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Assist Prof. Taner AKARSU Ph.D

PIONEER AND CONTEMPORARY STUDIES HEALTH SCIENCES

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EDITOR

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Pioneer and Contemporary Studies Health Sciences
Editor: Assist Prof. Taner AKARSU Ph.D

Editor in chief: Berkan Balpetek
Cover and Page Design: Duvar Design
Printing : JUNE-2023
Publisher Certificate No: 49837
ISBN: 978-625-6945-87-6

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1 This study was published as a summary presentation at the International Istanbul Congress Of Multidisciplinary Scientific Research congress on 16-17 May 2022

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ABSTRACT

Pain is an uncomfortable feeling and emotional state that accompanies or at least can be explained by actual or potential tissue damage. Pain sensation is essential for the body to protect itself from factors that can cause tissue damage. The process in the physiology of pain begins with the activation of specialized receptors called nociceptors by the stimulus that can cause pain. Although pain is very common, it is a subjective and personal experience. One of the most common cases that cause children to apply to the hospital is the universal experience of pain. The facial expressions of children experiencing pain, the position of their body and the words they use to describe their pain are important signals that show how much they are in pain. There are many factors that affect children's perception of pain and their reactions to pain. These; biological factors (age, cognitive development level, gender, temperament, genetics, fatigue), cultural factors (meaning of pain, ethnicity), social factors (attention, previous experiences, family support, social support) psychological factors (fear-anxiety) -anxiety, coping style). In clinics, health workers often face pain problems in children and they need to measure pain before starting pain treatment. It is necessary for nurses to know that many situations are effective on pain perception threshold and pain tolerance, and pain behavior is determined by facial expression, body position and the words to be used to explain pain, and the necessary features should be considered in the care of pediatric patients with pain. However, pain perception and expression differ according to culture and age; Due to their developmental characteristics, children do not have the communication skills to express their pain like adults. Therefore, evidence-based practices for pain show positive results, especially in identifying and reducing pain in children and expressing themselves about pain. The aim of this study includes information on pediatric acute pain and evidence-based practices.

Keywords: Pain, pediatric pain, evidence-based practice.

Introduction

Although pain is a universal phenomenon, it is seen in all societies and in all age groups, its perception is extremely subjective. Because the process of perception of pain varies from person to person, depending on many personal characteristics, especially age and culture. As a result of this subjectivity, the most reliable way to understand pain is possible with the narration of the person experiencing the pain. However, this process, which is stressful and complex even in adults, becomes more complicated because children cannot express themselves adequately and cannot provide reliable self-report.

What is Pain?

It is a physical and emotional disturbance that accompanies or refers to existing or impending tissue damage (Świeboda et al., 2013). International Association for Pain Studies, pain; It defines it as an unpleasant sensory and emotional experience or tissue damage that occurs in relation to actual or potential tissue damage (Loeser and Treede, 2008). The Neuman Systems Model states that each person has a dynamic and specific perception of pain, and this perception is shaped by the combination of cognitive, developmental, emotional, sensory and cultural factors with the pain context in different forms and degrees (Milani et al., 2011; Neuman and Fawcett, 2010;). This particular and dynamic combination results in the perception of pain being significantly personal and subjective, and also causes pain to be a multifaceted and independent perception. Pain is a problem that can occur anywhere and can affect all areas of life widely (Nair & Nell, 2013; Cohen et al., 2008).

Acute Pain

Acute pain serves a biological protective function that alerts the body of impending danger, which is usually initially and time-limited (Jungquis et al., 2017). It is always nociceptive and indicates the presence of an event that harms the body. There is a close relationship between the causative lesion and the pain in terms of location, severity and time. Causes include trauma, infection, tissue hypoxia, and inflammation. Postoperative acute is the best example of acute. Acute pain shows the characteristics of chronic pain after 3-6 months (Raj, 2000).

Pediatric Pain

Newborns, children and adolescents relieve pain; Beginning with needle insertion and insertion of intravenous catheters, continuing through more stressful medical procedures such as lumbar puncture, bone marrow aspiration, biopsies, chest tube insertion, cardiac catheterization, surgical operations, and burn

dressing. Pain in children is one of the most difficult side effects to be managed by healthcare professionals, and it is seen as the most stressful and feared illness and hospital process by children (Franck et al., 2004; Howard, 2003). As noted in the previous sections, pain is a very personal and subjective experience. This personal perception can be understood most accurately and reliably with the individual's own self-description, which is considered the "gold standard" of pain assessment methods (Hadjistavropoulos & Craig, 2002).

Factors Affecting Pain

There are many factors that affect children's perception of pain and their response to pain. These; biological factors (age, cognitive development level, gender, temperament, genetics, fatigue), cultural factors (meaning of pain, ethnicity), social factors (attention, previous experiences, family support, social support) psychological factors (fear-anxiety) -anxiety, coping style) (Yılmaz Kurt et al., 2019).

Physiopathology of Pain

The process in the physiology of pain begins with the activation of specialized receptors called nociceptors by the stimulus that can cause pain. These receptors are mechano, thermo and polymodal nociceptors. These nociceptors carry messages to the laminae in the posterior horn of the spinal cord. Aδ fibers are fast and C fibers are slow. Fibers carried to eight laminae reach the thalamus and cerebral cortex through 5 separate pathways in the spinal cord with different neurons in these laminae (Gürel, 2011).

Although the response to the perception of pain is given emotionally and cognitively, a defense process is created with various sympathetic activities. The pain is often burning, sharp and sometimes described as an electric shock. The occurrence of pain even with non-painful stimuli such as touch, wind, heat or cold is typical of neuropathic pain. Pain can last for months or even years.

Evidence-Based Practices for Pediatric Acute Pain

Although research on pain in children has increased in recent years, pediatric pain management and treatment is still insufficient. Because adequate and effective pain management and treatment requires a comprehensive pain assessment.

It emphasizes that children with pain should be listened to and believed, and they should be encouraged to express their feelings and ideas about pain. For this reason, healthcare professionals need to learn the language of pain and how they

express pain according to the age and culture of children (Royal Collage Nursing, 2009).

However, children cannot express pain like adults because of language development and cognitive deficiencies. This makes pain in children a difficult and complex phenomenon to understand. This results in inadequate communication between healthcare professionals and children, and limits accurate and reliable pain assessment in children. As a solution, it recommends the use of pain assessment methods and evidence-based practices that do not require communication skills, such as language or dialect, when healthcare professionals experience communication difficulties due to developmental and cultural differences (Hadjistavropoulos & Craig, 2020; Kristjánsdóttir et al., 2012; Royal Collage). Nursing, 2009).

Examples of Evidence-Based Practices for Pediatric Acute Pain;

Table 3 Validated pain tools for neonates

Tool name	Features	Suitable for setting	Suitable for (gestational age):	
			Preterm neonates	Term neonates
COMFORT[3,4]		Postoperative and periprocedural pain		✓
CRIES[5,6]		Postoperative pain	✓	✓
Neonatal Facial Coding System (NFCS)[†7–10]		Postoperative pain	✓	✓
Nepean NICU Pain Assessment Tool (NNICUPAT)[11]		Periprocedural pain		✓
Neonatal Infant Pain Scale (NIPS; from Children's Hospital of Eastern Ontario Pain Scale (CHEOPS) for neonates)[3,9,12]		Postoperative pain	✓	✓
Objective Pain Scale (OPS)[5]		Postoperative pain	✓	✓
Pain Assessment Tool (PAT)[13,14]		Postoperative pain	✓	✓
Premature Infant Pain Profile (PIPP)[15]		Periprocedural pain	✓	✓

[†]NFCS has been adapted for preschool-aged children to create the Child Facial Coding System. We found no assessment of interrater reliability for this derived tool.[16]

Table 5 Validated pain tools for non-verbal children with cognitive impairment

Tool name	Features	Suitable for (setting)	Suitable for (age (years))										
			<3	3	4	5	6	7	8	9	10	11	12	12+
FLACC (Face, Legs, Activity, Cry, Consolability)[52,53]		Postoperative pain 4–18 years			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PPP (Paediatric Pain Profile)[54,55]		All settings 1–18 years	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
NCCPC-R (Non-communicating Children's Pain Checklist – Revised)[56†]		All settings 3–19 years		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
NCCPC-PV (Non-communicating Children's Pain Checklist – Post-operative Version)[57]		Postoperative pain 3–19 years		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

†Both NCCPC-PV and NCCPC-R bundle some observations under a physiological heading (shivering, changes in colour, sweating, tears); these do not fall under our definition of physiological assessment.

Stapelkamp, C. et.al. Assessment of acute pain in children: development of evidence-based guidelinesjbr_1. Int J Evid Based Healthc 2011; 9: 39.

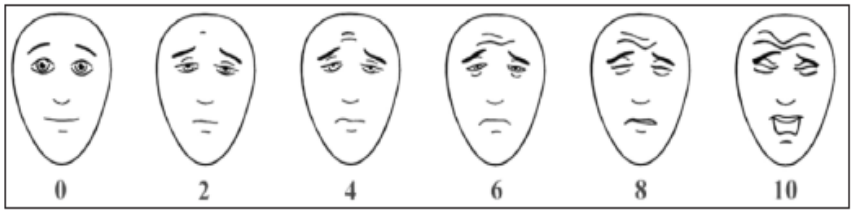
ITEM	BEHAVIOR	SCORE	DEFINITION
Cry	*No Cry*	1	Child is not crying
	Moaning	2	Child is moaning or quietly vocalising; silent cry
	Crying	2	Child is crying, but the cry is gentle or whimpering
	Scream	3	Child is in a full-lunged cry; sobbing: may be scored with/without complaint
Facial	*Composed*	1	Neutral facial expression
	Grimace	2	Score only if negative facial expression
	Smiling	0	Score only if definite positive facial expression
Child verbal	*None*	1	Child not talking
	Other complaints	1	Child complaints, but not about pain
	Pain complaints	2	Child complaints about pain
	Both complaints	2	Child complaints about pain and about other things
	Positive	0	Child makes any positive statement or talks about other things without complaint
Body	*Neutral*	1	Body (not limbs) is at rest, torso is inactive
	Shifting	2	Body is in motion in a shifting or serpentine fashion
	Tense	2	Body is arched or rigide
	Shivering	2	Body is shuddering or shaking involuntarily
	Upright	2	Child is in a vertical or upright position
	Restrained	2	Body is restrained
Touch	*Not touching*	1	Child is not touching or grabbing at wound
	Reach	2	Child is reaching for but not touching wound
	Touch	2	Child is gently touching wound or wound area
	Grab	2	Child is grabbing vigorously at wound
	Restrained	2	Child's arms are restrained
Legs	*Neutral*	1	Legs may be in any position but are relaxed
	Squirming/Kicking	2	Definitive uneasy or restless movements in the legs or striking out with feets
	Drawn up/Tensed	2	Legs tensed and/or pulled up thigtly to body and kept there
	Standing	2	Standing, crouching, or kneeling
	Restrained	2	Child's legs are being held down

Figure 1. CHEOPS Score: SUM (points for all 6 parameters). Minimum score: 4 (min pain); Maximum score: 13 (max pain).

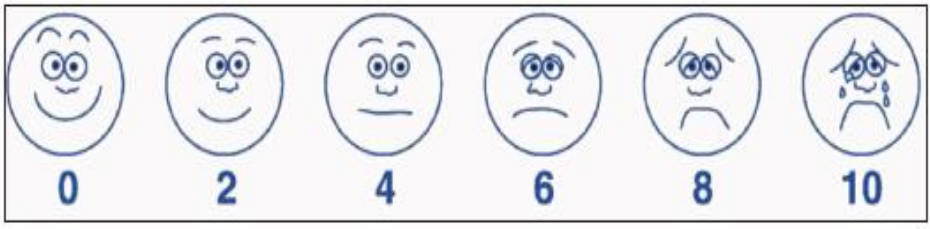
Figure 2. Objective Pain Scale (OPS) Minimum score: 0; Maximum score: 10 Maximum score if too young to complain of pain: 8. The higher the score the greater the degree of pain.

Parameter	Finding	Points
Systolic blood pressure	increase < 20% of preoperative blood pressure	0
	increase 20-30% of preoperative blood pressure	1
	increase > 30% of preoperative blood pressure	2
Crying	not crying	0
	responds to age appropriate nurturing (tender loving care)	1
	does not respond to nurturing	2
Movements	no movements relaxed	0
	restless moving about in bed constantly	1
	thrashing (moving wildly)	2
	rigid (stiff)	2
Agitation	asleep or calm	0
	can be comforted to lessent the agitation (mild)	1
	Cannot be comforted (hysterical)	2
Complains of pain	Asleep	0
	states no pain	0
	Cannot localize	1
	localizes pain	2

Chiratti, A. et.al. Current Practice and Recent Advances in Pediatric Pain Management. Eur Rev Med Pharmacol Sci Suppl. 2013; 1:112-26.

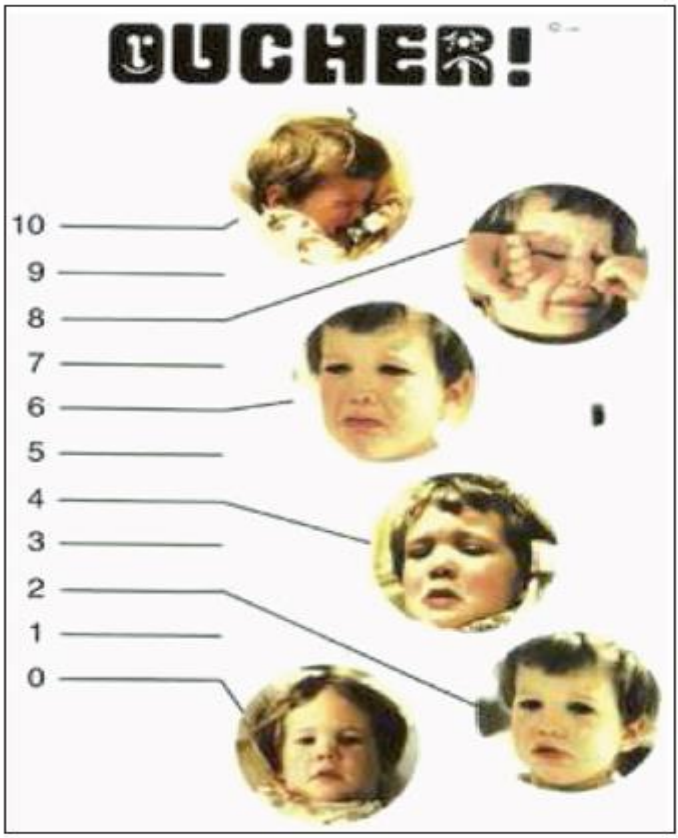


ŞEKİL 1: Gözden geçirilmiş yüzler ağrı ölçeği.

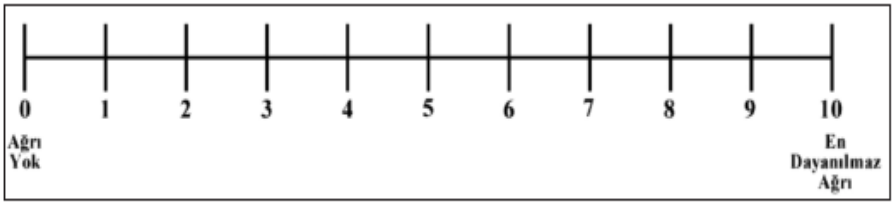


ŞEKİL 2: Wong ve Baker Yüzler Ağrı Ölçeği.

Tomlinson D, von Baeyer CL, Stinson JN, Sung L. A systematic review of faces scales for the self-report of pain intensity in children. Pediatrics 2010;126(5):e1168-98. act; Copper, E. (2017). Pain Assessment and Scales in Children: Effects of Culture and Age on Pain Assessment. Turkey Clinics J Nurs Sci 2017;9(4):299-314.



ŞEKİL 3: Orjinal Oucher.



ŞEKİL 4: Görsel analog skala.

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CHEOPS variables	Score 0	Score 1	Score 2
Cry	No	Crying, moaning	Scream
Facial	Smile	Neutral	Grimace
Verbal	Positive statement	Negative statement	Suffering from pain
Torso	Neutral	Variable, upright	Stretched
Legs	Neutral	Continuous move kicking	Stretched

PARENTS' POSTOPERATIVE PAIN MEASURE (PPPM)

Children sometimes have changes in behavior when recovering from surgery. The following is a list of behaviors that your child may or may not have exhibited while recovering from surgery between _____ and _____ today. For each of the behaviors below, circle the appropriate response, *yes* or *no*.

When your child was recovering from surgery between _____ and _____ today, did s/he . . .

