9
9. Dicker, F., Haferlach, C., Sundermann, J., Wendland, N., Weiss, T., Kern, W., Haferlach, T., & Schnittger, S. (2010). Mutation analysis for RUNX1, MLL-PTD, FLT3-ITD, NPM1 and NRAS in 269 patients with MDS or

secondary AML. *Leukemia*, *24*(8), 1528-1532. https://doi.org/10.1038/leu.2010.124

10. Dohner, H., Weisdorf, D. J., & Bloomfield, C. D. (2015). Acute Myeloid Leukemia. *N Engl J Med*, *373*(12), 1136-1152. https://doi.org/10.1056/NEJMra1406184
11. Duployez, N., Marceau-Renaut, A., Boissel, N., Petit, A., Bucci, M., Geffroy, S., Lapillonne, H., Renneville, A., Ragu, C., Figeac, M., Celli-Lebras, K., Lacombe, C., Micol, J. B., Abdel-Wahab, O., Cornillet, P., Ifrah, N., Dombret, H., Leverger, G., Jourdan, E., & Preudhomme, C. (2016). Comprehensive mutational profiling of core binding factor acute myeloid leukemia. *Blood*, *127*(20), 2451-2459. https://doi.org/10.1182/blood-2015-12-688705
12. El Achi, H., & Kanagal-Shamanna, R. (2021). Biomarkers in Acute Myeloid Leukemia: Leveraging Next Generation Sequencing Data for Optimal Therapeutic Strategies. *Front Oncol*, *11*, 748250. https://doi.org/10.3389/fonc.2021.748250
13. Gaidzik, V. I., Paschka, P., Spath, D., Habdank, M., Kohne, C. H., Germing, U., von Lilienfeld-Toal, M., Held, G., Horst, H. A., Haase, D., Bentz, M., Gotze, K., Dohner, H., Schlenk, R. F., Bullinger, L., & Dohner, K. (2012). TET2 mutations in acute myeloid leukemia (AML): results from a comprehensive genetic and clinical analysis of the AML study group. *J Clin Oncol*, *30*(12), 1350-1357. https://doi.org/10.1200/JCO.2011.39.2886
14. Jakobsen, J. S., Laursen, L. G., Schuster, M. B., Pundhir, S., Schoof, E., Ge, Y., d'Altri, T., Vitting-Seerup, K., Rapin, N., Gentil, C., Jendholm, J., Theilgaard-Monch, K., Reckzeh, K., Bullinger, L., Dohner, K., Hokland, P., Fitzgibbon, J., & Porse, B. T. (2019). Mutant CEBPA directly drives the expression of the targetable tumor-promoting factor CD73 in AML. *Sci Adv*, *5*(7), eaaw4304. https://doi.org/10.1126/sciadv.aaw4304
15. Jan, M., Snyder, T. M., Corces-Zimmerman, M. R., Vyas, P., Weissman, I. L., Quake, S. R., & Majeti, R. (2012). Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Sci Transl Med*, *4*(149), 149ra118. https://doi.org/10.1126/scitranslmed.3004315
16. Kayser, S., & Levis, M. J. (2019). Clinical implications of molecular markers in acute myeloid leukemia. *Eur J Haematol*, *102*(1), 20-35. https://doi.org/10.1111/ejh.13172
17. Kuykendall, A., Duployez, N., Boissel, N., Lancet, J. E., & Welch, J. S. (2018). Acute Myeloid Leukemia: The Good, the Bad, and the Ugly. *Am*

Soc Clin Oncol Educ Book, *38*, 555-573. https://doi.org/10.1200/EDBK_199519

18. Lachowiez, C. A., Loghavi, S., Kadia, T. M., Daver, N., Borthakur, G., Pemmaraju, N., Naqvi, K., Alvarado, Y., Yilmaz, M., Short, N., Ohanian, M., Pierce, S. R., Patel, K. P., Qiao, W., Ning, J., Sasaki, K., Takahashi, K., Jabbour, E., Andreeff, M., . . . DiNardo, C. D. (2020). Outcomes of older patients with NPM1-mutated AML: current treatments and the promise of venetoclax-based regimens. *Blood Adv*, *4*(7), 1311-1320. https://doi.org/10.1182/bloodadvances.2019001267
19. Levis, M. (2013). FLT3 mutations in acute myeloid leukemia: what is the best approach in 2013? *Hematology Am Soc Hematol Educ Program*, *2013*, 220-226. https://doi.org/10.1182/asheducation-2013.1.220
20. Li, H. Y., Deng, D. H., Huang, Y., Ye, F. H., Huang, L. L., Xiao, Q., Zhang, B., Ye, B. B., Lai, Y. R., Mo, Z. N., & Liu, Z. F. (2015). Favorable prognosis of biallelic CEBPA gene mutations in acute myeloid leukemia patients: a meta-analysis. *Eur J Haematol*, *94*(5), 439-448. https://doi.org/10.1111/ejh.12450
21. Liu, W. J., Tan, X. H., Luo, X. P., Guo, B. P., Wei, Z. J., Ke, Q., He, S., & Cen, H. (2014). Prognostic significance of Tet methylcytosine dioxygenase 2 (TET2) gene mutations in adult patients with acute myeloid leukemia: a meta-analysis. *Leuk Lymphoma*, *55*(12), 2691-2698. https://doi.org/10.3109/10428194.2014.893308
22. Luck, S. C., Russ, A. C., Du, J., Gaidzik, V., Schlenk, R. F., Pollack, J. R., Dohner, K., Dohner, H., & Bullinger, L. (2010). KIT mutations confer a distinct gene expression signature in core binding factor leukaemia. *Br J Haematol*, *148*(6), 925-937. https://doi.org/10.1111/j.1365-2141.2009.08035.x
23. Matynia, A. P., Szankasi, P., Shen, W., & Kelley, T. W. (2015). Molecular genetic biomarkers in myeloid malignancies. *Arch Pathol Lab Med*, *139*(5), 594-601. https://doi.org/10.5858/arpa.2014-0096-RA
24. Metzeler, K. H., Maharry, K., Radmacher, M. D., Mrozek, K., Margeson, D., Becker, H., Curfman, J., Holland, K. B., Schwind, S., Whitman, S. P., Wu, Y. Z., Blum, W., Powell, B. L., Carter, T. H., Wetzler, M., Moore, J. O., Kolitz, J. E., Baer, M. R., Carroll, A. J., . . . Bloomfield, C. D. (2011). TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol*, *29*(10), 1373-1381. https://doi.org/10.1200/JCO.2010.32.7742