1)	Whine or complain more than usual?	Yes	No
2)	Cry more easily than usual?	Yes	No
3)	Play less than usual?	Yes	No
4)	Not do the things s/he normally does?	Yes	No
5)	Act more worried than usual?	Yes	No
6)	Act more quiet than usual?	Yes	No
7)	Have less energy than usual?	Yes	No
8)	Refuse to eat?	Yes	No
9)	Eat less than usual?	Yes	No
10)	Hold the sore part of his/her body?	Yes	No
11)	Try not to bump the sore part of his/her body?	Yes	No
12)	Groan or moan more than usual?	Yes	No
13)	Look more flushed than usual?	Yes	No
14)	Want to be close to you more than usual?	Yes	No
15)	Take medication when s/he normally refuses?	Yes	No

www.google.com/, CHEOPS Scales visuals; date of access; 07 May 2023
www.google.com/ , PPPM Scales visuals; date of access; 07.05.2023

Conclusion

Evidence-based practices related to reducing acute pain in pediatric age groups have started to attract attention in the field of nursing, but the number of evidence-based studies should be increased in order to find a wider application area and to be more accepted in nursing. It is important for children to experience less pain in painful procedures and to ensure their comfort. In addition, it guides nurses about evidence-based practices related to the definition of pain that they can apply during painful intervention.

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Chapter 2

Is the Prognosis Predictable in Covid-19? Can It Contribute to the Treatment?

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ABSTRACT

Objective: The clinical course of the Coronavirus-2019 (Covid-19) disease is very variable and constituted an important part of health expenditures.It is not known which patient has a severe course, which results in death. Our study aim was to determine poor prognosis in patients with Covid-19 infection.

Methods: Information of hospitalized patients with Covid-19 infection confirmed by polymerase chain reaction between March and December 2019 were recorded from the hospital system. It were evaluated as good prognosis who were discharged. In addition who received to Intensive Care Unit (ICU), and died were evaluated with poor prognoses.Neutrophil, leukocyte, lymphocyte, neutrophil/lymphocyte ratio, D-dimer, C-reactive protein, and Aspartate-Aminotransferase levels of the two groups were compared. Non-contrast computed tomography reports were evaluated. Logistic multivariate regression models adjusted for age and sex were constructed to analyze independent predictive factors associated with good or poor prognosis.

Results:In this study, 336 Covid 19 patients were recorded. The mean age was 60.5±15.4 and 60.1% were male. The mean duration of hospitalization was 6.9±5.0 days.Our rate of recovery/discharge was 75%, and 21 patients(6.3%) were transferred to the relevant services due to comorbid disease; 58 patients were admitted to the ICU(17.3%) and five patients died(1.5). The rate of pneumonia cases was 70.5%, and 82.4% had a comorbid disease.A significant positive correlation was found between neutrophil, leukocyte, C-reactive protein, D-dimer, Aspartate-Aminotransferase levels neutrophil/lymphocyte ratio, and poor prognosis.

Conclusions:It should be considering some parameters in determining the prognosis of Covid-19 patients. Thus, it will be possible to which group the expenditures will be provided.

Keywords: Covid-19, prognoses, neutrophil count, markers, health expenditures

INTRODUCTION

Descriptions of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have focused on patients with severe disease (Ahmet, 2021:246). Coronavirüs (Covid-19) is a global pandemic caused by severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) (Ahmet, 2021:246). The 2019-nCoV infection caused clusters of severe respiratory illness similar to severe acute respiratory syndrome coronavirus and was associated with Intensive care unit (ICU) admission and high mortality (Huang, 22029:498). Major gaps in our knowledge of the origin, epidemiology, duration of human transmission, and clinical spectrum of the disease need fulfillment by future studies (Huang, 22029:499).

The mortality rate of this coronavirus has been reported at 3-4% (Guan, 2020: 1862). Although mortality due to Covid -19 is around 2%, this rate can rise to 40% in patients with a severe course, especially at those risk and requiring hospitalization, and up to 80% in the critical patient group followed up due to mechanical ventilation in the ICU (Tekin, 2021:153, Grasselli, 2020:1348, Yang, 2020:478). The case fatality rate in Turkey has been reported as 2.2% (Tekin, 2021:154). For more than two years, the covid-19 pandemic and its negative consequences have been experienced all over the world. When a new disease emerges, it is very important to know which patient group is most at risk (Udwadia, 2020:57). Predicting which patient will have a severe illness will be beneficial in terms of follow-up and mortality (Siso´-Almirall, 2020:3). It has been reported that clinical, biological, radiological features, evolution, and prognostic factors of patients with Covid-19 disease are important (Siso´-Almirall, 2020:5).

The most common signs of infection are respiratory symptoms such as fever, cough, and shortness of breath. In more severe cases, the infection can also cause pneumonia, severe acute respiratory syndrome, kidney failure, and death (Guan, 2020:1862). As the morbidity, mortality, and effects on health systems of Covid-19 patients are observed, it is clear that clinical laboratories will play an important role and contribute to prognosis, follow-up, and treatments (Ahmed, 2021:350). Studies have been conducted describing the characteristics of an outpatient population tested for SARS-CoV-2.

This study aimed to describe the poor prognosis of hospitalized patients with Covid-19 infection with clinical, biological, and radiological signs and to identify the most important predictors of poor prognosis.

MATERIAL AND METHODS

Ethic: The necessary approval for the study was obtained from the Akdeniz University's Clinical Research Ethics Committee (Decision number: 28.04.2021/221) and Helsinki Declaration ethical principles were followed. In addition, it was also approved by the Ministry of Health. (https://bilimselarastirma.saglik.gov.tr/_layouts/15/FormServer.aspx?XmlLocation =/2021-03-03T15_05_06.xm)

Study design: Information of hospitalized patients with Covid-19 infection confirmed by polymerase chain reaction between March and December 2019 was recorded from the hospital system. Firstly, in initial admission to the hematological/biochemical parameters were recorded.

In order to evaluate the changes in the examinations during the hospitalization, the second measurements made before discharge/ex were also recorded in the database.

Laboratory parameters: Neutrophil, leukocyte, lymphocyte, monocyte levels, neutrophil/lymphocyte ratio (NLR) as hematological parameters, and D-dimer, C-reactive protein (CRP), Aspartate-Aminotransferase (AST), troponin levels as biochemical parameters were recorded. In our study, the systemic immune inflammation index (SII) was calculated (neutrophil × platelet count)/lymphocyte by formula (Wang, 2021:155). In addition, NLR was also recorded. In order to determine the change in these parameters, the difference between the last measurement and the first measurement was evaluated.

Those who were discharged with recovery were evaluated as good prognosis, those who went to ICU and those who died were evaluated as poor prognoses. Charlson Comorbidity Index was used for comorbidities (Charlsona, 2022:15). Patients (n=21) taken over by the relevant services due to comorbid disease were excluded from this study.

The changes in hematological/biochemical parameters in the good and poor prognosis groups were compared.

Chest computed tomography: In addition, non-contrast computed tomography (CT) scans of Covid-19 patients were evaluated. Ground glass appearance Involvement of more than two lobes was evaluated as diffuse, if there was the involvement of one lobe, it was considered as localized involvement. Consolidation area, atelectasis, and emphysema findings were recorded radiologically.

Statistical analysis: Analyzes were made in SPSS 22.0 program; Categorical variables are presented as absolute frequencies and percentages (%) and continuous variables as means and standard deviations (SD). The t-test was applied to the dependent groups to compare the means, and hematological/biochemical parameters that correlated significantly with prognosis were determined, and the

chi-square test was performed for categorical variables. Findings were compared with logistic regression analysis. Prognoses status was included as the "dependent" variable in the model. Age, gender, number of comorbidities, ICU admission and observed changes in hematological/biochemical parameters were included as independent variables.

RESULTS

Data of 336 patients were evaluated. The mean age in the study was 60.5±15.4 years, 60.1% male. The mean ages of male and female patients were similar (60.2 and 60.7, respectively). Only 23.8% of the cases had a history of contact. History of contact with a patient with Covid-19 was higher in women than in men (32.4% vs 18.0%), (p=0.009). There was at least one comorbid disease in 82.4% of cases and at least two comorbid diseases in 51.8% of cases (Table 1).

Table 1. According to clinical characteristics distribution of Covid-19 patients hospitalized in the pandemic clinic

		n	%
Gender	Female	134	39.9
	Male	202	60.1
Contact history	Presence	60	23.8
Pneumonia	Presence	237	70.5
Comorbid diseases	At least 1	277	82.4
	≥2	174	51.8
Complaint	Cough	156	47.3
	Dyspnea	149	45.2
	Fever	131	39.7
	Nausea, Vomiting	55	16.7
	Sore throat, runny nose	33	10.0
	Chest pain	8	2.4

The respiratory system was evaluated. The mean respiratory rate of the patients was 24.2±5.8 (min 16-max 48) and the mean oxygen saturation was 92.9±5.8 (min 60-max 100).

The general condition of 81.8% of patients was good when hospitalized. The most frequent clinical symptoms were dyspnoea (45.2%), and fever (39.7%). Taste/smell disorder, which is one of the symptoms of Covid- 19, is only 5.2%.

Physical examination was shown in 49.0% of patients with auscultatory alterations (46.0% ral and 6.0% rhonchi).

Non-contrast CT was performed on 294 of 336 patients. When non-contrast CT findings were evaluated, only 1.4% were normal. The most important factors for ICU admission and death were determined diffuse (23.5%) and localized (51.0%) ground glass appearance. Radiologically, consolidation area was observed in 10.9%. atelectasis was 1.7% and emphysema 1.4%. Pneumonia was found in 70.5% of the patients, and 42.7% of the oxygen requirement was found. The presence of pneumonia is close to each other in men and women (67.9% and 72.3%, respectively; p=0.39).

The mean hospitalization period of the patients in the pandemic clinic was 6.9±5.0 days (min 2-max 48, median=5). Totally 252 patients were discharged with recovery (75.0%), 21 patients (6.3%) were taken over by the relevant services due to comorbidity; 58 patients were taken to the ICU (17.3%)

The poor prognosis group consisted of 63 patients, five of whom died (1.5%), and 58 patients who were taken to the ICU. The first and second measurement results of the examinations of the patients were compared and the data were given in Table 2. When the data were examined, a significant change was observed in only eight parameters. A significant increase in the glomerular filtration rate, thrombocyte, lymphocyte, and monocyte levels in the second measurement compared to the baseline; On the other hand, a significant decrease was observed in CRP, troponin, Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) levels.

Table 2. Changes in first and last hematological/biochemical parameters

		First measurement	Last measurement	
				p
İncrease	GFR*	77.31	80.67	0,000
	Platelet	218.7	288.7	0,000
	Lymphocyte	1.31	1.56	0,000
	Monocyte	0.58	0.65	0,001
Decrease	CRP*	87.18	52.57	0,000
	MPV*	10.58	10.44	0,000
	PDW*	12.28	12.01	0,011
	Troponin	322.31	104.60	0,015

*GFR: Glomerular filtration rate, CRP: C-Reaktif Protein, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width

The first and last measurement results in the parameters examined in both groups were presented and compared in Table-3. Changes between the last and first measurements were also compared between the good and poor prognosis groups. When the changes in hematological parameters were examined; a significant increase in neutrophil and leukocyte levels was observed in the group with a poor prognosis. In addition, a significant increase in SII and NLR rates was observed in the same group. In the good prognosis group, a significant increase was observed in lymphocyte levels, whereas a significant decrease was observed in neutrophil levels, NLR ratio, and CRP and D-dimer levels. The two groups were compared in terms of changes in hematological and biochemical parameters (Last measurement-first measurement) (Table 3). The increase in neutrophil and leukocyte counts, and in SII and NLR were remarkable findings of poor prognosis. The change in D-dimer level was borderline. In summary, in addition to hematological parameters, CRP, AST and D-dimer levels were associated with prognosis.

Table 3. Comparison of the changes in the biochemical tests of the patients according to the prognosis status

	Measurement	**Good Prognosis** (n=252)		**Poor Prognosis** (n=63)		Comparison of changes (p)
		mean	change	mean	change	
Neutrophil	First	5.73	**-0.56**	6.62	**2.68**	**0,000**
	Last	5.17		9.31		
	p	**0,013**		**0,000**		
Leukocyte	First	7.77	**-0.10**	8.47	**2.66**	**0,000**
	Last	7.66		11.14		
	P	0,660		**0,000**		
Lymphocyte	First	1.36	**0.30**	1.21	**-0.64**	**0,000**
	Last	1.66		1.14		
	p	**0,000**		0,553		
SII*	First	1541.39	**-79.76**	1701.18	**1819.84**	**0,000**
	Last	1461.61		3521.02		
	p	0,667		**0,001**		
NLR*	First	6.91	**-2.31**	8.28	**5.61**	**0,000**
	Last	4.59		13.89		
	p	**0,003**		**0,001**		
CRP*	First	79.93	**-41.86**	111.62	**0.60**	**0,000**
	Last	38.07		112.22		
	p	**0,000**		0,952		
AST*	First	52.60	**-14.67**	39.05	**57.76**	**0,016**
	Last	37.92		96.81		
	p	0,224		0,094		
D-dimer	First	3.35	**-1.68**	3.72	**0.99**	0,065
	Last	1.67		4.71		
	p	**0,045**		0,432		

*SII: Systemic Immune Inflammation Index, NLR: Neutrophil/Lymphocyte Ratio
CRP: C-Reactive Protein, AST: Aspartate Aminotransferase

In order to determine the hematological/biochemical factors affecting the prognosis, the correlation was evaluated between the changes first and last examination of the parameters. When the data were examined; a significant positive correlation was found between neutrophil, leukocyte levels, NLR and SII ratios, CRP and AST levels and poor prognosis. A negative correlation was found between lymphocyte and oxygen saturation levels and poor prognosis (Table 4)

Table 4. Factors significantly correlated with poor prognosis

With poor prognosis		p	r*
Parameters that change in the direction of increase	**Neutrophil**	0,000	,306
	Leukocyte	0,000	,263
	NLR **	0,000	,253
	CRP **	0,000	,251
	SII **	0,000	,230
	AST **	0,016	,138
Parameters that change in the direction of decrease	**Saturation**	0,001	-,297
	Lymphocyte	0,000	-,204

* Pearson correlation coefficient

**SII: Systemic Immune Inflammation Index, NLR: Neutrophil/Lymphocyte Ratio
CRP: C-Reactive Protein AST: Aspartate Aminotransferase

When logistic regression analysis was performed to determine the parameters affecting the prognosis, four variables that had a significant effect on the poor prognosis were determined. The high age of the patient and the increase in the first neutrophil, AST, and CRP levels were determined as factors that significantly increased the poor prognosis.

The rate of patient recovery was 75.0% in our study, and 63 patients with poor prognoses were admitted to the ICU (18.75%) or 5 patients (1.5%) died. When the parameters associated with poor prognosis were examined, increases in the neutrophil and AST levels take the first two places. Gender, number of comorbidities, GFR, creatinine, PDW, MPV, lymphocyte levels, and SII and NLR were not found to have significant effects on prognosis.

DISCUSSION

Identification of risk factors that will be decisive for the severe course and ICU follow-up is very important in the correct management of Covid-19 patients and the pandemic (Huang, 2020:503). In order to use the health service, which is struggling in the face of the increasing patient load, in the most effective way, it is necessary to clearly distinguish the risky patients (Huang,

2020:503). More than two years have passed, and many parameters to be used to identify patients at risk have also been defined. This study summarizes the clinical, biological, and radiological characteristics, evolution, and prognostic factors of patients with Covid-19 disease.

Clinically, in previous studies, dyspnea and fever have been reported as the main symptoms, and anosmia is less common (Siso´-Almirall, 2020:5). In our study, cough, dyspnea and fever were the most common findings in patients, and anosmia was observed less frequently. However, in the other study, it has been reported nearly 20% of patients had anosmia (Be´ne´zit F, 2020:1015). The anosmia rate was 5.2% in our study.

In studies conducted in large series, it has been reported that older age and male gender are predisposed to a higher mortality rate (Guan, 2020:1861, Richardson, 2020:2055). In our study, advanced age was found among the causes that increase mortality**.** The same comorbidities were identified, with hypertension and diabetes being the two most common, in the USA and Italy (CDC, 2020:345, Sorbello, 2020:727). In our study hypertension (%32.4) and diabetes (%27.7) rates were most high for comorbidity.

Lymphocytes and CRP have been reported as strong predictors of death in these patients (Richardson, 2020:2057). Several laboratory indicators have been shown to predict a higher risk of patient mortality (Udwadia, 2020:58). It has been reported that leukocytosis and lymphocytopenia are more common in patients who died in the second and third weeks of the disease than in survivors (Wang, 2020:1065). In another study biologically, lymphopenia and increased CRP, LDH and D-dimer were usually constant and similar in previous studies and were associated with an increased risk of mortality (Siso´-Almirall, 2020: 6). In our study, high leukocyte and CRP levels were observed in the poor prognosis group. In addition, a decrease in lymphocyte levels was remarkable in the poor prognosis group. It has been reported that NLR was observed as an independent risk factor for poor outcomes (Yang, 2020:6). In our study, a significant increase in NLR was observed in the poor prognosis group.

In one study, D-dimer was shown to be one of the best parameters to predict in-hospital mortality in Covid-19 patients. In Covid -19 cases, D-dimer concentrations were markedly greater (4.6 μg/mL) as compared to patients who recovered (0.6 μg/mL), emphasizing the role of D-dimer as a predictor of death in Covid-19 (Zhang, 2020:1327). In our study, D-dimer concentrations were markedly greater (3.35 μg/mL) as compared to patients who recovered (1.67 μg/mL), emphasizing the role of D-dimer as a poor prognosis group.

An international consensus states that radiological assessment is not necessary for asymptomatic patients or those with a mild disease but is required

in patients with moderate or severe disease, regardless of whether a definite diagnosis of Covid-19 has been made (Chen, 2020:368). In our study, non-contrast CT was performed in 294 of 336 patients who were positive for Covid PCR. Only 1.4% were normal. Bilateral pneumonia is the most common radiological finding, is present in more than half the cases, and is a factor in a poor prognosis and mortality (Chen, 2020:368). In our study, pneumonia was detected in 70.5% of the patients. Diffuse (23.5%) and localized (51.0%) ground-glass appearance, which may be an indicator of poor prognosis, was determined. Our findings were compatible with the literature.

In previous studies, ICU admission rates have been reported to be 10-15% (Siso´-Almirall, 2020:8, CDC 2020:346, Rubin, 2020:110). In our study, this rate was 17.3%, and our results were closely the other studies. In previous studies, it has been reported the mortality rate was 5.6%, and 10.2% (Siso´-Almirall, 2020: 6, Rubin, 2020:114). In our study, this rate was 1.5%. In our study, mortality was quite low compared to the literature.

Limitations: The study had some limitations due to the observational, retrospective design. It was not sufficiently representative of the population with confirmed Covid-19 to better identification of the factors of a poor prognosis of the disease from a clinical perspective. Even though in real clinical practice these percentages may be expected, the results corresponding to laboratory parameters should be interpreted with caution.

Conclusion: The clinical, biological, and radiological characteristics of probable cases of Covid-19 infection will be key to determining prognostic factors in the prognosis of the disease. Besides clinical findings, biological parameters such as neutrophil, lymphocyte, NLR, SII, and CRP levels can help predict disease progression and predict poor outcomes. In addition, non-contrast computed tomography will also give important clues in determining the prognosis in moderate and severe patients.

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Chapter 3

The Low-Frequency Mutation Significantly Affect Gene Expression of Isocitrate Dehydrogenase1 and Isocitrate Dehydrogenase2 in the Common Human Cancers

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ABSTRACT

Isocitrate dehydrogenase (IDH) enzyme converts isocitrate to α-ketoglutarate (α-KG) (or vice versa) and manages crucial control points in the TCA cycle. There are three protein isoforms, IDH1, IDH2, and IDH3. In addition to wild-type isoforms, the mutant IDH enzymes perform oncogenic activities. Mutations in the IDH1 and IDH2 genes lead to a new function, the production of 2-hydroxyglutarate (2-HG) oncometabolite. This mutation frequency is different according to cancer type. The aim of present study was to explore the mutation frequency of IDH1 and IDH2 in the ten common cancer types by using a bioinformatic tool, cBioportal for Cancer Genomics. In addition, the impact of IDH1 and IDH2 mutation status in gene expression pattern was examined in publicly available data deposited at the TCGA datasets. The results showed that cumulative alteration rate in IDH isoforms was in the range of 5.8-0.2%. Most of the mutations are missense, as well as truncating, inframe, and splice mutations are also seen. The gene expression level of IDH1 or IDH2 was increased or decreased after mutation in all studied cancer types. In conclusion, this study revealed that the mutation frequency in the IDH1 and IDH2 are low and cancer type-specific and directly affect the gene expression level in the ten common cancer types.

Keywords: Cancer, expression, gene, isocitrate dehydrogenase, mutation.

INTRODUCTION

Isocitrate dehydrogenase (IDH) is a key metabolic enzyme in the TCA cycle (D'Adamo and Haft, 1965:613-617). The essential role of IDH carries out the conversion reaction of α-ketoglutarate (α-KG) to citrate in mitochondria and cytoplasm. In addition to α-KG decarboxylation, IDH is involved in essential reactions in the metabolism, such as lipid synthesis, cell defence against oxidative stress, and oxidative respiration (Reitman and Yan, 2010:932-941). Importantly, this enzyme has a role in biomass and NAD^+/$NADP^+$ production (Barnes et al., 1971:3939-3944). There are three IDH isoforms, IDH1, IDH2, and IDH3 and three IDH3 subunits in eucaryotic cells (Dalziel, 1980:45-55). IDH1 and IDH2 (EC code: 1.1.1.41 and 1.1.1.42) show similarity in the gene sequence (70% identity in humans), molecular structure, catalytic mechanism and cofactor requirement ($NADP^+$), but their subcellular localization is different (Gabriel et al., 1986:661-667; Pollard and Ratcliffe, 2009:192-194). The homodimeric IDH1 and IDH2 enzymes reversibly catalyze decarboxylation reactions in cytoplasm and mitochondria, respectively (Pollard and Ratcliffe, 2009:192-194). The heterotetrameric IDH3 is an NAD^+-dependent enzyme and catalyzes the irreversible reaction in the mitochondrial matrix (Gabriel et al., 1986:661-667).

Point mutations in arginine residues (Arg132, Arg140, and Arg172) of IDH enzymes (IDH1/2) lead to changes in enzymatic activity. Thus, mutant IDH enzymes acquire neomorphic enzymatic activity and reduce α-KG to 2-Hydroxyglutarate (2-HG) (Dang et al., 2009:739-744). The incidence of these mutations is very high (~70-80%) in glioma (grade II-III) and secondary glioblastoma multiforme, is less frequent (~20%) in acute myeloid leukaemia (AML) and is low (<10%) in some cancer types such as colon, lung, and prostate (Parsons et al., 2008:1807-1812; Mardis et al., 2009:1058-1066; Sjöblom et al., 2006:268-274; Sequist et al., 2011:2616-2624; Kang et al., 2009:353-355). Some characteristics of mutations in IDH1 and IDH2 are prevalent in different cancer types. These mutations are somatic, heterozygous, one allele has gain-of-function and the other allele remains wild-type (wt), and they share a common biochemical mechanism (Ward et al., 2010:225-234). The concentration of 2-HG is quite low in a healthy cell. However, this metabolite is highly produced in various cancer types and affects many metabolic and epigenetic pathways in cell metabolism. Because of the structurally similar to α-KG, 2-HG competitively inhibit α-KG-dependent dioxygenase responsible for biosynthesis, post-translational modifications, and epigenetic modifications (Xu et al., 2011:17-30; Lu et al., 2012:474-478). Thus, 2-HG promotes cancer initiation or/and progression (Xu et al., 2011:17-30; Atalay and Kayalı, 2022:191-199).

In cancer disease, cells undergo metabolic and behavioral changes due to mutations in a multistage process. (Hanahan and Weinberg, 2000:57-70). In addition to protooncogenes or oncogenes, oncogenic mutations in metabolic enzymes such as IDH play a role in cancer pathogenesis (Nadhan et al., 2023:618). Cancer incidence has increased in recent years and has become the most common disease worldwide after significant advances in the treatment of heart disease (Twombly, 2005:330-331). As is known, there are more than 200 types of cancer. Breast, lung, cervix, colon, liver, prostate, esophageal and stomach cancer are the most common types of cancer that cause death in man and women (Sung et al., 2021:209-249; Chhikara et al. 2023:451). However, the gene expression pattern changes after mutation in the IDH was not studied in the most common cancer types. The present study analyzed mutation frequency in the IDH1 and IDH2 genes on the ten common human cancers using a bioinformatic tool, cBioPortal for Cancer Genomics (Cerami et al. 2012:401-404). Then, the effect of mutation on gene expression pattern was investigated in these cancer types. The results showed that even mutation frequency is low among all studied cancer types, the gene expression level of IDH1 or IDH2 is significantly affected by the mutation in these cancers.

MATERIAL AND METHODS

Analyze the mutation status

The genetic alterations ratio of IDH1 and IDH2 were examined on the cBioPortal for Cancer Genomics (Cerami et al. 2012:401-404) of the ten common cancer types. BLCA (Bladder Urothelial Carcinoma), BRCA (Breast Invasive Carcinoma), COAD (Colon adenocarcinoma), ESCA (Esophageal carcinoma), KIRC (kidney renal clear cell carcinoma), LUAD (Lung Adenocarcinoma), LUSC (Lung Squamous Cell Carcinoma), PAAD (Pancreas adenocarcinoma), PRAD (Prostate Adenocarcinoma), and STAD (Stomach adenocarcinoma) samples in the TCGA PanCancer Atlas were chosen as samples queries. Totally 4954 samples were analyzed. Genetic alterations, which include both mutations and copy numbers, were given in Figure 1A-B and include only mutations were given in Figure 1C-D. The mutation types and locations in the IDH1 and IDH2 in ten common cancer types were investigated using the “Oncoprint” function of the cBioPortal for Cancer Genomics, and results are given in Figure 2. In addition, the mutation frequencies in the IDH1 and IDH2 in ten common cancer types were also determined using the “Mutation” function of the cBioPortal for Cancer Genomics, and results were given in Figure 3.

Gene expression analysis according to mutation status

The "Plot" function of the cBioPortal for Cancer Genomics was used to analyze the effect of mutations on the gene expression of IDH1 and IDH2 in ten common cancer types. All boxplots that contain expression comparisons between mutation and non-mutant cases were combined in Figure 4.

RESULTS

The mutations frequency in IDH1 and IDH2 isoforms were low in the ten cancer types

The genetic alteration status (including mutations and copy numbers) of IDH1 and IDH2 in the ten common cancer types was examined using PanCancer Atlas data (4954 samples) in cBioPortal for Cancer Genomics (Cerami et al. 2012:401-404). In addition, mutation types and locations on IDH1 and IDH2 genes were investigated using the same web tool.

The cumulative alteration rates revealed that the mutations frequency in both IDH isoforms were low in all studied cancer types. Among the two isoforms, the highest mutation and copy number alterations were observed in the IDH2, with a cumulative alteration rate of 5.8% in stomach cancer (Figure 1B). Cumulative alteration rate in the IDH1 was observed in the range of 3.6-0.98% (Figure 1A). Focusing on only mutation, the mutation rate of the IDH1 gene is 2.2-0.5% (Figure 1C). Similar to the IDH1 gene, the highest mutation rate of IDH2 is 2.2% (in colorectal cancer). There is no mutation in breast and prostate cancer types in the IDH2 gene (Figure 1D). It has been found that IDH1 mainly contains missense mutations (30) (Figure 2; Figure 3A). Most of the IDH2 mutations are missense (16), and truncating (4), inframe (1), and splice (2) mutations are also seen (Figure 2; Figure 3B). The locations of mutations in the IDH isoforms are also included in Figure 2. There had been mutational overlaps in the IDH isoforms (Figure 2).

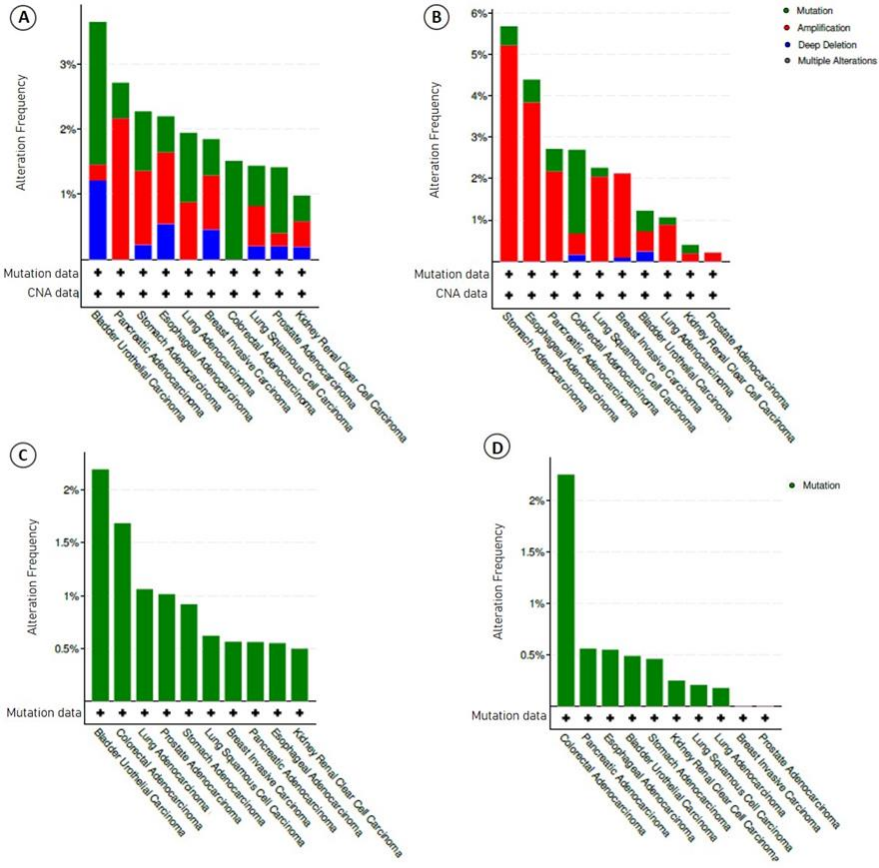

Figure 1: Alteration frequency of IDH isoforms in TCGA PanCancer datasets. The cumulative alteration rate of IDH1 (A) and IDH2 (B) was analyzed through cBioPortal web tool by using samples (*n*=4954) in the TCGA PanCancer Atlas Study. The only mutation frequency in the IDH1 (C) and IDH2 (D) isoforms were analyzed through cBioPortal web tool by using samples (*n*=4954) in the TCGA PanCancer Atlas Study.

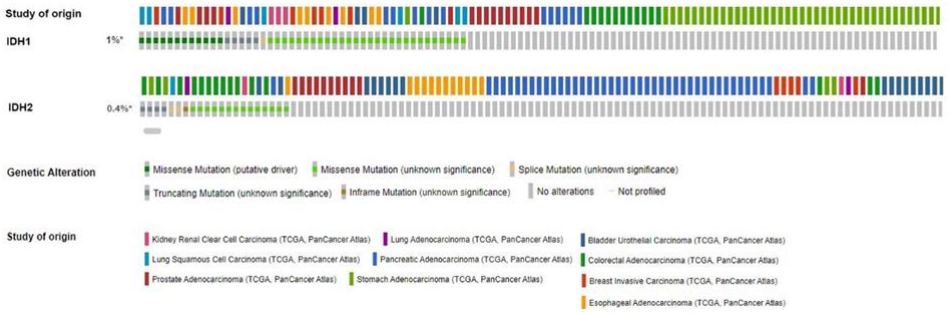

Figure 2: Genetic alterations of IDH1 and IDH2 in the ten human cancer types. The mutation types and locations on IDH1 and IDH2 genes were shown in the Oncoprint image.

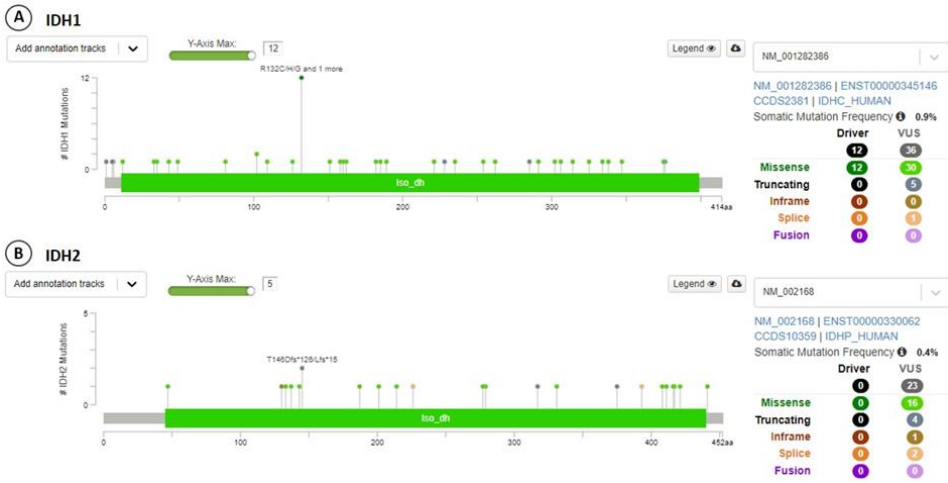

Figure 3: Mutation types and frequencies on IDH1 and IDH2 in the ten common cancer types. The figures represent the mutation frequencies of the most common mutations found on the IDH1 (A) and IDH2 (B) isoforms.