25. Mill, C. P., Fiskus, W., DiNardo, C. D., Qian, Y., Raina, K., Rajapakshe, K., Perera, D., Coarfa, C., Kadia, T. M., Khoury, J. D., Saenz, D. T., Saenz, D. N., Illendula, A., Takahashi, K., Kornblau, S. M., Green, M. R., Futreal, A. P., Bushweller, J. H., Crews, C. M., & Bhalla, K. N. (2019). RUNX1-targeted therapy for AML expressing somatic or germline mutation in RUNX1. *Blood*, *134*(1), 59-73. https://doi.org/10.1182/blood.2018893982
26. Nibourel, O., Kosmider, O., Cheok, M., Boissel, N., Renneville, A., Philippe, N., Dombret, H., Dreyfus, F., Quesnel, B., Geffroy, S., Quentin, S., Roche-Lestienne, C., Cayuela, J. M., Roumier, C., Fenaux, P., Vainchenker, W., Bernard, O. A., Soulier, J., Fontenay, M., & Preudhomme, C. (2010). Incidence and prognostic value of TET2 alterations in de novo acute myeloid leukemia achieving complete remission. *Blood*, *116*(7), 1132-1135. https://doi.org/10.1182/blood-2009-07-234484
27. Oren, M., & Rotter, V. (2010). Mutant p53 gain-of-function in cancer. *Cold Spring Harb Perspect Biol*, *2*(2), a001107. https://doi.org/10.1101/cshperspect.a001107
28. Patel, J. P., Gonen, M., Figueroa, M. E., Fernandez, H., Sun, Z., Racevskis, J., Van Vlierberghe, P., Dolgalev, I., Thomas, S., Aminova, O., Huberman, K., Cheng, J., Viale, A., Socci, N. D., Heguy, A., Cherry, A., Vance, G., Higgins, R. R., Ketterling, R. P., . . . Levine, R. L. (2012). Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*, *366*(12), 1079-1089. https://doi.org/10.1056/NEJMoa1112304
29. Pelcovits, A., & Niroula, R. (2020). Acute Myeloid Leukemia: A Review. *R I Med J (2013)*, *103*(3), 38-40. https://www.ncbi.nlm.nih.gov/pubmed/32236160
30. Perl, A. E., Martinelli, G., Cortes, J. E., Neubauer, A., Berman, E., Paolini, S., Montesinos, P., Baer, M. R., Larson, R. A., Ustun, C., Fabbiano, F., Erba, H. P., Di Stasi, A., Stuart, R., Olin, R., Kasner, M., Ciceri, F., Chou, W. C., Podoltsev, N., . . . Levis, M. J. (2019). Gilteritinib or Chemotherapy for Relapsed or Refractory FLT3-Mutated AML. *N Engl J Med*, *381*(18), 1728-1740. https://doi.org/10.1056/NEJMoa1902688
31. Prada-Arismendy, J., Arroyave, J. C., & Rothlisberger, S. (2017). Molecular biomarkers in acute myeloid leukemia. *Blood Rev*, *31*(1), 63-76. https://doi.org/10.1016/j.blre.2016.08.005
32. Rasmussen, K. D., Jia, G., Johansen, J. V., Pedersen, M. T., Rapin, N., Bagger, F. O., Porse, B. T., Bernard, O. A., Christensen, J., & Helin, K. (2015). Loss of TET2 in hematopoietic cells leads to DNA

hypermethylation of active enhancers and induction of leukemogenesis. *Genes Dev*, *29*(9), 910-922. https://doi.org/10.1101/gad.260174.115

33. Shlush, L. I., Zandi, S., Mitchell, A., Chen, W. C., Brandwein, J. M., Gupta, V., Kennedy, J. A., Schimmer, A. D., Schuh, A. C., Yee, K. W., McLeod, J. L., Doedens, M., Medeiros, J. J., Marke, R., Kim, H. J., Lee, K., McPherson, J. D., Hudson, T. J., Consortium, H. P.-L. G. P., . . . Dick, J. E. (2014). Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*, *506*(7488), 328-333. https://doi.org/10.1038/nature13038
34. Stein, E. M., DiNardo, C. D., Fathi, A. T., Pollyea, D. A., Stone, R. M., Altman, J. K., Roboz, G. J., Patel, M. R., Collins, R., Flinn, I. W., Sekeres, M. A., Stein, A. S., Kantarjian, H. M., Levine, R. L., Vyas, P., MacBeth, K. J., Tosolini, A., VanOostendorp, J., Xu, Q., . . . de Botton, S. (2019). Molecular remission and response patterns in patients with mutant-IDH2 acute myeloid leukemia treated with enasidenib. *Blood*, *133*(7), 676-687. https://doi.org/10.1182/blood-2018-08-869008
35. Walker, A., & Marcucci, G. (2012). Molecular prognostic factors in cytogenetically normal acute myeloid leukemia. *Expert Rev Hematol*, *5*(5), 547-558. https://doi.org/10.1586/ehm.12.45
36. Weissmann, S., Alpermann, T., Grossmann, V., Kowarsch, A., Nadarajah, N., Eder, C., Dicker, F., Fasan, A., Haferlach, C., Haferlach, T., Kern, W., Schnittger, S., & Kohlmann, A. (2012). Landscape of TET2 mutations in acute myeloid leukemia. *Leukemia*, *26*(5), 934-942. https://doi.org/10.1038/leu.2011.326
37. Yohe, S. (2015). Molecular Genetic Markers in Acute Myeloid Leukemia. *J Clin Med*, *4*(3), 460-478. https://doi.org/10.3390/jcm4030460
38. You, E., Cho, Y. U., Jang, S., Seo, E. J., Lee, J. H., Lee, J. H., Lee, K. H., Koh, K. N., Im, H. J., Seo, J. J., Park, Y. M., Lee, J. K., & Park, C. J. (2017). Frequency and Clinicopathologic Features of RUNX1 Mutations in Patients With Acute Myeloid Leukemia Not Otherwise Specified. *Am J Clin Pathol*, *148*(1), 64-72. https://doi.org/10.1093/ajcp/aqx046

Pain Physiology

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Pain center and peripheral neuronal structures are complex but multidimensional mechanisms that protect the bodies from external protection. This mechanism has sensory, emotional, and operational registers (Cranfill and Luo, 2021, Chen and Wang et al., 2023). When evaluated in terms of the world,it is a clinical, social,and economic problem(Henschke et al., 2015).Age and gender are among themost common risk factorsfor pain in the future (Zobdeh et al., 2022). Perception is a sensation; however, when expressed, it is described as perception it is wrong. We can understand that pain exists as it is felt. That's why the feeling of suffering in pain is seen on the roads (Brodal P, 2017). The difference in pain perception, which can change with many factors,such as the person's culture, situation, and past experiences with this pain, depends on such variables. By monitoring the findings of the causative agent or pathology, we ensure that the protocols are applied to achieve more successful results (Wang and Mullally, 2020).At the same time, pain also has acircadian rhythm that can vary according to thetype of disease and thelocation of the person (Bruguerolle and Labrecque, 2007; Chu et al., 2022). Especially in rheumatoid arthritis patients, maximum pain is observed in theearly morning hours(Lee Y, 2021).

Pain is classified according to duration, pathological, and physiological conditions (Figure 1). When pain is classified according to duration, it is evaluated in category I as transient, acute, and chronic,while it is classified in category2 as pathological and physiological pain. In category III, physiological pain is classified as physiological and nociceptive pain, while pathological pain is classified as inflammatory, neuropathic, and cancer. While no tissue damage can be given as anexample of transient pain class, tissue damage is anexample of acute pain.Nerve damage and tissue damage are examples of chronic pain. Examples of nociceptive physiological pain include acute trauma and surgical pain. Physiological pain can be seen as touching a pin and hot plate. Post-operation and arthritis are examples of pathological inflammatory pain, while peripheral or central nervous system lesionsand diabetic neuropathy are included in the neuropathic pain classification. Bone cancer and glioblastoma are cancer pain in category III in the pathological pain classification (Xiao et al., 2021). Neuropathic pain develops due to various disorders affecting theperipheral or central nervous system.When we look at theetiology of neuropathic pain, we see that it has abroad perspective ranging from mechanical, metabolic or ischemic, inflammatory, radiation, and even heredity. We see neuropathic pain in traumatic or mechanical etiology such as pressure, for example, carpal tunnel syndrome. Diabetic polyneuropathy and vitaminB12

deficiency are frequently encountered in metabolic or ischemic etiology (Rosenberger et al., 2020).

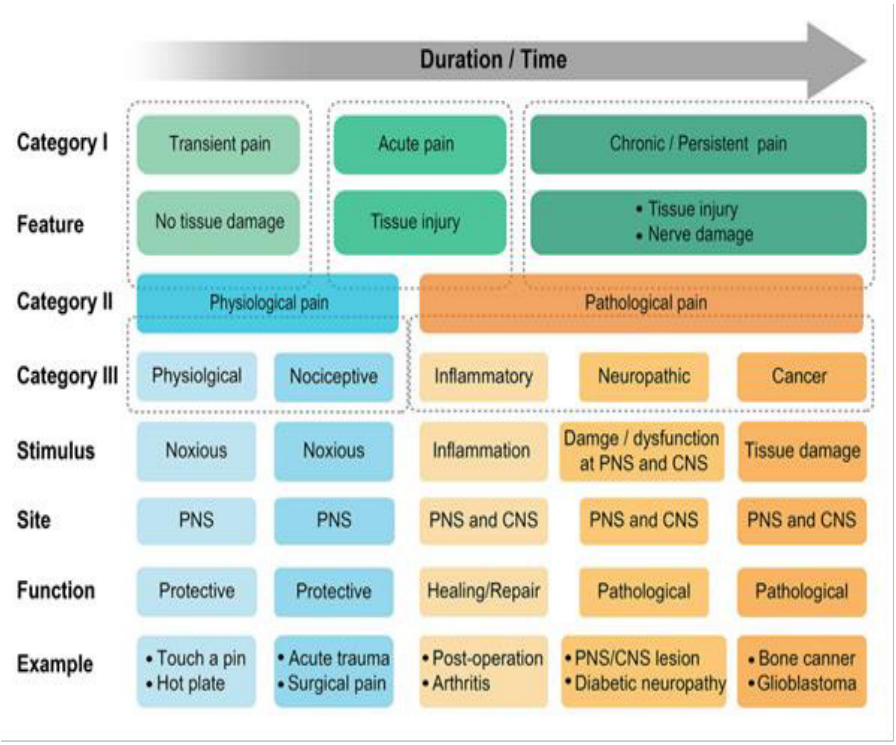

Figure 1. Classification of pain (Xiao et al., 2021).
PNS; peripheral nervous system, CNS; central nervous system

Within the broad spectrum of the category, there are several different approaches to thetreatment of pain,such as opioids, non-steroidal anti-inflammatory drugs(NSAIDs),antidepressants or anticonvulsants, and corticosteroids (Zobdeh et al., 2022).It is very important to provide quality pain treatment because it affects the prognosis and changes the quality of lifeof the patients (Wang and Mullally, 2020).

In a person with normal liver function, drug-assisted pain management is performed according totheWorld Health Organization(WHO) pain ladder and taking into account the American Pain Society guidelines(Figure 2).In simple terms, the WHO defines pain management as "by the clock, by the mouth, by the ladder"; this means regular and timely ("by the hour") use of the safest and simplest structure ("orally").In the first step of treatment, acetaminophen and aspirin are started. In the second stage, more active drugs with more severe side effects,such as fentanyl and tapentadol, are included.Finally, strong opioids

such as morphine and oxycodone should be used,that is,a ladder should be used.It should betaken into consideration that addiction does not develop at this stage (Zobdeh et al., 2022).

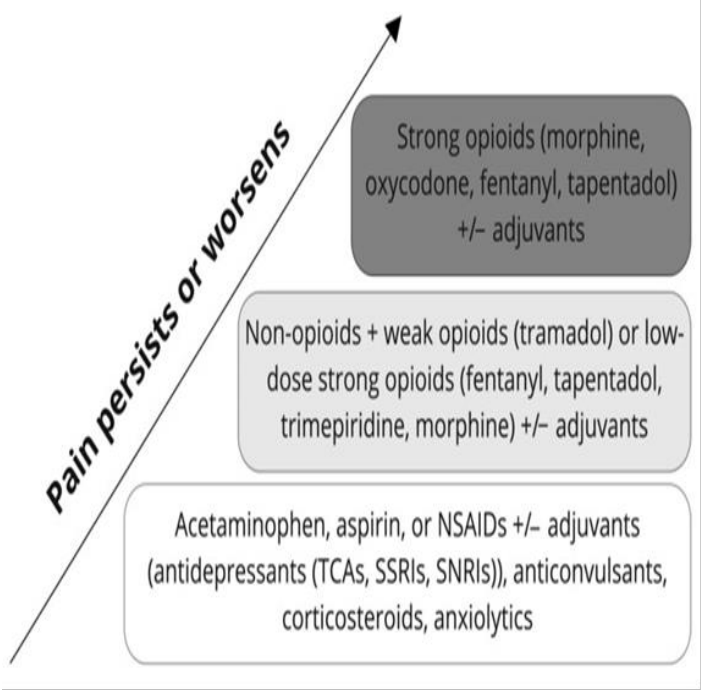

Figure 2. The WHO analgesic ladder (Zobdeh et al., 2022).

Pain perception processes of brain tissue start with nociceptive stimuli. Free nerve endings that convert these stimuli into electrical signals are nociceptors that are located in all internal organs, muscles, and skin (Figure 3).Cell bodies of nociceptors are located inthe dorsal root ganglia(DRG). Nociceptors can detect mechanical and chemical changes such as inflammatory cytokines, temperature,and pressure. As a result ofthis stimulation, three types oftransient receptor potential ion channels(TRP) or G-protein-coupled receptors with nociceptive properties are activated. These TRP channels are TRPV (vanilloid), TRPA(ankyrin),and TRPM(melastatin) (Sontheimer H., 2021).