The mutations in the IDH isoforms directly affect the gene expression

In the next step, the effect of mutations on the gene expression of IDH isoforms was examined through the cBioPortal for Cancer Genomics. The IDH1 expression was moderately increased after mutations in the BLCA, COAD, KIRC, and STAD (Figure 4A, C, E, J), and it was highly increased in ESCA (Figure 4D). The IDH1 expression was moderately decreased after mutations in the LUAD, LUSC, and PRAD (Figure 4F, G, I). Mutations in the IDH1 isoforms did not affect the expression of the IDH1 gene in the BRCA and PAAD (Figure 4 B, H). The IDH2 expression was highly increased after mutations in the BLCA (Figure 4K), and it was highly decreased in the ESCA, LUAD, and STAD (Figure

4M, O, S). The IDH2 expression was moderately decreased after mutations in the KIRC and PAAD (Figure 4N, R). Mutations in the IDH2 isoforms did not affect the expression of the IDH2 gene in the COAD and LUSC (Figure 4L, P).

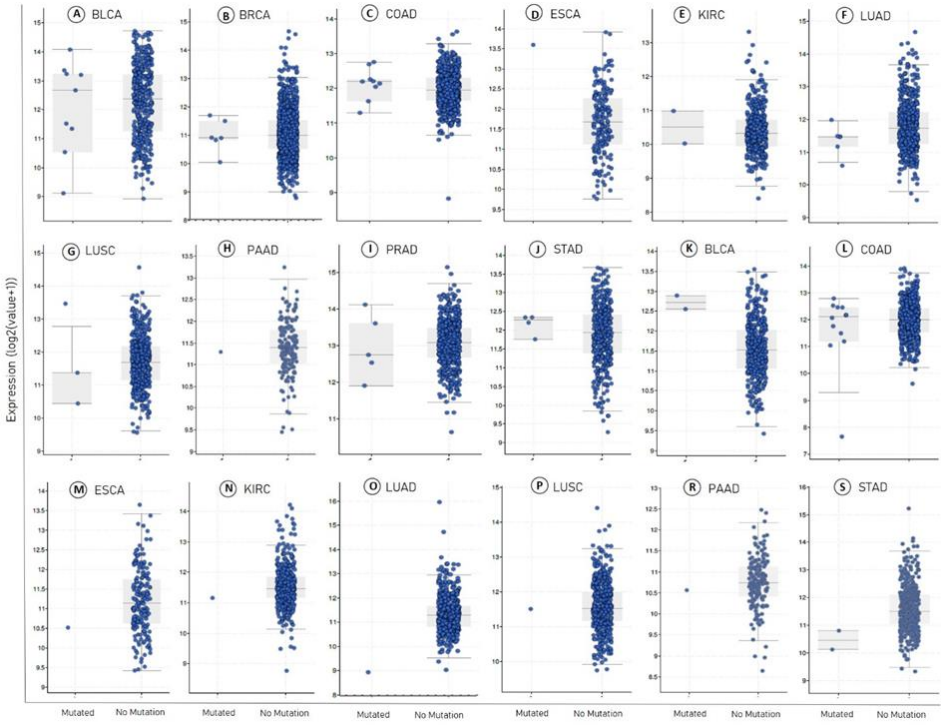

Figure 4: The mutations in the IDH1 and IDH2 genes directly affect the gene expression. Box plots exhibit the expression level of IDH1 (A-J) and IDH2 (K-S) in IDH1 (A-J) and IDH2 (K-S) mutant and non-mutant bladder, breast, colorectal, esophageal, kidney, lung, pancreas, prostate, and stomach cancers.

DISCUSSION

IDH carry out reversible interconversion of isocitrate to α-KG in the TCA cycle (D'Adamo and Haft, 1965:613-617). This enzyme lies at a key branch point in carbohydrate metabolism (Hurley, 1989:8635-8639). In addition to its potential role in the TCA cycle, mitochondrial and cytosolic IDH isoforms serve diverse biological functions in cellular defence against oxidative damage, as a source of NADH and NADPH and play a key role in lipid metabolism (Jo et al., 2001:16168-16176; Koh et al., 2004:39968-39974). Metabolic rearrangement is one of the hallmarks of cancer (Hanahan and Weinberg, 2000:57-70). IDH is one of the disease-causing gene in the metabolism. The literature shows that wild-type IDH isoforms (IDH1 and IDH2) have a role in the growth and progression

of tumors such as breast, colon, esophageal, lung, and pancreas (Atalay et al. 2023:1-17; Špačková et al. 2021:1709; Li et al. 2023:11031; Chen et al. 2017:700; Zarei et al. 2023:03). Our previous study showed that wild type IDH1 can be a potential therapeutic target for colon cancer (Atalay et al., 2023:1-17). In this study, we studied mutant IDH isoforms (IDH1 and IDH2), not their wild-type counterparts. The mutation frequency and gene expression pattern changes in the IDH1 and IDH2 were investigated on the 10 common human cancers using a publicly available dataset. According to bioinformatic data, we showed that the mutation frequency was low in all studied cancer types (Figure 1) and the gene expression of IDH1 or IDH2 was significantly changed (increased or decreased) after mutations in all studied cancer types (Figure 4).

In addition to protooncogenes or oncogenes, oncogenic mutations in metabolic enzymes such as IDH play a role in cancer pathogenesis (Nadhan et al., 2023:618). The heterozygous point mutation was first discovered in colon cancer in 2006 (Sjöblom et al., 2006:268-274). Then, this mutation was found in glioblastoma as a result of a cancer genome project (Parsons et al., 2008:1807-1812). In the following years, mutant IDH1/2 enzymes were detected in different cancers, such as lung and prostate (Sequist et al., 2011:2616-2624; Kang et al., 2009:353-355). Tumor-based mutations in the active sites of IDH1/2 affect the catalytic activity of the enzyme, and mutant enzymes catalyze the conversion from α-KG to 2-HG, which plays a role in carcinogenesis. In this case, 2-HG role as oncometabolite and mutant IDH role as an oncogene (Xu et al., 2011:17-30). In addition, IDH acts as a tumor suppressor where a mutation cause loss of function (Pollard and Ratcliffe, 2009:192-194). The mutation in the IDH3 isoform does not occur at an appreciable frequency (Krell et al., 2011:19868). Therefore, in our study, the mutation frequency were analyzed in IDH1 and IDH2 isoforms. The results showed that cumulative alteration frequency is low (5.8-0.2%), and mutation was found to be different among all studied cancer types (Figure 1). The highest mutation in IDH1 was found in bladder cancer (2.2%), but there wasn't any mutation in the IDH2 gene in this cancer type (Figure 1). After analysing amplification in addition to mutation, the cumulative alteration rate was increased to 5.8% in stomach cancer (Figure 1).

IDH1 and IDH2 mutations are very critical for metabolism because they change the structural organization of the active site, and enzyme affinity increases to NADPH, not $NADP^+$. Diminished IDH1 activity result in a decrease in α-KG and NADPH levels and deficient carbon flux into the lipid mechanism. Correspondingly, the level of ROS and histone methylation increases in metabolism (Calvert et al., 2017:1858-1873). In our study, the changes in IDH1 and IDH2 gene expression levels were investigated, and significant results were

obtained. The gene expression of IDH1 and IDH2 was significantly decreased in PRAD, ESCA, KIRC, LUAD, and STAD cancers (Figure 4 M, N, O, R, S) and increased in ESCA, STAD, and BLCA cancers (Figure 4 D, J, K).

In conclusion, it was determined that IDH1 and IDH2 mutations are very low in the breast, bladder, colorectal, esophageal, kidney, lung, prostate, pancreas, and stomach cancers. However, the level of IDH1 or IDH2 gene expressions significantly changed after mutation in these cancer types. In the future, IDH1 and IDH2 genes can be knocked out with target mutant points to decrease gene expression in ESCA, STAD, and BLCA cancers.

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Chapter 4

Adaptive Immunity In Type Two Diabetes Mellitus (T2dm) Patients With Sars-Cov-2 (Covid-19) Infection

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ABSTRACT

Introduction

The very first incidence of the coronavirus infection-2019, which brought on by the SARS-CoV-2 (severe acute respiratory syndrome) virus, and reported in Wuhan, China, in the end of December 2019. SARS-CoV-2 is a phylogenetic relative of the coronavirus 2002 (Kong et al. 2020). According to an epidemiological research carried out in China, the majority of Covid-19 patients experience moderate or uncomplicated illness. However, 14% of patients experience severe disease that requires hospitalization and oxygen assistance, and 5% need to be admitted to an intensive care unit (Team 2020). To date, 10 May 2022, there have been 515.748.861 cases of Covid-19 confirmed, including 6.255.835 deaths, reported to WHO. A total of 11.593.897.033 vaccine doses administered (WHO 2021). For 80% of symptomatic patients, the condition presents as moderate flu-like symptoms (such as fever and cough) and upper respiratory tract virus infection. For the remaining 20% of individuals, however, SARS-CoV-2 may spread to the lower respiratory tract, causing serious diseases such as pneumonia and acute respiratory distress syndrome (ARDS) (Lauer et al. 2019). Several studies have revealed that advanced age and male sex, as well as the comorbidity of chronic conditions such as hypertension, diabetes (type 1 and 2), cardiovascular and respiratory disorders, might raise the risk of severity and death in such a way that these susceptibilities can be correlated with the pathogenesis of Covid-19 infection. Notably, chronic diseases have certain characteristics, including an inflammatory state, immune response issues, and an increased propensity to contracting contagious diseases (Bloomgarden 2020, Holman et al. 2020, Chávez-Reyes et al. 2021, Rahmani-Kukia and Abbasi 2021).

Patients with diabetes, however, also had distinct immunological characteristics from those without diabetes (Han et al. 2021). Diabetes was the second highest incidence rate among chronic Covid-19 comorbidities (7.4 % to 19.0%), following hypertension (15% to 30%) (Xu et al. 2020). Several pathophysiological pathways behind the relationship between diabetes mellitus and SARS-CoV-2 are yet to be investigated. Few early investigations have demonstrated that underlying cardiovascular disease and diabetes are high among Covid-19 hospitalized to intensive care units (Piva et al. 2020). Limited data are available about the immunological and inflammatory response to Covid-19 in diabetic individuals. Diabetes patients with Covid-19 infection have been found to have increased vulnerability to develop an inflammation and severity of infection, which associated with a rise of critical care unit hospitalization. The cytokine storm usually accompanied with glucose

metabolism dysregulation, resulting in metabolic failure (Van Wyngene et al. 2018).

Both diseases characterized by increased obesity accompanied by persistent systemic low-grade inflammation, which induces immunological dysregulation and increases susceptibility to infection. However, the impact of high glucose level or DM on innate and aduptive immune response in Covid-19 patients is yet unclear, and more research needed. An increased in lymphocytopenia, immune system dysfunction, and cytokine storm was found in earlier studies on Middle East respiratory syndrome (MERS) and SARS-CoV (Yin and Wunderink 2018). According to study comparing patients with diabetes with healthy individuals during the Covid-19 infection, diabetic individuals were more likely to experience high blood glucose and lymphocytopenia and coupled with increased level of myoglobin, ferritin, D-dimer, and urea nitrogen. Diabetes patients also exhibited obviously raised mortality and cytokine levels (Han et al. 2021). In accordance with, a study on Covid-19 patients, the infected individuals had significantly decreased T cell counts, and the remaining T cells, seeming functionally fatigued (Diao et al. 2020). Some research on these patients showed impaired glucose metabolism and homeostasis (Korytkowski et al. 2020, Müller et al. 2021). Alteration in white blood cell population, and other immunity members (Kwiecień et al. 2021), in addition to the secretion of cytokines including interleukin (IL)-6, IL-1β and TNF-α (Viurcos-Sanabria and Escobedo 2021), leading to more susceptibility to infection, disease severity, and ultimately death. In addition, a research on convalescent patients with diabetes and other comorbidities indicated that Covid-19 patients had lower absolute counts and proportions of lymphocytes, monocytes, eosinophils, and basophils than convalescent patients in the study cohorts. Furthermore, changes were also observed in neutrophils activity (Kwiecień et al. 2021). According to another research of convalescents, patients at the early recovery stage (ERS) of Covid-19 patients had higher levels of monocytes; also, there was a larger proportion of classical CD14++ monocytes with high inflammatory gene expression as well as a higher number of CD14++ IL1+ monocytes (Wen et al. 2020).

Concerning all of the above-mentioned data, it is essential to explore the role and percentage of cellular immunity, especially members of innate immunity, which are considered to be the first line of defense against Covid-19 infection and other viral infections. Using quantitative real-time polymerase chain reaction (qRT-PCR), the aim of this study was to examine the expression of proinflammatory cytokine (IL-1 β), as an inflammatory mediator during the course of inflammation, from monocyte gene in Type 2 diabetes mellitus

(T2DM) recovered patients from SARS-CoV-2 infection in comparison to those without previous infection.

The characterization of the key cellular subsets and their states in Covid-19 is a crucial step in developing critical insights on the immune clearance mechanism, and understanding the pathophysiology of Covid-19 infection on the immunity and its association to T2DM, as well as the development of new Covid-19 treatments. Although, the mechanism by which immune cell subsets change during Covid-19 is still largely unknown.

Background

SARS-CoV-2 (Covid-19)

Since the beginning of the 21st century, coronaviruses have triggered lethal pneumonia outbreaks in people. SARS-CoV emerged in 2002 and caused an epidemic with a 10% death rate that spread to five continents before being contained in 2003 (with additional cases reported in 2004). MERS has emerged in 2012, in the Arabian Gulf, and has produced repeated outbreaks with a death rate of 35%. SARS and MERS are zoonotic viruses that crossed the species barrier with the help of bats/palm civets and dromedary camels, respectively (Tortorici and Veesler 2019).

Coronaviruses are members of the Nidovirales family Coronaviridae. Corona displays crown-like spikes on the outer surface of the virus; hence, it was designated a coronavirus. The family of Coronaviruses have genitic material as a single-stranded RNA with a size 26 to 32 kbs and tiny in diameter (65-125 nm) (Figure 2.1). The subgroups of the family of coronaviruses are alpha (α), beta (β), gamma (γ), and delta (δ), as well as omicron coronavirus. The transmission rate of SARS-CoV-2 is higher than that of SARS-CoV, and a genetic modification at the spike (S) protein in the receptor-binding domain (RBD) region of Covid-19 virus may be responsible for this kind of spreading rate (Shereen et al. 2020).

The initial step in infection is the binding of a virus to its target receptor on a host cell. Earlier, literature on SARS-CoV2 revealed that this virus primarily targets airway epithelial cells, alveolar epithelial cells, vascular endothelial cells, and macrophages in the lung, which all express the angiotensin-converting enzyme 2 (ACE2). Multiple studies have demonstrated that excessive expression of ACE2 in the intestinal epithelium results in viral shedding via feces (Xu et al. 2020). Although ACE2 does not appear to be expressed by immune cells. SARS-CoV target receptor on the host as SARS-CoV-2 employs the same entrance receptor, these cell subgroups are possible targets of this virus (Tay et al. 2020).

Response of innate immunity to infection with SARS-CoV-2 virus

The earliest line of defense against viruses is the innate immunity reaction, and it is vital for protection from viruses' infection (Vabret et al. 2020). The humoral components of antiviral innate immunity include complement and coagulation-fibrinolysis system components, soluble proteins that detect glycan on cell surfaces (e.g. mannose binding lectin (MBL)), chemokines, interferons (IFN), and naturally occurring antibodies (mainly IgM but also IgA and IgG). It also contains a number of biological components, including natural killer (NK) cells, other innate lymphoid cells (ILCs), and γ, δ, and T cells, which limit the spread of viral infection by cytotoxic action on target cells, cytokine synthesis, and activation of an adaptive response (Boechat et al. 2021).

In the majority of cases, the immune response eliminates the infection and has no lasting repercussions; nevertheless, the immune system, in some individuals, becomes overstimulated resulting in a cytokine storm and severe lung injury (Hasan et al. 2021). One of the basic causes of Covid-19 pathophysiology is the cytokine storm, which results in severe inflammation, lung injury, ARDS, and other organ failure. In addition, elevated levels of IL-10, CXCL-10, IL-7, TNF-α, granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1A (MIP-1A) levels, indicate the critical role of "cytokine releasing hormone" in pathogenesis of Covid-19 virus (Chen et al. 2020). The IFN response is an important early line of defense against viruses. IFN types I and III (the so-called "innate" IFNs) appear to have a role in limiting infection for many respiratory viruses, including SARS-CoV-1, by developing a cellular state of viral resistance and initiating adaptive immune responses (Park and Iwasaki 2020).

SARS-CoV-2, like SARS-CoV and MERS, is an RNA virus with a single strand. Once entering a target cell, the virus is identified by pattern recognition receptors (PRRs) such as toll-like receptors (TLR), viral-infection sensors melanoma differentiation-associated gene 5 (MDAG5), and retinoic acid-inducible gene I (RIG-I), leads to the viral recognition induces the type I IFN response program and IFN-stimulated genes (Figure 2.3). The TLR3 response induces transcription of the NLR family pyrin domain containing 3 (NLRP3) gene, which, along with other cellular responses to viral infection, such as the formation of reactive oxidative species, calcium flux from cytoplasmic stores, protein aggregation, and the release of danger-associated patterns, helps to the activation of the NLRP3 inflammasome and possibly other inflammasome complexes (Brodin 2021). The NLRP3 inflammasome triggers caspase-1-dependent cleavage and release of major proinflammatory cytokines IL-1 β and

IL-18, as well as pyrophoric cell death mediated by gasdermin-D. The degree of NLRP3 activation corresponds with the severity of Covid-19 illness (Rodrigues et al. 2021). As a consequence of pyrophoric cell death, LDH is secreted. Patients with Covid-19 have elevated LDH levels in their blood, and these enzyme levels associate with disease severity (Huang et al. 2020). These findings imply that inflammasome activation is an essential characteristic of Covid-19 (Han et al. 2020).