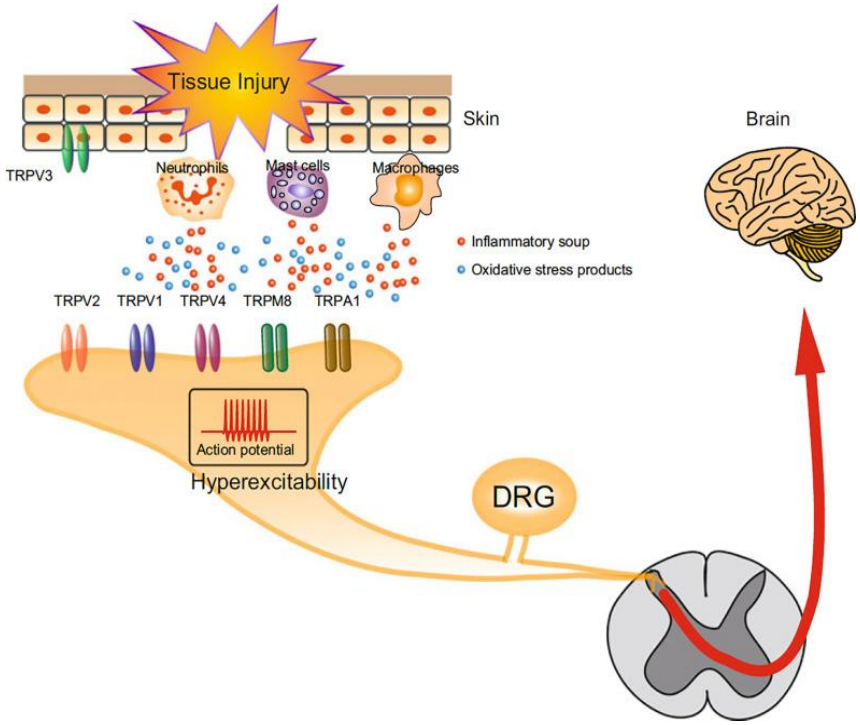

Figure 3. Nociceptors (Sontheimer H., 2021)
TRP; transient receptor potential channels, TRPV (vanilloid), TRPA (ankyrin), TRPM (melastatin), DRG; dorsal root ganglion

The process that starts with thesensitivity of the pain is completed with thetransmission of thebody to the dorsal horn via the peripheral nerves and finally reaching the central nervous system(Yam et al., 2018). The primary afferents in conducting the nociceptive medium are the Aσ and C fibers.The unmyelinated C fibers are the afferents of delayed pain with small permanent localization. In contrast, the medium-sized myelinated Aσ fibers are responsible for thesensation of acute, localized sharp pain (Fig. 4). However, some afferents transmit mechanical information, such as myelinated light touch, of which Aβ fibers are the most extensive walking.Positioning in the deep dorsal horn makes it challenging to interlock with stimuli and inhibitory, excitatory interneurons (Tsuda M, 2016).

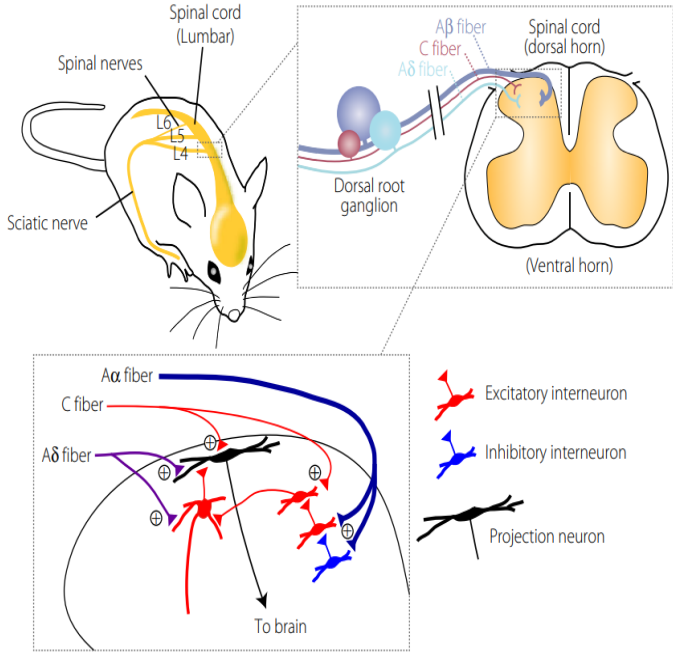

Figure 4. Primary afferent sensory fibers and neuronal circuits (Tsuda M, 2016).

Stimuli from afferent neurons enter the spinal cord at thelumbar and cervical levels and are transmitted to the primary somatosensory cortex of thecerebrum.Then, signals from thecentral nervous system are transmitted to the effector structures, muscles, and glands, by efferent neurons. There are many neurotransmitters and neuromodulatory substances responsible for its system (Figure 5). The main ones are: Adenosine Triphosphate(ATP),Artemin and glial cell-line derived neurotrophic factors(GDNF), Brain-derived nerve growth factor(BDNF), Calcitonin Gene-Related Peptide(CGRP), Cytokines and chemokines, Gamma-aminobutyric acid(GABA), Nerve Growth Factor, Norepinephrine. There are also Tachykinins such as Substance P,neurokinin A,neurokinin B, neuropeptides such as corticotropin-releasing hormone, and urocortin 1–3 (Yam et al., 2018).

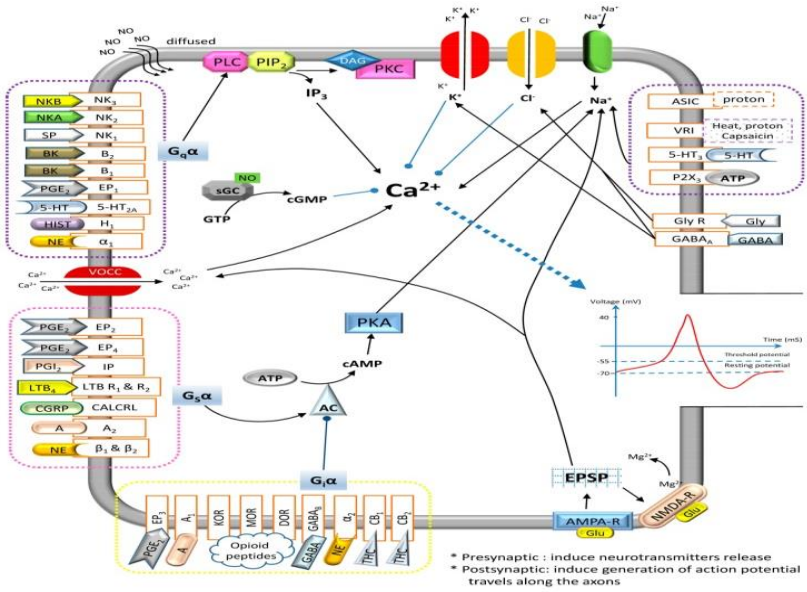

Figure 5. The signaling mechanism pathways of pain-associated neurotransmitters (Yam et al., 2018).

As a result of glial and neuronal modulation with these mediator molecules, pain signals regulate the integrative cognitive processes that will affect the individual's perception andexperience of pain (Figure 6).The limbic system plays anactive role in these processes (Murray et al., 2021).

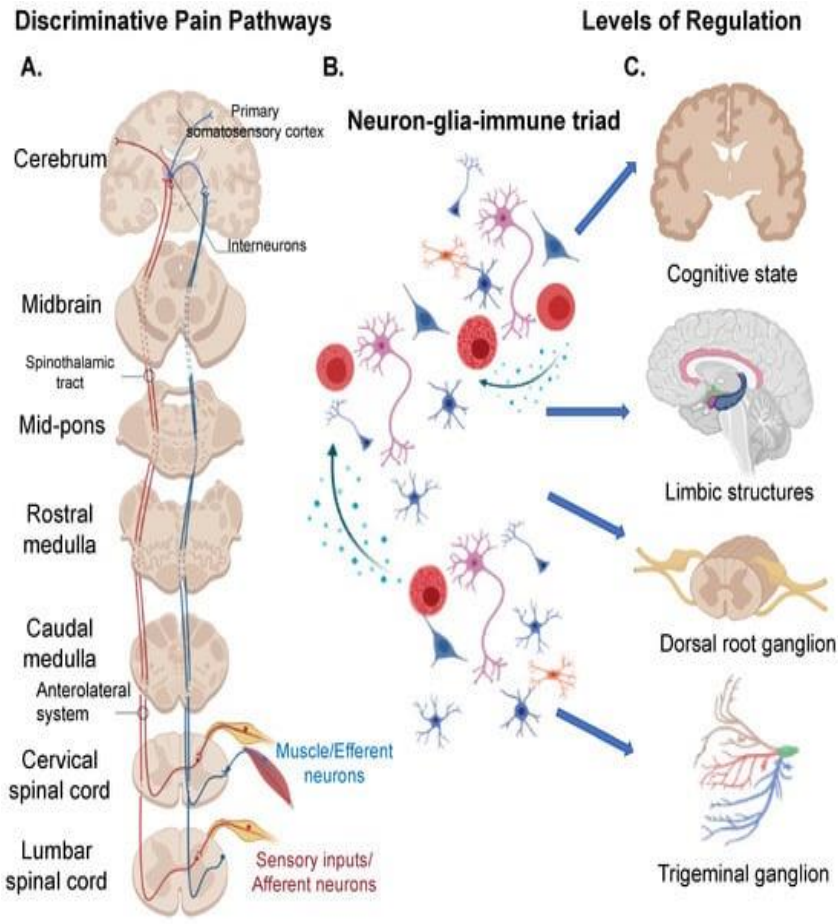

Figure 6. Pain pathways (Murray et al., 2021).

Unfortunately, today, a specific type of treatment is not applied to the person in the same way in terms of showing the changes of the person it contains depending on the age and gender as a result of the experiences, level of consciousness, and beliefs of the pain. Therefore, combined pain management protocols are chosen. This requires cost and transitions in pain management. Further studies must examine the mechanisms underlying this incompletely understood difference between individuals.

REFERENCES

1. Brodal P, (2017). A neurobiologist's attempt to understand persistent pain. Scandinavian Journal of Pain 15; 140–147.
2. Caroline E.P., Edita N., Frank P., (2021). Cognition in the Chronic Pain Experience: Preclinical Insights, Trends in Cognitive Sciences, 25, 5, 365-376.
3. Chu Y., He H., Liu Q., Jia S., Fan W., Huang F., (2022). The Circadian Clocks, Oscillations of Pain-Related Mediators, and Pain. Cell Mol Neurobiol.
4. Cranfill S.L, Luo W., (2021). The development of somatosensory neurons: Insights into pain and itch. Curr Top Dev Biol.,142:443-475.
5. Henschke N., Kamper S.J., Maher C.G., (2015). The epidemiology and economic consequences of pain. Mayo Clin. Proc.;90:139–147.
6. Lee Y, (2021). Circadian Rhythms and the Treatment of Rheumatoid Arthritis. Archives of Rheumatology & Arthritis Research.1:3.
7. Murray I, Bhanot G, Bhargava A., (2021). Neuron-Glia-Immune Triad and Cortico-Limbic System in Pathology of Pain. Cells, 10(6):1553.
8. Rosenberger DC, Blechschmidt V, Timmerman H, Wolff A, Treede RD., (2020). Challenges of neuropathic pain: focus on diabetic neuropathy. J Neural Transm (Vienna)., 127(4):589-624.
9. Sontheimer H., (2021). Pain, Editor(s): Harald Sontheimer, Diseases of the Nervous System (Second Edition), Academic Press, 325-356.
10. Tsuda M., (2016). Microglia in the spinal cord and neuropathic pain. J Diabetes Investig, 7: 17–26.
11. Wang V.C, Mullally W.J., (2020). Pain Neurology. Am J Med.,133(3):273-280.
12. Yam MF, Loh YC, Tan CS, Khadijah Adam S, Abdul Manan N, Basir R., (2018). General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. International Journal of Molecular Sciences. 19(8):2164.
13. Zhang, Y. Q., (2021). Role of the anterior cingulate cortex in translational pain research. Neuroscience Bulletin, 37(3), 405-422.
14. Zobdeh F, Eremenko II, Akan MA, Tarasov VV, Chubarev VN, Schiöth HB, Mwinyi J., (2022). Pharmacogenetics and Pain Treatment with a Focus on Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and Antidepressants: A Systematic Review. Pharmaceutics,14(6):1190.

Evaluation of The Efficiency of Animal Models Used in Depression Studies

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INTRODUCTION

Depression is a serious neuropsychiatric diseases affecting quality of life. Depression, such as feeling, thinking, sleeping, eating, or working It is a mood disorder that badly affects daily life. It is also called "clinical depression" or "depressive disorder" (1). Depression affects more than 350 million people worldwide. Persistent sadness is characterized by a lack of interest and pleasure in rewarding or enjoyable activities. It can disrupt sleep and appetite (2), as well as cause fatigue and insufficient concentration. In addition to being prolonged or recurrent, depression can significantly affect an individual's daily functioning and ability to live a rewarding life (3).