Diabetes Mellitus

Based on the WHO, DM is a long-term disease marked by hyperglycemia that eventually cause damage to the eyes, kidneys, nerves, blood vessels, and heart. About 422 million people worldwide have diabetes, the majority living in low and middle-income countries, and 1.5 million deaths are directly attribute to diabetes each year. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades. Over 90% of diabetes mellitus cases are type 2 diabetes, a condition marked by deficient insulin secretion by pancreatic islet cells, tissue insulin resistance (IR) and an inadequate compensatory insulin secretory response. Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin by itself. For people living with diabetes, access to affordable treatment, including insulin, is critical to their survival. There is a globally agreed target to halt the rise in diabetes and obesity by 2025. However, diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. Genital yeast infections frequently occur. The most severe clinical manifestations are ketoacidosis or a non-ketotic hyperosmolar state that may lead to dehydration, coma and, in the absence of effective treatment, death (Sapra et al. 2021).

Type one diabetes mellitus

T1DM is one of the most frequent chronic diseases among children, but it can present at any age. The occurrence and ubiquity of T1DM had been gradually increasing, accounting for about 5% to 10% of all diabetics. The most typical age of onset is in early puberty, between the ages of 4 and 6 (10 to 14 years). There is also a lot of variance in incidence across the globe. Finland and other Northern European countries had the highest documented incidences, with rates 400 times higher than China and Venezuela, which have the lowest reported incidences. T1DM in children characterized by hyperglycemic symptoms, with diabetic ketoacidosis accounting for one-third of all cases. The onset of symptoms, particularly in adolescence, might be abrupt at the time of

diagnosis. It can turn into a medical emergency if not evaluate and treated immediately (Lee et al. 2018).

Type two diabetes mellitus

The incidence and prevalence of T2DM varies by geographic area, with more than 80% of patients residing in low- to middle-income nations, which presents additional therapeutic issues. When compared to people without DM, people with T2DM have an average mortality risk about 15% greater than those without DM, while cardiovascular disease (CVD) now being the main cause of morbidity and mortality in this population (IDF 2019). In elderly (>40 years) obese people, T2DM appears in a less acute way; many patients have definitely had the condition for some time (even years) before diagnosis. T2DM is uncommon in younger individuals, but is on the rise due to the rising frequency of obesity in this age range. Insulin levels are measurable, and the metabolic deficiency appears to be either inadequate insulin secretion or insulin resistance. Insulin therapy is often unnecessary for the prevention of ketosis in these individuals, since them, are resistant to its development. However, insulin may be required to fix blood glucose abnormalities (Beckett et al. 2010).

Insulin therapy during the course of T2DM

Patients with T2DM have insulin resistance and increasing pancreatic β-cell failure, resulting in inadequate insulin production, hyperglycemia, and an elevated level of free fatty acids. In response to hyperglycemia or oral hypoglycemic medications, glucotoxicity and lipotoxicity create a vicious cycle that further impairs the ability of the β-cell to release insulin. In addition, the inevitable decline of pancreatic β-cell activity in T2DM leads eventually to failure in oral agents as a therapeutic modality (Nyenwe et al. 2011). Glycemic control is measured by screening HbA1c (A1C indicates average glycaemia over about 3 months), performing continuous glucose monitoring, and self-monitoring blood glucose levels. A1C is the current statistic utilized in clinical trials proving the advantages of better glycemic management (American Diabetes Association 2021). Due to the continuous reduction in β-cell activity throughout the course of the disease, many T2DM patients will ultimately require insulin therapy (Inzucchi et al. 2012). Insulin remains the most powerful glucose-lowering drug, particularly for patients with high HbA1c levels, despite the presence of several challenges to initiating insulin therapy, such as time restrictions, patient discomfort with self-injections, and inadequate knowledge of novel insulin formulations (Thrasher 2017). However, different forms of insulin vary in terms of how fast they act, when they reach their peak, and how

long they remain. These include rapid-acting/postprandial (e.g., lispro, aspart, glulisine), short- acting (e.g., human regular), intermediate-acting (e.g., human isophane neutral protamine Haledon), and premixed formulations (American Diabetes Association 2017). When compared to human insulin, quick and long-acting insulin analogs produce less hypoglycemia and weight gain, but are more costly (Nyenwe et al. 2011).

The basal insulin alone is the most practical starting insulin regimen, and it can be combined with metformin and other oral medications. Initial dosages can be approximated based on body weight (0.1–0.2 units/kg/day) and the severity of hyperglycemia, with individual titration over days to weeks as necessary. The primary function of basal insulin is to inhibit hepatic glucose synthesis and control hyperglycemia overnight and between meals (American Diabetes Association 2021). However, individuals with T2DM are often more resistant to insulin than individuals with T1DM, require larger daily dosages (1 unit/kg), and have a lower incidence of hypoglycemia. Patients who remain hyperglycemic despite intensified basal insulin treatment may require the addition of prandial insulin based on postprandial glucose levels. Clinical investigations have demonstrated that the administration of prandial insulin in a stepwise fashion reduces HbA1c levels with a minimal risk of hypoglycemia. Alternately, guidelines recommend combining basal insulin with a GLP-1 RA instead of prandial insulin, with studies demonstrating comparable efficacy and the advantages of weight reduction and less hypoglycemia with GLP-1 RA therapy vs prandial insulin therapy (Garber et al. 2017).

Hyperglycemia and susceptibility to infection. In normal circumstances, the human body uses remarkable mechanisms to protect itself against thousands of viruses, fungi, bacterial infections, parasites, and toxins. In ideal circumstances, microorganisms are unable to cross this protective shield, but the immune system can fail to do so, and pathogens struggle under normal conditions to penetrate this protective barrier due to the diverse of reasons. Unfortunately, diabetes disrupts the host's immune response. T2DM can impair the cells of the system, along with the possibility of natural protective walls disruption from nerves damages. Insulin insufficiency and hyperglycemia are the prior causes of this condition (Tessaro et al. 2017).

Effect of hyperglycemia on the immune response in patients with diabetes. Generally, in hyperglycemia, immune system changes seem to be connect to a number of mechanisms, such as decreased release of proinflammatory cytokines, reduction of T cell and neutrophil function, in

addition to reductions in humoral immunity. Additionally, it has been shown that hyperglycemia may hinder tissue recovery, which could raise the vulnerability of this tissue to develop second infections. Another changes in the immune reaction caused by hyperglycemia can be described by biochemical or/and cellular mechanisms including: (1) reduced migration of polymorphnuclear leukocytes, and/or phagocytosis impairment, chemotaxis production, (2) the formation of advanced glycation end-products, (3) risen apoptosis of polymorphnuclear leukocytes and decreased movement across endothelium, and (4) inhibition of glucose-6-phosphate dehydrogenase (Chávez-Reyes et al. 2021).

Patients with T2DM are more likely to get infections that can lead to sepsis. Although a few uncommon infections, such as Klebsiella liver abscesses, malignant otitis externa, and emphysematous cholecystitis closely linked to T2DM, most infections that affect people with T2DM are also common among the population. In contrast, T2DM, worsens infection prognosis, with these individuals experiencing higher sepsis morbidity and death than the rest of the population (Frydrych et al. 2018). Furthermore, respiratory and urinary tract infections are strongly associated to T1DM and T2DM. This vulnerability to infection may lead to a challenges in controlling for diabetes patients, including; sepsis, after operative infections, and chronic periodontitis (Joshi et al. 1999, Casqueiro et al. 2012).

It is important to note that foot infections are very prominent in diabetic individuals; it has been hypothesized that vascular endothelium damage driven by oxidative stress and inflammation may result in changes in microcirculation and, ultimately, nerve injury. Such infections frequently result in ischemia at wounds, which eventually necessitates the amputations (Eleftheriadou et al. 2020).

Gestational Diabetes

Gestational diabetes mellitus (GDM) characterized as glucose intolerance developing during pregnancy in women without a diabetes background. Thus, by definition, it differs from both T1DM and T2DM in that it is temporary and gender-specific. Moreover, it only happens during pregnancy, a unique physiological situation. However, GDM and T2DM share many characteristics. These include insulin resistance and metabolic syndrome components. Due to its brief onset during pregnancy, GDM has been hypothesize to represent a pre-diabetic state or a temporary manifestation of a T2DM-like syndrome caused by pregnancy. GDM lacks an auto-immune component, unlike T1DM (Vokalova et al. 2018). In many countries, GDM has diagnosed clinically by a variety of

methods. In general, the techniques utilize at least one of the following procedures: A) evaluation of clinical risk, B) glucose tolerance screening, and C) formal glucose tolerance testing. The procedures are used on pregnant women who have not been diagnosed with diabetes previously (Buchanan et al. 2007).

SARS-CoV-2 and T2DM

The global outbreak of SARS-CoV-2 has significant implications for the treatment of common metabolic disorders including T2DM. It has well known that diabetic individuals, particularly those with hyperglycemia, glycemic fluctuation, and metformin usage, have an increased risk of infection and T-cell dysfunction in their immune system. This tendency is highly correlated with HbA1C levels. In practical medicine, diabetic individuals have a higher prevalence of yeast infections, foot infections, urinary tract infections, and infections at the site of operation (Abu-Ashour et al. 2018).

Several host factors, spanning from local bacterial invasion to systemic alteration of the immune response, might explain this result. Diabetes, for instance, affects the release of cytokines by macrophages and T-cells, which restricts neutrophil recruitment (Delamaire et al. 1997). Both in in vitro and in vitro, alteration seen with humoral innate immunity, and innate immune responses (Mazucanti and Egan 2020). In individuals with either T1DM or T2DM, IFN-α synthesis by dendritic cell reduced. Reduction of NK cell activation and altered dendritic cell activity restrict effective adaptive immune responses (Summers et al. 2006). Moreover, it is recognized that obese individuals are at a higher risk for influenza-related complications, and obesity is emerging as a key comorbidity for illness severity in the context of SARS-CoV-2 (Ryan et al. 2020).

The respiratory epithelium is composed of several cell types, including ciliated and non- ciliated epithelial cells, goblet cells, which create mucus that serves as the first line of defense against invading viruses, and club cells, which produce proteases. When virus particles inhaled or come into direct touch with the nose or throat mucosa, the virus can infect the cells of the respiratory mucosa and cause viral respiratory infections (Subbarao and Mahanty 2020). The lung's cells, including pneumocytes, are important cellular locations for coronavirus entrance and inflammation (Hamming et al. 2004). Some of these pulmonary cells may also express vital proteins that facilitate coronavirus entrance into cells, including ACE2, transmembrane protease serine 2 (TMPRSS2), and dipeptidyl peptidase-4 for some viral strains (DPP4). ACE2 and DPP4 have demonstrated pleiotropic metabolic activities that contribute

directly to the physiological and pharmacological regulation of cardiovascular and glucose homeostasis, and DPP4 inhibitors are commonly using to treat T2DM (Drucker 2020).

Association between ACE2 receptors and infection by covid-19 in patients with diabetes

SARS-CoV-2 entrance across the membrane of the virus mediated by S glycoprotein, which produces protruding homotrimers on the viral surface. (Figure 2.7). The S glycoprotein is composed of (2) functional subunits involved for either fusion of the viral and cell membranes (S2 subunit) or attachment to the infected cells receptor (S1 subunit such as the RBD). According to latest publications, Angiotensin converting enzyme-2 also serves as a receptor for the novel coronavirus (Yang et al. 2020).

Three coronavirus strains (SARS-CoV, NL63, and SARS-CoV-2) enter cells via ACE2 receptors. The heart, arteries, gut, lung (especially in type 2 pneumocytes and macrophages), kidney, testis, and brain all have widespread and widespread expression of ACE2 receptors. Only a little amount of ACE2 found in the circulation in a soluble form since it has mostly linked to cell membranes. Angiotensin II is broken down to angiotensin-1-7 by membrane-bound and soluble ACE2, which is a crucial therapeutic function. Therefore, the negative consequences of angiotensin II binding to AT1 receptors, such as vasoconstriction, increased inflammation, and thrombosis, are limited by ACE2 receptors (Verdecchia et al. 2020). ACE2 has been found to be the receptor for Covid-19 proteases, including TMPRSS2, which also cleave the ACE2 C-terminal region, in particular, compounds 697-716, therefore facilitating S protein-driven viral entry (Yan et al. 2020). Because of, it consider the host cellular entry receptor, Covid-19 virus attaches to the ACE2, the expression of which has shown to be greater in smokers and chronic obstructive pulmonary disease (COPD) patients. It has hypothesized that greater pulmonary ACE2 mRNA levels in diabetic individuals lead to increased SARS-CoV-2 infectivity. Despite the fact that they are supported by results in non-obese diabetic mice. There are a little investigations on human lung tissue and ACE2 protein levels (Wijnant et al. 2020).

The ACE2 dysfunction and inflammatory destruction caused by Covid-19 seem to be increase in all cells with ACE2 expression, including pancreatic islets. The elevated pancreatic enzymes observed in Covid-19 patients are supportive of such damage. An organoid investigation demonstrated that the expression of ACE2 receptors on β cells of the pancreas, induces Covid-19 sensitivity, which triggers b β cell apoptosis, cytokine secretion and

inflammation, and eventually decreased insulin production (Ardestani and Maedler 2020). Another study that utilized postmortem examinations of Covid-19 infected individuals, and cell cultures of pancreatic islets, revealed the existence and multiplication of the virus in the islets. In addition to decreased insulin that depend of glucose, along with diminution of insulin secretory granules (Müller et al. 2021). Two other recent researches using autopsy specimens revealed a dual impact on beta cells, causing apoptosis or trans-differentiation in the case of those that survive. In mice fed a high-fat diet, ACE2 deficiency results in β-cell dedifferentiation, while deletion of ACE2 receptors in mice with DM causes, oxidative stress on β cells, decrease in the synthesis of insulin, and high blood glucose. Preclinical findings also indicate the involvement of ACE2 in β-cell homeostasis. Finally but not least, eradication of ACE2 in fat mice inhibits β-cell proliferation, reduces beta cells density, and causes β-cell malfunction (Kazakou et al. 2022). Evidence in mice with DM, ACE2 activity was higher in pancreas cells. While in individuals without DM, the binds of SARS-CoV to ACE2 receptors shows to damaging islets, induce high blood glucose, and reducing insulin production. Nevertheless, the high blood levels may be transient. Despite the fact that no such impact was documented yet for Covid-19, These data show the significance of glucose monitoring both during the severe phase of illness and throughout follow-up. Additionally, unmanaged blood glucose may result in modifications in glycosylation of the virus (s) protein and ACE2 receptors, which in turn might alter how the S protein binds to ACE2, and the efficacy of the immune response to the virus (Yin et al. 2021).

Role of diabetes in severity and susceptibility to infection with covid-19 and other viruses

Diabetes can exacerbate infectious diseases and put people at higher risk of developing serious conditions. In a matched group, recently, research assessed the rates of infection in 306.003 individuals (102.493 patients with T1DM and T2DM) and found that people With DM, especially type one, much more likely to contract infectious diseases (Carey et al. 2018). Furthermore, diabetes patients are more vulnerable to bacterial, fungal, and viral infections, than in individuals without DM, and their outcomes are much worse when the infection occurs (Erben et al. 2013). One of most prominent comorbidity linked to severe or deadly infections that elevates admissions among individuals with DM is respiratory tract infections (Klekotka et al. 2015). Furthermore, patients with DM are at higher risk of hospital admission due to pneumonia, which is the main reason for acute lower respiratory tract disease (Jensen et al. 2017).

Emerging global health researches indicate that, in addition to pneumonia, viral agents causing additional chest infections in people with DM and higher fatality rate. Viruses such as, MERS, Influenza, SARS-CoV, and, the latest one covid-19 are among them (Chávez-Reyes et al. 2021).

Role of hyperglycemia in patients with DM during covid-19 infection

Hyperglycemia is the most prevalent metabolic complication of T2DM and defined as persistently elevated glucose levels in blood. The immune response and high blood glucose seem to have significant overlap, which directly worsens the pathogenesis of the Covid-19 infection. T2DM individuals and SARS-CoV-2 infection have had elevated serum levels of inflammatory cytokines such as IFN-1, IL-1, 6, 8, and TNF-α. This is so- called cytokine storm and accompanied by other inflammatory markers such as ferritin, CRP, and D-dimer, and is linked to the acuteness of SARS-CoV-2 infection (Viurcos-Sanabria and Escobedo 2021).

Analysis of more than 500 patients hospitalized in China with SARS-CoV found an association between elevated fasting glucose levels and higher mortality rates (Yang et al. 2010). In addition, accumulating data suggests that high blood glucose is a poor indicator in SARS-CoV-2 due to, endothelial dysfunction, blood clotting, reactive oxygen species production (ROS), and high inflammatory mediators releasing (Bode et al. 2020). Chronic hyperglycemia enhances ROS production and oxidative stress of mitochondria, which ultimately leads to, malfunction of the β-cells, vasculature damages, and reduced insulin releasing (Zhang et al. 2019).

Simultaneously, increased lactate production and LDH action enhanced by elevated blood glucose. This occurrence is especially significant inside this scenario of SARS-CoV-2, in which it discovered that high lactate dehydrogenase values accurately predict mortality in individuals who had serious Covid-19 disease. Obviously, LDH levels in T2DM individuals may delay Covid-19 virus removal by suppressing the RIG-1-like receptor through the antiviral-signaling peptide of mitochondria, hence preventing interferon synthesis and reducing antiviral response (Zhang et al. 2019).

In T2DM, high blood glucose have a negative influences on a multiple neutrophil functions, such as, phagocytic activity, and the eliminate infection by bacteria, and the migration of cells (Alba-Loureiro et al. 2007). Considering the immunological implications of the observed mechanisms, may possibly clarify why T2DM individuals are more vulnerable to acute SARS-CoV-2 infection and negative consequences such sepsis, elevated viral load, and fatality in the course of Covid-19. Therefore, unmanaged high blood glucose should always

be considere to play a significant factor in the pathogenesis of Covid-19, in individuals with T2DM (Viurcos-Sanabria and Escobedo 2021).

Role of obesity in DM patients during covid-19 infection

Individuals with DM, hypertension, and extreme obesity (BMI 40 kg/m2) are higher vulnerability to have Covid-19 infection and have a greater risk of developing complications and mortality (Guan et al. 2020). Obesity is also a risk factor for the intensification of SARS-CoV-2-associated disease. Analysis of 124 recurrent Intensive care unit (ICU) admissions at a single institution in Lille, France, between February 27 and April 5, 2020, found higher rates of obesity and severe obesity among SARS-CoV-2 patients compared to non-infected control individuals (Simonnet et al. 2020). In this observational investigation, the prevalence of obesity was 47.5%, compared to 25.8% in a historical control group of ICU participants with no infection of Covid-19. Morover, the need for intubation in addition to mechanical ventilation was greater in obese individuals. In a retrospective review of 3615 SARS-CoV-2 patients who admitted to the emergency room of a single medical hospital in New York from March 4 to April 4, 2020, related observations were reported that 60-year-olds with obesity or severe obesity were more likely to seek emergency medical treatment and ICU hospitalization (Lighter et al. 2020).

Obesity is a commonly related with T2DM and may have detrimental influence on T-cell function. Indeed, obese Covid-19 patients had a considerably greater risk of hospitalization, ICU admission, and death rates than in non-obesity Covid-19 patients, highlighting the need to evaluate BMI as a confounding risk factor. Obesity linked to a decreased T-cell response to influenza virus infection, with prolonged T-cell activation resulting in T-cell dysregulation. In particular, increasing adipose tissue in obese patients inhibits T-cell and macrophage function, producing persistent inflammation and reducing antiviral response. A study found that obese patients have impaired dendritic cells, indicating decreased T-cell responses to influenza antigens. Obesity was also associated with increased T-cell and immune cell degeneration (Tong et al. 2021). In adipose tissue of mice models with T2DM and human patients, anti-inflammatory and\or regulatory macrophages “M2” and regulatory “T-cells” converted for Th17 CD4+, Th1 T-cells, and “M1” proinflammatory macrophages. Thus, late hyperinflammatory response and prolonged Th1 response seen frequently in DM. Those changes to the immunological composition certainly play a role within the occurrence and intensity of infections in diabetic individuals (Mazucanti and Egan 2020).

Innate immunity in T2DM patients with covid-19 infection

Innate immune response is essential for containing and eliminating an invading virus. There is evidence that disorders such as obesity and T2DM modify the innate immune system, leaving patients more vulnerable to infection (Martín-Cordero et al. 2011). Despite substantial production of chemokines such as the chemokine C-C motif ligand (CCL) 2 and IFN-induced protein-10, innate system are unable to initiate a rigorous IFN- III and I, antivirus defence in severe Covid-19 infections. To conclude that, according to many studies, individuals who have at least one precomorbid condition for examples: T2DM, CVD, and obesity are more vulnerable to serious Covid-19 infections and have higher levels of inflammatory mediator's secretion. The majority of authors recommend quick and specialized anti-inflammatory treatment to prevent catastrophic outcomes. An efficient innate response and adequate management of the proinflammatory may limit the infection from continuing to develop, which might explain the limited prevalence of contagion rate in some people, including patients without obvious symptoms (Pérez-Galarza et al. 2021).

Adaptive immunity in T2DM patients with covid-19 infection

Adaptive immune system is crucial to eliminating pathogens and clear it from the body, like SARS-CoV-2 virus. If a virus, such as Covid-19 infects obese or T2DM individuals, it is hypothesized that preexisting abnormalities in their immune cells such as B and T cells may increase their chance of experiencing poor clinical outcomes. The association between the activation and inactivation in adaptive immune cells during the infection with Covid-19 virus are yet to be studying. The features T-lymphocytes residents' throughout the medical phase of Covid-19 infection seems to be in a predictable pattern. In the same context, several investigations demonstrated substantial indication of an overall decreases in T-lymphocyte (lymphocytopenia) in T2DM and obese individuals as the infection going to precede (Pérez-Galarza et al. 2021). Another study reported that hospitalized individuals with underlying conditions had more severe lymphocytopenia than those who did not have comorbidities (Zhao et al. 2020).

In contrast, to previous data, regarding the statistical characteristics of T-lymphocytes number during Covid-19 incubation, it is not well characterized if T-cell response may contribute positively or adversely on to severity of this disease (Chen and John Wherry 2020). In this regard, in a few research, it was observe that the proportion of SARS-CoV- 2-specific CD4+ to CD8+ T-cells was greater with severely affected patients, along with morphological and functional alterations. Flow cytometry study on Covid-19 specific CD4+ T-

lymphocyte indicated that the population of lymphocytes capable of generating IFN-γ, TNF-α and IL-2, and seemed to be much lesser in people with acute infection than in convalescent patients. In addition, the CTLA-4 expression values of CD4+ T- lymphocyte in Covid-19 infection from critical care unit patients were considerably greater than in convalescent patients. In addition, CD8+ T-cells specific for SARS-CoV- 2 in UCI patients displayed decreased levels of granzyme B (Schub et al. 2020).

Despite the only few B-lymphocyte found in severe cases, it was reported that these patients have higher amounts of specific antibodies such as “IgA” and “IgG” than in recovered patients. Surprisingly, not all infected individuals can have these antibodies during Covid-19 infection. (Schub et al. 2020) found IgG-specific antibodies against SARS-CoV-2 in 83 percent of cases, but “IgA”specific antibodies in just 69% of participants. It is, thought that among individuals who are severely ill, the function of CD4+ lymphocyte in inducing humoral immunity is changed. The proportion of CD4+ T lymphocytes specific for SARS-CoV-2 correlated significantly with both specific IgG and IgA antibodies (Schub et al. 2020). This finding is important because it shows a correlation between the synthesis of IL-17A and the initiation of ARDS (Xu et al. 2020). Contradictory data has emerged about the absence of Covid-19 antibodies in mild infection. (Ibarrondo et al. 2020) showed a significant decline in antibodies which found in people whom have treated from SARS-CoV-2. nevertheless, according to other research, antibodies against Covid-19 were elevate between (35-40) days following the beginning of illness (Wang et al. 2020). In agreement, (Yang and Ibarrondo 2020) observed that antibody values against Covid-19 has been raised for 50-60 days following the start of illness, while the “IgG” values of antibodies remains higher with only a modest decline at 120 days after the beginning of symptoms. As a result of these discoveries, it is required to do more research to determine if this occurrence indicates a changed immunomodulation in Covid-19 patients, particularly in individuals with greater danger of a life-threatening disease (Pérez-Galarza et al. 2021).

Conclusion

T2DM characterized by both insulin resistance and relative insulin insufficiency. While insulin resistance is always present in the early stages, the rate at which the illness manifests itself determined by β-cell failure. The development of medications that target inflammatory cytokines has been sparked by mounting evidence that T2DM is a disease in which the immune system is engaged and plays a significant role (Eguchi and Manabe 2013).

Inflammatory reactions' potential significance in the pathophysiology of T2DM has been supported by growing research in recent years. Failure of β-cells has been linked to IL- 1β. Upon stimulation with glucose, "β-cells" themselves produce IL-1β. Further, IL-1β attracts macrophages, which can serve as an additional source of IL-1β and other cytokines, and increases IL-1β production in β-cells. Therapies that target IL-1β have demonstrated significant progress, but with mixed outcomes in various clinical studies, despite the fact that it is still unknown whether inflammatory responses are a fundamental cause or a subsequent impact in the evolution of T2DM (Zhao et al. 2014). Additionally, the raised glucose concentrations that result in elevated levels of IL-1 within cells of the pancreas and lower levels of IL-1R antagonist in islets of T2DM individuals leading to inhibiting the cell differentiation, reduced insulin production, and increase β cells demise (Böni-Schnetzler and Donath 2013). An earlier epidemiological reserch found that the increase of each of cytokines IL-6 and IL-1 was linked to an approximately three-fold greater incidence of T2DM, and furthermore, IL-1β may cause resistance in body cells to insulin by triggering the IB kinase-β. (Spranger et al. 2003) reported elevated IL-1β levels significantly correlated with reduced insulin concentration, decreased β-cell activity, and increased fasting hyperglycemia.

On the other hand, they discovered that the C allele of IL-1β-511 enhanced the the incidence of T2DM in a research that examined the connection of IL-1β-511 genotypes with T2DM in an endogamous Northern Indian community. The TC and CC genotypes increased T2DM risk as compared to TT, while the T allele had a protective impact. In bronchial alveolar lavage fluid samples from individuals with acute Covid-19, high expression values of cytokines such IL-1β have seen. Because of the inflammatory response caused by cytokines such IL-1β, the SARS-CoV-2 mostly causes lung injury (Doody et al. 2017).

There is growing evidence with indicate that people during acute Covid-19 infection; experienced cytokine storm. VEGF, TNF-α, PDGF, MIP1-B,MIP-1A, MCP-1, IP-10, IL- 1β, IL-7, IL-8, IL-9, IL-10, IFN-γ, GM-CSF, G-CSF, and FGF, levels were found to be higher in patients admitted to the ICU than in individuals who were not, as compared to healthy people. All trial participants had pneumonia, and 1/3 of these patients died after being brought to the ICU (Huang et al. 2020). According to a different research, patients with serious Covid-19 had a tendency to develop a strongly correlated (and extended) cluster of IL-1 cytokines, as well as a tendency for an IFN-γ/IL-6 cluster to develop correlation with TNF-α. Overall, research suggests that the severity of Covid-19 illness may be distinguished by its highly pro-inflammatory cytokine co association profile (Vanderbeke et al. 2021). Additionally, IL-1β, IL-6, TNF-α,

and CXCL-10 levels were considerably greater in early-phase infected persons and asymptomatic individuals in an Indian investigation, while they were significantly lower in recovered patients after 45- 65 days. The study conducted by (Tripathy et al. 2021) suggests that IL-6, IL-1β, TNF- α, CXCL-10, and decreased antiviral cytokines may employed as biomarkers of Covid- 19 marker infection, and immune systems of those individuals with Covid-19 may take longer to normalize.

During SARS-CoV-2 infection, T2DM or hyperglycemia reduced the number of immune cells, such as granulocytes, monocytes, and phagocytic activity, which might exacerbate the intensity of Covid-19, particularly among T2DM people who had the impairment of immune response to infections. The results revealed that infection with SARS-CoV-2 in T2DM.R affected glucose homeostasis, altered cellular immunological status, hemoglobin, and increased IL-1β expression from monocyte cells compared with diabetic individuals without infection T2DM, and controls. However, it is still unknown how long immune dysregulation continues following Covid-19 infection and if this may change the immunological response to future infections. Similarly, persistent neutrophil and monocyte activation may have a role in the development or worsening of other inflammatory diseases, such as cardiovascular disease and rheumatoid arthritis.

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Chapter 5

An Overview of Carbamazepine and Carbamazepine-Loaded Nanoparticle Drug Delivery Systems

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ABSTRACT

Epilepsy is one of the most common neurologic conditions and affects around 65 million people of all social classes, ages, races, and geographical locations worldwide. Epilepsy treatment approaches include a special diet (ketogenic diet), vagus nerve stimulation, surgery, or using antiseizure drugs (ASDs). ASDs are selected considering seizure and epilepsy types, epilepsy syndrome and drug-related adverbs effects. Carbamazepine (CRB) is used for epilepsy management and treatment. CRB, a tricyclic compound, is a sodium channel blocker. It is thought that the release of synaptic glutamate and possibly other neurotransmitters is inhibited by the blockade of sodium channels. CRB has poor water solubility. Its solubility and dissolution rate are significant determinants of the oral bioavailability of CRB. In the literature, nano-sized systems (polymeric nanoparticles, solid lipid nanoparticles, etc.) for CBR have been developed for various reasons like increasing its solubility, improving its oral bioavailability, and enhancing its anticonvulsive effect. Our aim is to provide a brief overview of the studies on the CRB-containing polymeric, lipid and polymeric lipid hybrid nanoparticles.

Keywords: antiseizure drugs, carbamazepine, epilepsy, polymeric nanoparticle, lipid nanoparticle

EPILEPSY AND ASDs

Epilepsy, which globally affects around 65 million people of all ages, social classes, races, and geographical locations, is one of the most common neurologic conditions and has many causes, each reflecting underlying brain dysfunction. It is a chronic disease of the brain characterized by recurrent and unprovoked seizures and the cognitive, neurobiological, social, and psychological consequences of repetitions of these seizures (Beghi, 2019; Devinsky et al., 2018; Stafstrom & Carmant, 2015). There are three diagnostic levels as epilepsy syndrome, epilepsy type, and seizure type in the "International League Against Epilepsy 2017 Classification," and it is important to consider the etiology and comorbidities at each level (Wirrell, Tinuper, Perucca, & Moshé, 2022). The epilepsy types are classified as focal, generalized, combined generalized and focal, and unknown (Devinsky et al., 2018; Sarmast, Abdullahi, & Jahan, 2020). An epileptic seizure is defined as the sudden onset of temporary signs and symptoms caused by abnormal and excessive/synchronized neuronal activity in the brain (Kanner & Bicchi, 2022). The majority (about 80%) of people with epilepsy live in low- and middle-income countries. It is estimated that with proper diagnosis and treatment, about 70% of people with epilepsy can live without seizures ("Epilepsy," 2023). Epilepsy treatment approaches include a special diet (ketogenic diet), vagus nerve stimulation, surgery, or using ASDs (Green, Nguyen, Kaalund-Hansen, Rajakulendran, & Murphy, 2020). Seizures can be controlled in epilepsy treatment, and the seizures can be prevented in about 70% of people with epilepsy with the appropriate use of ASDs ("Epilepsy," 2023). ASDs are selected considering seizure and epilepsy types, epilepsy syndrome and drug-related adverbs effects (Kanner & Bicchi, 2022). The risk of adverse effects from ASDs is considerable and includes potential behavioral and cognitive impacts. Therefore, the occurrence of a single seizure does not always require the starting of ASDs. The risk of recurrent seizures is significant. Key risk factors for recurrence in adults are two unprovoked seizures (occurring more than 24 hours apart), abnormal brain imaging, epileptiform abnormalities on electroencephalography, nocturnal seizures, or a seizure-related epileptic syndrome, while in children, these risk factors are seizure-related epileptic syndrome, abnormal electroencephalography results, severe head trauma, and cerebral palsy. In the absence of risk factors and the seizure does not recur, physicians should consider postponing the use of ASDs until a second seizure occurs (G. Liu, Slater, & Perkins, 2017). The mechanisms of action of clinically used ASDs are summarised in four broad classes: 1. enhancement of GABA-mediated inhibitory neurotransmission (vigabatrin, tiagabine, benzodiazepines,

etc.); 2. modulation of voltage-gated ion channels [a. the modulation of voltage-gated calcium channels: e.g., ethosuximide; b. the modulation of voltage-gated sodium channels: e.g., lamotrigine, CRB, phenytoin; c. the modulation of voltage-gated potassium channels: such as ezogabine (retigabine)]; 3. modulation of neurotransmitter release via a presynaptic action (a. "α2δ subunit of voltage-gated calcium channels" is the molecular target of action for the ASDs such as pregabalin, gabapentin; b. "SV2A" protein is the molecular target of action for the ASDs such as brivaracetam, and levetiracetam); 4. attenuation of glutamate-mediated excitatory neurotransmission (such as perampanel) (Löscher, Potschka, Sisodiya, & Vezzani, 2020; Sills & Rogawski, 2020). Besides, there are ASDs (topiramate, valproate, rufinamide, cenobamate, cannabidiol, etc.) with mixed/unknown mechanisms of action and mechanism-targeted agents (with disease-specific mechanism) such as everolimus (Löscher et al., 2020; Sills & Rogawski, 2020). Despite new ASDs that are highly chemically diverse and have relatively various mechanisms of action, the overall outcome for seizure freedom in epilepsy treatment has not improved. There has been notable progress in understanding how ASDs affect excitability mechanisms at the cellular level. Unfortunately, this knowledge has not been effectually used in the development of the more effective agents (Sills & Rogawski, 2020). Due to the recurrent characteristic of the epileptic seizure requires long-term pharmacotherapy. However, this therapy has suffered from potential side effects (psychiatric problems, impaired cognition, and impairment of renal and liver functions, etc.) and limited efficacy because of untimely medication and blood-brain barrier (BBB) (Wu et al., 2022). Nanotechnology represents an attractive strategy to increase the therapeutic efficacy of ASDs in drug-resistant epilepsy and reduce the potential unwanted side effects of ASDs (Torre et al., 2014; Wu et al., 2022). Nano-sized drug delivery systems are a versatile platform for drug delivery. These carriers can improve the efficacy of existing drugs due to their features such as controlled/sustained release, better pharmacokinetic properties, targeting specific organs/tissues/cells, passing biological barriers such as BBB, and reducing drug dose and unwanted side effects (Bennewitz & Saltzman, 2009; Malam, Lim, & Seifalian, 2011; Yavuz, Yildirim, & Yilmaz, 2021).