It is reported that Jarvis conducted the first epidemiological study of depression in history by indirectly investigating the prevalence of insanity and mental retardation in 1855. Epidemiological studies conducted at the beginning of the 20th century are based on hospital and treatment records. In some of these studies, the psychological and social causes of mental disorders were discussed. Others focused more on genetic factors, diagnosis, follow-up, statistics on mental health, and various treatment processes, and these studies were eventually named "First Generation Research" (4).With the discovery of monoamines in 1968, a hypothesis was put forward establishing a link between depression and monoamines. From the middle of the 20th century, neurophysiological studies have provided more information about depression (5). Studies conducted from 1990 to 2006 include studies using DSM (Diagnostic and Statistical Manual of Mental Disorders) diagnostic criteria (4). Depression according to DSM diagnostic criteria; five or more of the symptoms of dysfunction and dysphoric mood, loss of pleasure, suicidal thoughts or actions, agitated or slow movements, guilty or self-humiliating feelings, fatigue, and sleep disorder for two or more weeks (6).

Mood swings are defined by the following symptoms: Symptoms of a depressive episode, of which at least five must meet diagnostic criteria and last at least two weeks (7).

1. Almost constantly depressed mood for most of 24 hours
2. Lack of interest in pleasure or activity most of the day, almost all week (anhedonia).
3. Feelings of worthlessness or unreasonable guilt almost every day.
4. Sleep disturbance almost every day (insomnia or oversleeping).
5. Fluctuations in weight or appetite that change daily.
6. Daily psychomotor tension or retardation.
7. Fatigue or loss of energy continually.
8. Decreased ability to concentrate or think nearly continually.
9. Repetitive suicidal thoughts or thoughts of passing away.

Environmental stress factors cause Hypothalamo-Pituitary-Adrenal (HPA) axis dysregulation, resulting in excessive glucocorticoid release. Stress can affect biological systems together with genetic polymorphisms by affecting the physiological response. Morever to depressive symptoms, it may trigger changes in cortical brain regions and limbic system (7). Continuous exposure to stressful life events is a risk factor for major depression (8). In rodents, chronic uncalculable stress or chronic mild stress (CMS) has been found to increase cognitive impairment and behavioral despair. These effects may be mediated by HPA hyperactivity and increased corticosterone release (8). Under stress, the hypothalamus plays an important role in the psychosocial stress reaction by regulating the secretion of corticotropin-releasing factor (CRF) (9). The hypothalamus induces the release of adrenocorticotropic hormone (ACTH) from the anterior side of the pituitary gland under the influence of CRF (10,11)

It is known that a number of biological, environmental and psychological factors play a role in the improvement and run of the disease (12). The exist of certain signs can be operationally identified (eg, sleep disturbance, changes in cognitive and psychomotor activity, loss of appetite and weight variability) and therefore evaluated in experimental animals (2). Despite the widespread effects of depression in mankind, what is known about its physiopathology is limited. Various studies have been conducted to understand the mechanism of depression and to improve cure methods. Experimental models of depression have been of great help in understanding the causes and deep mechanisms of depression. The most used animals in the studied models are mice and rats. Behaviors of depressed animals such as exploring the area they are in, recognizing and adapting to their environment are examined. Determining the changes in behavior after the administration of therapeutic drugs or the administration of therapeutic agents forms the basis of the research. In this chapter, an evaluation of efficacy in animal models of depression was conducted to better understand depression.

The most frequently cited principles regarding animal models were proposed by McKinney and Bunney (13) about thirty-five years ago. The criteria that the animal model of depression should meet (Table 1) are listed by the authors as showing some analogy to the human disorder in terms of appearance or symptoms, behavioral changes that can be observed and evaluated objectively, reversibility of behavioral changes with interventions that are effective in humans, and repeatability of the model by different researchers.

Table 1. Criteria that animal models used in depression studies must meet (13)

1. Analogy in appearance or singular symptoms to a human disorder
2. Behavioral changes that can be objectively evaluated and observed
3. Reversibility of behavioral changes with interventions that are effective in humans
4. The model can be repeated by different researchers

Experimental Models of Depression

1. Learned Helplessness

This model is one of the oldest paradigms first discovered in the 1970s and used to model depression (14; 15). In this model, experimental animals are randomly exposed to unavoidable shock and depression-like behaviors occur after shock application. In the two-compartment mechanism, an electric shock is given on one side and no shock is applied on the other.Or, in the single-section model, the animal stops the shock by means of a pedal (16).

In the learned helplessness model, a decrease in the motor activity of the animals, a decrease in eating and drinking behavior and weight loss were observed. These symptoms are very similar to the methods used to diagnose depression (15). The extreme and extreme stress that animals are exposed to is also controversial in terms of both ethics and depression modeling (16). However, it has been proposed to be a reliable model for investigating the pathophysiology of post-traumatic depression (15).

Figure 1. Learned Helplessness Experiment (17)

2. Tail Suspension Test

The tail hang test is another behavioral test used to measure the response to stress. Rodent tails are hung on a horizontal bar with adhesive tape for 6 minutes and the immobility time is observed. It is expected that the period of inactivity in the experimental animal will increase. It is not used in rats due to their weight and size, it is only used in mice. This test is mostly used to determine the response to antidepressants (18). Despite the problems with construct validity, its high predictive validity and ease of use made it frequently preferred in drug development studies that require testing a large number of compounds (19).

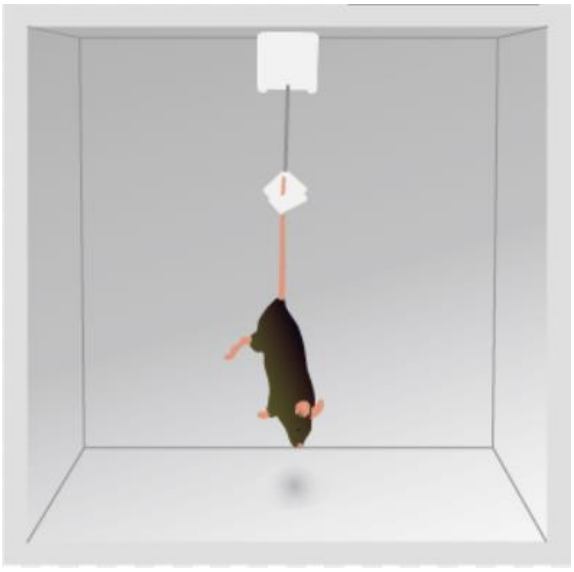

Figure 2. Tail Suspension Test (20)

3. Forced Swimming Test (FST)

It is a test defined by Porsolt in 1977 (21). In FST, a rodent's behavior is evaluated based on how it responds to an unpleasant environment. In FST, a 15 cm part exceeding the length of the experimental animal (as a minimum, the diameter must be 18 cm and the height 40 cm) is left in a cylinder filled with water. A rat or mouse released into the water typically tries to escape. However, the subject has to swim for a while, and after realizing that he cannot escape, he exhibits a more depressive behavior and hangs on the water surface without attempting to escape (22; 15). After a while, a deep despair develops. This suspension (immobilization) is called despair. The swimming, struggling and immobile times of the experimental animal are recorded. It is a highly preferred method in evaluating the effects of antidepressant treatment in rats (22).