CRB and nano-sized systems prepared for CRB

CRB is used for epilepsy (for mixed seizure patterns, generalized tonic seizures, and complex partial seizures) management and treatment. Also, it is used to treat acute manic and mixed episodes in bipolar I disorder and trigeminal neuralgia. CRB, a tricyclic compound, is a sodium channel blocker.

It is thought that the release of synaptic glutamate and possibly other neurotransmitters is inhibited by the blockade of sodium channels (Lo, 2014). CRB modulates voltage-gated sodium channels and also binds to other voltage-gated ion channels (e.g., calcium channels). It inhibits the generation of action potentials and causes decreased synaptic transmission (Beydoun et al., 2020; Maan, Duong, & Saadabadi, 2023; Osuntokun et al., 2021). CRB was discovered and developed by Swiss chemist Walter Schindler and others around 1954 at the labs of J.R. Geigy AG in Basel, Switzerland (Motika & Smith, 2010). It was first marketed in 1962 to treat trigeminal neuralgia. In addition, it has been used as an ASD in the UK since 1965. However, CRB was approved for use in epilepsy (Tegretol) in 1974 in the US (Motika & Smith, 2010; Tolou-Ghamari, Zare, Habibabadi, & Najafi, 2013). According to the Biopharmaceutics Classification System, CRB is a Class II active substance (high permeability and low solubility) (Uzunović, Vranić, & Hadzidedić, 2010). The water solubility of CRB has been reported as less than 200 µg/mL (poor water solubility) (Halford, 2015). The dissolution rate of CRB limits its oral bioavailability. Therefore, its solubility and dissolution rate are significant determinants of the oral bioavailability of CRB (Uzunović et al., 2010). Oral absorption of CRB is slow and erratic. It has suboptimal bioavailability and a highly variable plasma concentration (H. Li, Zhang, Xiong, Feng, & Williams, 2020). It is approximately 75%-80% bound to plasma proteins (Tolou-Ghamari et al., 2013). CRB is metabolized in the liver to CRB-10,11-epoxide (active metabolite that leads to pharmacological action). Cytochrome P450 3A4 is the major isoform responsible for this metabolite formation (Maan et al., 2023; Pearce et al., 2008). Due to CRB being a potent cytochrome P-450 enzyme inducer, it is significantly more prone to drug-drug interactions (Beydoun et al., 2020). Furthermore, it is known that CRB induces its metabolism. Therefore, it has a shorter half-life (12 to 17 hours) after repeated doses compared with the half-life (25 to 65 hours) after single dosing (Song et al., 2016). Vomiting, dizziness, ataxia, nausea, and drowsiness are the most common side effects associated with using CRB. In addition, a few serious skin reactions are rarely seen (Maan et al., 2023). Looking at US Food and Drug Administration (FDA)-approved drug products for CRB in the Orange Book, CRB is available in the dosage forms of immediate-release tablets, extended-release tablets, chewable tablets, extended-release capsules, oral suspension, and i.v. solution ("Orange Book," 2023).

In the literature, nano-sized systems (polymeric nanoparticles, solid lipid nanoparticles, etc.) for CBR have been developed for various reasons like increasing its solubility, improving its oral bioavailability, and enhancing its

anticonvulsive effect. Our aim is to provide a brief overview of the studies on the CRB-containing polymeric, lipid and polymeric lipid hybrid nanoparticles.

Nanoparticles are colloidal carrier systems, which are the dispersion of particles smaller than 1000 nm (Scioli Montoto, Muraca, & Ruiz, 2020). They can be prepared using different materials such as lipids, metals, or polymers (natural or synthetic) (Pudlarz & Szemraj, 2018) and applied by various routes of administration (pulmonary, parenteral, oral, ocular and nasal, etc.) (Carissimi, Montalbán, Fuster, & Víllora, 2021).

Solid lipid nanoparticles, which were introduced in 1991 as an alternative drug delivery system to traditional colloidal systems, such as polymeric nanoparticles, and liposomes, are prepared by using lipids (tripalmitin, cetyl palmitate, cetyl alcohol, trimyristin, glyceryl monostearate, tristearin, stearic acid, etc.), which are solid at both room temperature and body temperature (Das & Chaudhury, 2011; Müller, Mäder, & Gohla, 2000). Solid lipid nanoparticles as delivery systems for hydrophobic active substances have attracted increasing attention (Musicanti & Gasco, 2012). They protect the active substance from environmental conditions, enhance the solubility of poorly water-soluble active substances and improve their bioavailability (Das & Chaudhury, 2011; Mukherjee et al., 2016). On the other hand, the drug loading capacity of solid lipid nanoparticles is limited due to the crystalline structure of the solid lipid, polymorphic transitions are possible during storage, and the drug release profile may vary with storage time (Das & Chaudhury, 2011). Consequently, solid lipid nanoparticles contain only solid lipids, making their applicability difficult due to low drug loading, internal rearrangement of the crystal lattice, and subsequent drug expulsion. Nanostructured lipid carriers developed as second-generation lipid nanoparticles contain both liquid and solid lipids to increase drug loading. This dual lipid structure creates a less ordered lipidic core. This imperfect nature of the internal arrangement helps to load more drugs. Thus, relative to solid lipid nanoparticles, nanostructured lipid carriers can encapsulate higher drug amounts, contain lower water content, and minimize drug leakage during storage (Chauhan, Yasir, Verma, & Singh, 2020; Elmowafy & Al-Sanea, 2021). In addition, nanostructured lipid carriers have advantages such as increased drug solubility, improved drug permeability and bioavailability, reduced undesirable side effects, and sustained drug release (Fang, Al-Suwayeh, & Fang, 2013). CRB-loaded lipid nanoparticles were prepared to enhance its anticonvulsant effect (Arya et al., 2023; Qushawy, Prabahar, Abd-Alhaseeb, Swidan, & Nasr, 2019; Scioli Montoto et al., 2018).

Besides, polymeric nanoparticles and polymeric lipid hybrid nanoparticles as drug delivery systems have been studied extensively. Synthetic [poly(lactide),

poly(lactide-co-glycolide) (PLGA), poly(A)caprolactone), etc.] and natural (chitosan, albumin, alginate, etc.) polymers are used in the production of polymeric nanoparticles with size in the range of 10–1000 nm (generally less than 200 nm) (Sardoiwala, Kaundal, & Roy Choudhury, 2018; Singh & Lillard, 2009; Zielińska et al., 2020). The active substance can be encapsulated within the nanoparticle core or adsorbed/conjugated to the nanoparticle surface. Some of the advantages of polymeric nanoparticles prepared by using generally biodegradable and biocompatible polymers can be summarized as follows: providing sustained/prolonged drug release, loading the hydrophilic and hydrophobic active substances into the nanoparticles, protecting the drug from environmental factors such as pH, and enzymes, targeting of nanoparticles actively and/or passively, the availability of many preparation methods (Kahraman, Güngör, & Özsoy, 2017; Sardoiwala et al., 2018; Zielińska et al., 2020). The disadvantages of these systems are the difficulty of scaling up and the lack of sufficient data for toxicological evaluations (Jesus et al., 2019; Kahraman et al., 2017). PLGA, commonly used in the preparation of polymeric nanoparticles, is a biocompatible and biodegradable FDA-approved copolymer due to its safety profile, low immunogenicity, and minimal toxicity (Kandilli et al., 2020; Swider et al., 2018). PLGA composed of lactic and glycolic acid monomers biodegrades into these monomers, readily metabolized via the tricarboxylic acid cycle and eventually eliminated from the body as water and carbon dioxide (Swider et al., 2018). The ratios of PGA and PLA forming the copolymer are changed to obtain PLGA with different physicochemical properties and molecular weights (Kandilli et al., 2020). For the treatment of epilepsy, ASDs-loaded PLGA nanoparticles have been prepared (Kandilli et al., 2020; Musumeci et al., 2018; Shah et al., 2021). In addition, ASDs-loaded chitosan nanoparticles have been prepared (S. Liu, Yang, & Ho, 2018; Yousfan et al., 2020). Chitosan, a positively charged linear polysaccharide (consists of β-(1–4)-linked d-glucosamine and N-acetyl-d-glucosamine), is another polymer often used in polymeric carrier preparation. It is a biocompatible and natural polymer with mucoadhesive properties. Chitosan is mainly produced from the deacetylation of chitin, widely found in the exoskeletons of arthropods (such as crustaceans), insect cuticles, and the cell wall of fungi. The significant biological and technological properties of this polymer (such as mucoadhesive, antimicrobial, and wound healing) are closely related to its physicochemical properties (mainly deacetylation degree and molecular weight) (Aranaz et al., 2021; J. Li et al., 2018). Its solubility in acidic aqueous solutions below pH 6.5 limits many of its potential applications. However, water-soluble derivatives of chitosan can be obtained by chemical modification (introducing hydrophilic or

carboxyalkyl groups to chitosan or quaternization, etc.) (S. Liu et al., 2018). Different dosage forms (such as gel and nanoparticle) have been prepared for various biomedical applications using chitosan and its derivatives (Kou, Peters, & Mucalo, 2022) .

In the literature, Qushawy et al.(2019) prepared CRB-loaded solid lipid nanoparticles by the modified hot high-shear homogenization and ultra-sonication method to enhance its anticonvulsant effect. For these nanoparticles preparation, they used stearic acid or glyceryl monostearate as the oil phase and Tween 80 or Poloxamer 188 (0.5% or 1% w/v) as the surfactant. They evaluated the effects of various factors (lipid type, surfactant type, and surfactant concentration) on the nanoparticle's characteristics (such as the encapsulation efficiency % (EE%), and particle size). The size, PDI, zeta potential, and EE% values were determined in the range of 45.11-760.7 nm, 0.196-0.419, (-)21.5-(-)38.4 mV and 39.66-71.91%, respectively. The EE increased when glyceryl monostearate was used as the oil phase in the formulation. In addition, they obtained a higher EE% value by using 1% of poloxamer as the surfactant. The long carbon chain structure of glyceryl monostearate and the use of the surfactant with a higher HLB value (1% of poloxamer) were notified as possible reasons for obtaining higher encapsulation efficiency values. It was emphasized that glyceryl monostearate causes a less ordered solid lipid nanoparticle structure, thus providing more space for CRB loading, and 1% of poloxamer use also increases the solubility of CRB in the lipid. For all prepared formulations, initial burst release due to the release of CRB adsorbed on the surface of solid lipid nanoparticles followed by a sustained CRB release was observed. Furthermore, they investigated the effects of pure CRB and the optimum formulation (its size, PDI, zeta potential, and EE% values: 45.11 nm, 0.277, (-)33.3 mV, and 71.91%, respectively) administrated orally on time to death after intraperitoneal administration of a lethal dose (70 mg/kg) of pentylenetetrazole and their impacts on mean seizure scores in pentylenetetrazole-kindled mice. They showed that the time of death in mice administered the pentylenetetrazole, CRB, or CRB-loaded solid lipid nanoparticles was approximately 120 s, 2340 s, and 3720 s, respectively. CRB-loaded solid lipid nanoparticles have been noted to significantly increase the time to death after pentylenetetrazole's a lethal dose compared to CRB. The percent reductions in final seizure scores in pentylenetetrazole-kindled mice treated with CRB or CRB-loaded solid lipid nanoparticles were 14.71% and 35.29%, respectively. It has been emphasized that these results can be attributed to the advantages (sustained drug release, nano-size, increased permeability

through biological membranes, etc.) provided by the nano-sized carrier (Qushawy et al., 2019).

In another study, CRB-loaded lipid nanocarriers (nanostructured lipid carriers and solid lipid nanoparticles) as a promising tool to improve the treatment of refractory epilepsy prepared using a homogenization technique followed by ultrasonication. During the preparation, CRB was added to the lipid/lipid+oil phase as a powder or its solution in DMSO. The size and zeta potential values of these nanocarriers were about 160 nm and −2.4 to −6.3 mV, respectively. The CRB-loaded lipid nanocarriers displayed an initial burst release followed by sustained release. In addition, they used "Madin–Darby canine kidney transfected with MDR1" epithelial cells to assess CRB permeability in vitro and found that CRB-loaded solid lipid nanoparticles or CRB-loaded nanostructured lipid carriers prepared using DMSO showed significantly improved permeability over pure CRB. However, CRB-loaded solid lipid nanoparticles without DMSO exhibited a lower permeability coefficient compared to pure CRB. It was emphasized that the presence of DMSO and liquid lipids alter the lipid matrix (making it more deformable) and can lead to nanoparticles passing through the cell monolayer more readily, thereby improving drug permeability. Furthermore, they evaluated the anticonvulsant activity of CRB-loaded nanostructured lipid carriers, prepared using DMSO, in mice using the maximal electroshock seizure model. The results revealed that CRB-loaded nanostructured lipid carriers showed protection against seizures for at least two hours (the percentage of mice protected at 2 hours: approximately 40%) after drug administration (intraperitoneally). In addition, no protection was observed with pure CRB at 4 hours, while CRB-loaded nanostructured lipid carriers protected against seizures in 1 out of 5 mice (20%). They stated that this formulation showed a comparable protective effect to pure CRB in the seizure model (Scioli Montoto et al., 2018).

Furthermore, in a study, different lipid carriers [solid trimyristin-nanosuspension, tristearin nanoparticles (α-, form), trimyristin-nanoemulsion, smectic cholesteryl myristate nanoparticles, and crystalline cholesteryl myristate nanoparticles] dispersions were exposed to different model drugs (CRB, ibuprofen, ubidecarenone, betamethasone-17-valate, griseofulvin, retinyl palmitate, flufenamic acid, diazepam) to evaluate the drug loading capacity of lipid nanoparticles by passive drug loading. The rank order of the passive CRB loading was trimyristin-nanoemulsion ≈ trimyristin-nanosuspension> smectic cholesteryl myristate nanoparticles> crystalline cholesteryl myristate nanoparticles> tristearin nanoparticles (α-form). It was found that the extent of

loading depended on the type of formulations and the model drug properties. CRB didn't have much affinity with the nanoparticle formulations. It was also reported that the passive loading procedure did not significantly affect the properties of nanoparticles, such as particle size (Rosenblatt & Bunjes, 2017).

For the treatment of refractory epilepsy, Zybina et al. (2018) investigated the effect of verapamil (a P-glycoprotein inhibitor) on the anticonvulsant effect of pure CRB and CRB-loaded PLGA nanoparticles coated with poloxamer 188 (CRB-PLGA nanoparticles-P188) in the rat model (seizures induced by isoniazid). They prepared CRB- or lipophilic fluorescent dye ("1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate")-loaded PLGA nanoparticles by using emulsification-solvent evaporation method. The size, PDI, and zeta potential values of the nanoparticles were about 130 to 150 nm (in nano-range), 0.2 (narrow size distribution), and −0.3 mV (approximately neutral), respectively. Before in vivo study, they coated the nanoparticles with a surfactant (P188) to help nanoparticles transport across the BBB and increase drug concentration in the brain. Zybina et al. (2018) determined that the effective doses of pure CRB and poloxamer-coated-nanoparticles were 30 mg/kg and 1 mg/kg (equivalent to CRB; intravenous injection), respectively, according to a significant reduction of the severity and duration of seizures in the isoniazid-induced seizure model. They stated that administration of higher doses (3 mg/kg-8 mg/kg; equivalent to CRB) of surfactant-coated nanoparticles did not over-enhance drug efficacy but produced a significant and stable anticonvulsive effect. Also, they showed that the impact of CRB-loaded nanoparticles without surfactant (uncoated nanoparticles; administered only at a dose equivalent to 3 mg/kg CRB) was similar to that of blank nanoparticles (without CRB). Furthermore, they also reported that lipophilic fluorescent dye-loaded PLGA nanoparticles-P188 were easily internalized in neurons. Therefore, they emphasized that the coating of nanoparticles with surfactant plays a significant role in drug transport at higher concentrations to the brain. In addition, they investigated the effect of verapamil application on the effective doses of CRB and CRB-loaded nanoparticles. They found that verapamil application to rats (seizures induced by isoniazid) before the administration of pure CRB resulted in a significant increase in the antiepileptic effect of CRB and a reduction of the minimum effective dose of CRB (from 30 mg/kg to 20 mg/kg). However, they also showed that the application of verapamil did not further increase the anticonvulsant effect of the surfactant-coated CRB-loaded nanoparticles. As a result, it was reported that the coating of CRB-loaded nanoparticles with P188 increased the anticonvulsive effect by 30 times

compared to the pure CRB, and a P-glycoprotein inhibition did not affect the effectiveness of CRB-loaded nanoparticles (Zybina et al., 2018).