Figure 3. Forced Swimming Test

4. Social Defeat Animal Model

Social stress plays an important role in the occur of depression and psychopathological conditions in mankind. In this model, social conflict is used to create psychological stress and emotional stress. Animals live in a certain hierarchical order. The dominant male rodent is left in the cage with the experimental animals to create a competitive environment. The intruder is attacked and defeated by the inhabitants. When a threat attack or physical attack occurs, the intruders are separated from the residents by a obstacle. Afterwards, the tested rodent is exposed to another attacker. After the physical attack, the trespasser is given various behavioral tests. A series of changes are observed in sexual behavior, locomotor and exploratory activity, increase in defensive behavior and anxiety, circadian rhythm (including sleep disorders), nutrition and body weight in ingested rodents (15).

Social stress causes an important problem in many living things. It is thought to play an important role in the occur of depression and other psychopathologies in mankind. The use of social challenge as a source of stress and the use of social interplay as a measurable outcome are valid for depression (23). This model has two major drawbacks. First, a short-term paradigm leads to more manifestations of anxiety, so a previous study has shown that 20 days of social stress are necessary for depression to develop. Another disadvantage is that only masculine

rodents can be used in this test, since woman rats or mice do not fight in a resident-trespasser encounter (24).

5. Chronic Mild Stress (CMS)

The CMS model is intended to more carefully mimic the daily life of mankind by exposing rats to mild stressors. It is known that exposure to chronic or acute stress causes depressive disorder (25). The CMS depression model is often considered a prototypical example. In this test, animals are chronically exposed to continuous administration of unpredictable micro-stressors (11).

The CMS model has its origins in articles published by Katz et al. in the early 1980s (26). In this first protocol, rats were sequentially exposed to a variety of severe stressors. These stress factors can be disruption of the light-dark cycle in the environment where animals are present, unexpected changes in housing conditions (application of slopes to their cages), keeping the cage floor wet, deprivation of feed and water, exposure to noise. These stressors are applied alternately for several hours. Animals are exposed to this treatment for 5 or 9 weeks and tested weekly (19; 11).

5.1. Chronic Mild Stress (CMS) Protocol

The chronic mild stress protocol used in the studies is a modified version of the model proposed by Willner in 2016 (11). Experimental planning is made according to how many days the study will last and how the drug administration will be. Various stressors are applied to animals in CMS applications. In order to avert animals from getting used to and anticipating the stressors, they are exposed to numerous stressors at several times. In wet cage application, 333 g of wood shavings in a cage is wetted with 1.5 liters of water. Rats are kept on wet sawdust for 7 hours, starting at different times in the morn.

In the curved cage application, the cage with the animals is placed at an angle of 60 degrees and the food part of the cage remains at the top and the rats are kept in this way for 7 hours. Under noise stress, a 60 dB ringing tone, which rings continuously for 1 second, once in 10 seconds, is applied for 4 hours. It is floated for 10 minutes in plexiglass cylinder containers (as a minimum, the diameter must be 25 cm, the height 60 cm and water level 39 cm) where the swimming stress FST is performed. Restraint stress is placed in 7x7x12-22 cm, 7x7x12-22 cm, motion-restricting transparent apparatus with ventilation holes on the front made of plexiglass, which can be adjusted according to the animals' own size, with their noses coming into the air holes in the front of the apparatus, then they are placed by adjusting the slides according to the size of the rat, with their tails out, and placed in the restraint apparatus. It is held for 45 minutes. For starvation stress,

only water containers are left in the cages of the rats, the rats are starved for 16 hours by collecting the food in the cages. The animals are kept in this chamber for 16 hours (16.00-08:00) by leaving the light of the room where the animals are located under uninterrupted lighting stress (27)

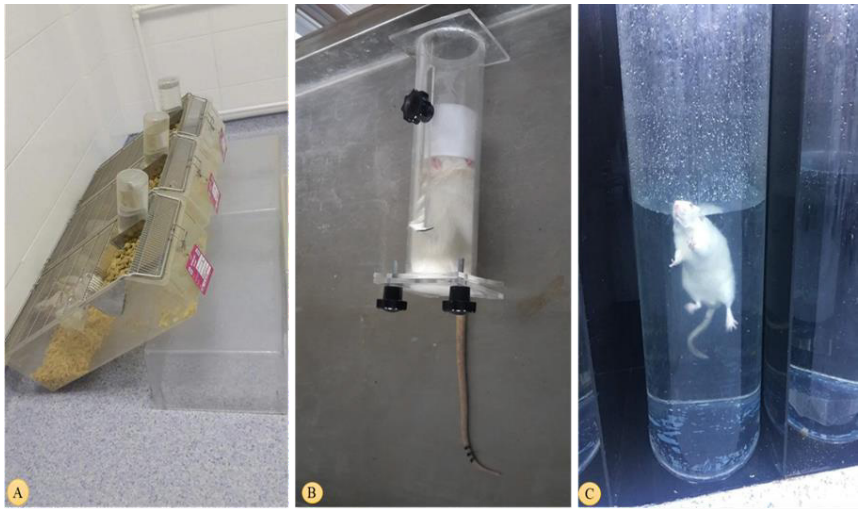

Figure 4. Stressors used in the creation of the CMS depression model A) Inclined cage, B) Restraint apparatus, C) Swimming apparatus

Table 2 Example Chronic mild model experiment scheme (27)

	MORNING			NOON			NIGHT		
Day	**Hour**	**Stressor**	**Duration**	**Hour**	**Stressor**	**Duration**	**Hour**	**Stressor**	**Duration**
1	09.00 am	Restraint stress	45 minutes	01.00 pm	Noise	4 hours	04.00 pm	Food deprivation	All night
2	10.00 am	Wet cage	7 hours				04.00 pm	Light on	All night
3	09.00 am	FST	10 minutes	03.00 pm	Restraint stress	45 minutes			
4	11.00 am	Inclined cage	7 hours						
5	09.00 am	Noise	4 hours	02.00 pm	FST	10 minutes	04.00 pm	Food deprivation	All night
6	09.00 am	Wet cage	7 hours				04.00 pm	Light on	All night
7	10.00 am	Inclined cage	7 hours						

CMS does not show an appearance of the anxiety profile on the elevated plus test and social interaction anxiety behavioral tests, suggesting that behavioral changes are specific to depression (24). Exposure to CMS leads to some other behavioral and physiological changes analogous to symptoms of depression, such as decreased self-care and sleep changes, and other anomalies such as increased hypothalamo-pituitary-adrenal (HPA) axis activation and defance system aberrancies support the superficial validity of this protocol (23). The advantage of the CMS model is that it has good predictive validity, visual validity, and structural validity. Therefore, the CMS protocol is probably the most precious and widely prefered depression model. The CMS protocol has two major disadvantages. First, the workload, attention spans, and long durations are the practical difficulties in performing CMS experiments. The second is the establishment of a new laboratory setup and the creation of similar data between laboratories (24).

CONCLUSION

Animal models have contributed significantly to the discovery of the deep mechanism of depression and the development of novel cure techniques. But it has some disadvantages. Two main problems with these models are that while chronic treatment is beneficial for human depression, acute treatment can yield results in animals, and when the observed condition is short-lived, it is difficult to conduct studies on the outcome of repeated treatment. In the light of the information obtained from human studies on depression to further develop the models, it seems possible to reach more valid and reliable animal models, especially with the development of genetics and laboratory techniques.

REFERENCES

1. National Institute of Mental Health. 2016; Publication No. 19-MH-8079
2. Schweizer, M. C., Henniger, M. S., & Sillaber, I. (2009). Chronic mild stress (CMS) in mice: of anhedonia, 'anomalous anxiolysis' and activity. PloS one, 4(1), e4326. https://doi.org/10.1371/journal.pone.0004326
3. World Health Organization, 2020. Investing in Mental Health. WHO, Geneva.
4. Kaya, B., Mine, Kaya. (2007). 1960' lardan Günümüze Depresyonun Epidemiyolojisi, Tarihsel Bir Bakış. Klinik Psikiyatri, 10: 3-10
5. Schildkraut J. J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. The American journal of psychiatry, 122(5), 509–522. https://doi.org/10.1176/ajp.122.5.509
6. Shi, J., Potash, J. B., Knowles, J. A., Weissman, M. M., Coryell, W., Scheftner, W. A., Lawson, W. B., DePaulo, J. R., Jr, Gejman, P. V., Sanders, A. R., Johnson, J. K., Adams, P., Chaudhury, S., Jancic, D., Evgrafov, O., Zvinyatskovskiy, A., Ertman, N., Gladis, M., Neimanas, K., Goodell, M., … Levinson, D. F. (2011). Genome-wide association study of recurrent early-onset major depressive disorder. Molecular psychiatry, 16(2), 193–201. https://doi.org/10.1038/mp.2009.124
7. Mahar, I., Bambico, F. R., Mechawar, N., & Nobrega, J. N. (2014). Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. Neuroscience and biobehavioral reviews, 38, 173–192. https://doi.org/10.1016/j.neubiorev.2013.11.009
8. Bambico, F. R., Nguyen, N. T., & Gobbi, G. (2009). Decline in serotonergic firing activity and desensitization of 5-HT1A autoreceptors after chronic unpredictable stress. European neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology,19(3), 215–228. https://doi.org/10.1016/j.euroneuro.2008.11.005
9. Dranovsky, A., & Hen, R. (2006). Hippocampal neurogenesis: regulation by stress and antidepressants. Biological psychiatry, 59(12), 1136–1143. https://doi.org/10.1016/j.biopsych.2006.03.082
10. David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., Drew, M., Craig, D. A., Guiard, B. P., Guilloux, J. P., Artymyshyn, R. P., Gardier, A. M., Gerald, C., Antonijevic, I. A., Leonardo, E. D., & Hen, R. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron, 62(4), 479–493. https://doi.org/10.1016/j.neuron.2009.04.017

11. Willner P. (2016). The chronic mild stress (CMS) model of depression: History, evaluation and usage. Neurobiology of stress, 6, 78–93. https://doi.org/10.1016/j.ynstr.2016.08.
12. Remus J. Neuroımmune mechanısms of an anımal model of recurrent depressıon. Kent State University. Degree of Doctor of Philosophy. Doctoral Thesis. Ohio, 2015
13. McKinney, W. T., Jr, & Bunney, W. E., Jr (1969). Animal model of depression. I. Review of evidence: implications for research. Archives of general psychiatry, 21(2), 240–248. https://doi.org/10.1001/archpsyc.1969.01740200112015
14. Seligman M. E. (1972). Learned helplessness. Annual review of medicine, 23, 407–412. https://doi.org/10.1146/annurev.me.23.020172.002203
15. Wang, Q., Timberlake, M. A., 2nd, Prall, K., & Dwivedi, Y. (2017). The recent progress in animal models of depression. Progress in neuro-psychopharmacology & biological psychiatry, 77, 99–109. https://doi.org/10.1016/j.pnpbp.2017.04.008
16. Başar, K., Ertuğrul, A. (2005). Depresyon araştırmalarında kullanılan hayvan modelleri. Klinik Psikiyatri Dergisi, 8:123-34
17. https://www.simplypsychology.org/learned-helplessness.html
18. Steru, L., Chermat, R., Thierry, B., & Simon, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology, 85(3), 367–370. https://doi.org/10.1007/BF00428203
19. Willner, P., Towell, A., Sampson, D., Sophokleous, S., & Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology, 93(3), 358–364. https://doi.org/10.1007/BF00187257
20. https://en.wikipedia.org/wiki/Tail_suspension_test
21. Porsolt, R. D., Le Pichon, M., & Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. Nature, 266(5604), 730–732. https://doi.org/10.1038/266730a0
22. Uzbay I.T. (2004). Psikofarmakolojinin Temelleri ve Deneysel Araştırma Teknikleri. Çizgi Tıp Yayınevi 1. Baskı, Ankara, 103-12.
23. Duman C. H. (2010). Models of depression. Vitamins and hormones, 82, 1–21. https://doi.org/10.1016/S0083-6729(10)82001-1
24. Yan, H. C., Cao, X., Das, M., Zhu, X. H., & Gao, T. M. (2010). Behavioral animal models of depression. Neuroscience bulletin, 26(4), 327–337. https://doi.org/10.1007/s12264-010-0323-7

25. Haridas, S., Kumar, M., & Manda, K. (2013). Melatonin ameliorates chronic mild stress induced behavioral dysfunctions in mice. Physiology & behavior, 119, 201–207. https://doi.org/10.1016/j.physbeh.2013.06.015
26. Katz R. J. (1982). Animal model of depression: pharmacological sensitivity of a hedonic deficit. Pharmacology, biochemistry, and behavior, 16(6), 965–968. https://doi.org/10.1016/0091-3057(82)90053-3
27. Solak, H., Gormus, Z. I. S., Koca, R. O., Gunes, C. E., & Kutlu, S. (2022). Does Sertraline Affect Hypothalamic Food Intake Peptides in the Rat Experimental Model of Chronic Mild Stress-Induced Depression?. Neurochemical research, 47(5), 1299–1316. https://doi.org/10.1007/s11064-022-03529-9