Kandilli et al. (2020) developed a combination formulation of CRB and levetiracetam for the treatment of epilepsy. Levetiracetam and CRB-loaded PLGA nanoparticles were prepared using the nanoprecipitation method, and their antiepileptic effect was evaluated in a rat epilepsy model. It has been reported that CRB and levetiracetam combination and their PLGA-NPs showed generally higher antiepileptic activities compared to CRB or levetiracetam (Kandilli et al., 2020).

Rodríguez-Cruz et al. (2009) prepared a CRB-containing drug delivery system based on infiltrated/adsorbed PLGA nanoparticles into the biodegradable porous membrane. To prepare the system, they first prepared the porous membrane by solvent casting and particulate leaching technique, using PLGA (50:50) as a biodegradable polymer and sodium chloride to create pores in the membrane. PLGA (50:50) nanoparticles were prepared using emulsification–diffusion technique. Later, they immersed (for 12 hours) the biodegradable porous membrane in a saturated aqueous solution (∼180 μg/mL) of CRB to ensure drug adsorption to the porous membrane. Moreover, the porous PLGA membrane was immersed (for 12 hours) in the aqueous medium containing CRB (∼180 μg/mL) and PLGA nanoparticles (different amounts: 50-600 mg) to obtain the porous membrane containing these nanoparticles and CRB. They showed that CRB release from the porous membrane containing both nanoparticles and CRB was slower than that from only CRB-containing porous membrane. The CRB release rate was also slowed significantly as the amount of adsorbed/infiltrated nanoparticles into the porous membrane increased. They reported that nanoparticles formed a film on the porous membrane surface and this film acted as an additional diffusion barrier for CRB release. As a result, as the amount of adsorbed/infiltrated nanoparticles into the porous membrane increases, a thicker diffusion barrier will form, resulting in a slower CRB release from the system (Rodríguez-Cruz et al., 2009).

In the literature, there are studies in which chitosan was used for the CRB-loaded polymeric nanoparticles preparation (Arya, Juyal, & Kunwar, 2015; Edityaningrum, Zulaechah, Putranti, & Arimurni, 2022; S. Liu et al., 2018) or coating of the CRB-loaded lipid nanoparticles (Ana et al., 2019). In a study on the preparation of CRB-loaded chitosan nanoparticles, chitosan nanoparticles were prepared by the ionic gelation method using sodium TPP. It has been shown that increasing the chitosan concentration increased the zeta potential and decreased the particle size but had no effect on the encapsulation efficiency (Edityaningrum et al., 2022).

Moreover, in another study, the researchers developed chitosan-coated lipid nanoparticles (solid lipid nanoparticles and nanostructured lipid carriers) for oral delivery of CRB. Lipid nanoparticles were prepared by the hot high-pressure homogenization method using different liquid and/or solid lipids (such as tripalmitin, Compritol®888 ATO, oleic acid, Transcutol® HP) and various surfactants (such as Tween 80, Vitamin E TPGS) (Ana et al., 2019). The sizes of all prepared formulations with/without chitosan (0.5% or 1% w/w) coated were found to be in the range of 142-1841 nm. The CRB-loaded nanostructured lipid carrier was prepared using tripalmitin (as solid lipid; 2.5%w/w) and Transcutol (as liquid lipid; 2.5% w/w), and Tween 80 (as a nonionic surfactant; 3% w/w) coated with chitosan (1% w/w) was optimized formulation. The size of this formulation with a zeta potential higher than 30 mV was 177 nm. They performed an ex vivo permeability study (using approximately 2 cm jejunum segments from mice) for the CRB-loaded nanostructured lipid carriers and found that the apparent permeability coefficient values for the non-coated and chitosan-coated formulations were about 1x10−6 cm/sec and 1.2x10−4 cm/sec, respectively (a 100-fold increase in the permeability coefficient value after the coating with chitosan). It was stated that positively charged chitosan causes an increase in drug permeation, probably due to its penetration-enhancing and mucoadhesive properties (Ana et al., 2019). In addition, they carried out in vivo studies in mice [oral administration by gavage] and determined the pharmacokinetic parameters. They stated that encapsulated CRB (in the chitosan-coated formulation) was absorbed more slowly than pure CRB, and the mean residence time (MRT) and elimination half-life determined for the chitosan-coated formulation were also extended relative to pure CRB, which was consistent with the observed modified release properties. They also noted that CRB-10,11-epoxide levels were significantly reduced due to the avoidance of hepatic first-pass metabolism with lipid nanoparticle use (preferential absorption via lymphatic route) (Ana et al., 2019).

In addition, chitosan-solid lipid nanoparticles were prepared by Nair et al. (Nair, Kumar, Priya, Yadav, & Raju, 2012). These nanoparticles were prepared by solvent injection method (ethanol used as organic solvent) using chitosan, tristearin, phospholipon R 80 H and CRB. It has been shown that the prepared nanoparticles have high encapsulation efficiency and high physical stability, and a prolonged CRB release from the nanoparticles is achieved (Nair et al., 2012).

The nose-to-brain delivery approach is substantial for the effective treatment of brain-related diseases. There are limitations in the delivery of drugs by the common routes of administration (such as oral and intravenous routes) to the

brain due to extensive first-pass metabolism and the presence of the BBB. Therefore, intranasal administration has attracted attention as a non-invasive route to bypass the BBB. This route has the advantage of direct and effective drug delivery to the brain, mainly via the olfactory and trigeminal nerve pathways, rather than through systemic circulation (Jeong, Jang, & Lee, 2023; Su et al., 2020). In addition, the nasal-brain lymphatic system is attracting attention for drug delivery to the brain. However, there are some limitations in drug delivery to the brain via intranasal administration, such as low drug permeability and the presence of nasal mucosa and mucociliary system. Therefore, mucoadhesive nanocarriers such as chitosan nanoparticles can be utilized to overcome these limitations (Jeong et al., 2023). In addition, lipid nanoparticles can improve drug permeability, absorption and delivery to the brain (nose-to-brain). These nanoparticles also have a potential to facilitate drug administration by the intranasal route (Costa, Moreira, Sousa Lobo, & Silva, 2021).

In a study on the preparation and optimization of solid lipid nanoparticles for intranasal delivery of CRB to the brain, a full factorial design was performed by to evaluate the effect of variables (surfactant concentration, sonication time, and lipid concentration) on the response variables (EE%, nanoparticle size, and CRB release) (Arya et al., 2023). The optimum CRB-loaded solid lipid nanoparticle formulation (mean particle size: 210 nm and the percent encapsulation efficiency: about 42%) was prepared and in vivo evaluated (on male Wistar Rats) by Arya et al. (Arya et al., 2023). They stated that a higher amount of CRB reached the brain through intranasal application than the intravenous administration (Arya et al., 2023).

Liu et al. (2018) synthesized carboxymethyl chitosan to improve the solubility of chitosan in the water by chemical modification. They reported that carboxymethylation occurred at both hydroxyl and amino groups of chitosan but mainly at its hydroxyl groups. After carboxymethyl chitosan synthesis and characterization, they developed CRB-loaded carboxymethyl chitosan nanoparticles for intranasal administration to overcome the BBB and increase the therapeutic efficacy of CRB. The size and entrapment efficiency (%) values of the CRB-loaded nanoparticles were in the range of about 217-221 nm and 78.48-81.92%, respectively. The prepared nanoparticles had negative zeta potential values [about (-) 32 mV- (-) 35 mV] and a narrow size distribution (PDI<0.3). CRB release from the nanoparticles showed a biphasic pattern characterized by a burst release (due to CRB on the surface of nanoparticles) in the first four hours, followed by a sustained release. Furthermore, they performed the intranasal administration of the CRB solution in water containing

2% hydroxypropyl-β-cyclodextrin or the CRB-loaded carboxymethyl chitosan nanoparticles (equivalent to 2 mg/kg CRB) in mice. They found that the plasma AUC0–∞ and brain AUC0–∞ values obtained for the nanoparticles were about 2.6 times and 12 times higher than the corresponding values obtained for pure CRB, respectively. They stated that the CRB-loaded nanoparticles significantly increased the CRB concentrations in plasma and the brain due to increasing the contact time between the nanoparticles and the nasal mucosa with chitosan use (S. Liu et al., 2018).

In another study, chitosan nanoparticles were prepared using the ionic gelation method for nasal administration of CRB. Their average particle size, zeta potential and EE% values were found to be approximately 124-580 nm, (+)21- (+)26.6 mV and 65%-72.7%, respectively. Researchers administered CRB-loaded nanoparticles to rats nasally and showed that CRB could be transported directly to the rat brain via the nose, and possible side effects can be minimized (Arya et al., 2015).

As a result, polymeric nanoparticles/lipid nanoparticles/chitosan-coated lipid nanoparticles containing CRB have been prepared for application by different administration routes (intranasal, oral, and parenteral) in the literature. In some of these studies, the CRB-loaded nanoparticles were evaluated in vivo (pharmacokinetic or pharmacodynamic study on rats/mice). These studies revealed that CRB-loaded nanoparticles may help improve the anticonvulsant activity of CRB. Nanoparticles allow for the reduction of drug dose and adverse effects, increasing the dosing interval and improving drug bioavailability. However, more studies are required to evaluate their biological behaviors and safety in detail.

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Chapter 6

Artificial Intelligence in Medicine and Healthcare

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ABSTRACT

Artificial Intelligence (AI) has emerged as a transformative technology with promising applications in the field of medicine and healthcare. This paper provides an overview of the current and potential use cases of AI in healthcare, highlighting its impact on diagnosis, treatment, patient care, and healthcare management. The study begins by introducing the concept of AI and its underlying technologies, such as machine learning and natural language processing. It discusses how AI algorithms can analyze vast amounts of data, including electronic health records, medical images, and scientific literature. This introduction sets the foundation for exploring the applications of AI in medical diagnosis and imaging. AI techniques, such as image recognition and pattern analysis, have shown promising results in interpreting medical images like radiology scans, mammograms, and pathology slides. These AI algorithms play a crucial role in early detection, accurate diagnosis, and personalized treatment planning for various diseases, including cancer, cardiovascular conditions, and neurological disorders. After discussing AI's applications in medical imaging, the study delves into personalized medical applications. It explores how AI can impact patient care and monitoring, including remote monitoring and wearable devices. AI-enabled systems facilitate real-time data collection, early detection of abnormalities, and proactive interventions, empowering patients to actively participate in their healthcare journey. Lastly, the study concludes by addressing the challenges associated with the adoption of AI in the healthcare domain. It acknowledges factors such as data privacy, algorithm transparency and interpretability, ethical considerations, and regulatory compliance. These challenges emphasize the need for developing robust frameworks to ensure the responsible and ethical use of AI in medicine and healthcare.

Keywords: artificial intelligence, healthcare, medicine, diagnosis, treatment, patient care, healthcare management, machine learning.

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INTRODUCTION

Artificial Intelligence (AI) has emerged as a powerful and transformative technology with the potential to revolutionize various sectors, including healthcare (Cabitza et al., 2017). In medicine, AI holds immense promise to improve patient outcomes, enhance clinical decision-making, and streamline healthcare processes (Rajkomar et al., 2018). By advanced algorithms and machine learning techniques, AI enables the analysis of large and complex datasets, extraction of valuable insights, and development of predictive models (Char et al., 2018). This study provides an overview of the applications of AI in medicine such as highlighting its impact on diagnosis, treatment planning, drug discovery, and patient care (Esteva et al., 2017; Schwab et al., 2018; Cuperlovic-Culf et al., 2018). Additionally, the article discusses the challenges and ethical considerations associated with AI implementation in healthcare (Obermeyer et al., 2019).

AI has a wide range of applications in the field of medicine, revolutionizing various aspects of healthcare. Here are some key areas where AI is being utilized:

I) Medical Imaging: AI algorithms can analyze medical images, such as X-rays, CT scans, and MRIs, to assist in diagnosing and detecting abnormalities. They can identify patterns and anomalies that may be missed by human observers, enabling earlier and more accurate diagnoses of conditions like cancer, cardiovascular diseases, and neurological disorders.

II) Disease Diagnosis: AI can help doctors in diagnosing diseases by analyzing patient data, such as medical records, symptoms, and test results. Machine learning algorithms can learn from vast amounts of data to identify patterns and predict the likelihood of certain diseases. This can aid physicians in making more accurate diagnoses and suggesting appropriate treatment plans.

III) Drug Discovery and Development: AI is being used to accelerate the drug discovery process. Machine learning models can analyze large datasets of biological and chemical information to identify potential drug candidates. AI algorithms can also simulate and predict the effectiveness and safety of drug compounds, reducing the time and cost involved in developing new medications.

IV) Personalized Medicine: AI algorithms can analyze patient data, including genetic information, medical history, and lifestyle factors, to provide personalized treatment recommendations. This can help doctors tailor treatments to individual patients, improving efficacy and reducing side effects.

V) Predictive Analytics: AI algorithms can analyze large amounts of data to predict health outcomes and disease progression. By considering factors such as

patient demographics, lifestyle, genetics, and medical history, AI can assist in identifying high-risk patients, predicting disease complications, and optimizing treatment plans.

VI) Robotic Surgery: AI and robotics are being combined to enhance surgical procedures. Surgeons can use robotic systems with AI capabilities to assist in performing precise and minimally invasive surgeries. AI algorithms can analyze real-time data, provide guidance during surgery, and improve the accuracy and safety of procedures.

VII) Health Monitoring and Wearable Devices: AI can leverage data from wearable devices and remote monitoring technologies to track and analyze patients' health parameters in real time. This enables continuous monitoring of vital signs, early detection of abnormalities, and timely intervention in case of emergencies.

AI in Medical Imaging

Medical imaging techniques, including X-rays, CT scans, MRI, and ultrasound, provide valuable insights into the internal structures of the human body. The integration of AI into medical imaging has revolutionized the field, introducing unprecedented advancements in diagnostics and patient care. AI algorithms, particularly machine learning and deep learning models, have showcased remarkable capabilities in analyzing medical images, detecting anomalies, and assisting radiologists in making accurate diagnoses. Applications of AI in Medical Imaging:

1. Automated Detection and Segmentation: AI algorithms have demonstrated remarkable capabilities in automatically detecting and segmenting anatomical structures, lesions, and abnormalities in medical images. Deep learning techniques, such as convolutional neural networks (CNNs), have shown great success in tasks such as detecting lung nodules in CT scans (Shen et al., 2017) and identifying skin cancer in dermoscopy images (Esteva et al., 2017).

2. Computer-Aided Diagnosis (CAD): AI-based CAD systems have become valuable tools in radiology, providing diagnostic support and assisting radiologists in the interpretation of medical images. These systems employ machine learning algorithms to analyze images and provide quantitative assessments, aiding in the detection of various conditions, such as breast cancer (Wang et al., 2016) and lung diseases (Lakhani et al., 2018).

3. Image Reconstruction and Enhancement: AI techniques have been utilized to enhance the quality of medical images, improving resolution, reducing noise, and reconstructing images from limited or low-dose data. Deep learning-based approaches, such as generative adversarial networks (GANs), have shown

promising results in improving image quality in CT (Kang et al., 2020) and MRI (Yang et al., 2020).

4. Quantitative Analysis and Prognostication: AI enables the extraction of quantitative data from medical images, facilitating the assessment of disease severity, treatment response, and prognostication. Radiomics, a field that leverages AI algorithms to extract and analyze a large number of quantitative features from images, has shown promise in predicting outcomes for various cancers (Aerts et al., 2014). Additionally, AI techniques can aid in functional imaging analysis, such as estimating myocardial perfusion from cardiac MRI (Tao et al., 2020).

5. Real-time Image Analysis and Interventional Radiology: AI algorithms are being developed for real-time image analysis to assist interventional radiologists during procedures. These algorithms can provide real-time guidance, tumor localization, and navigation support, improving the accuracy and safety of interventions (Klinkhammer et al., 2018; Treilhard et al., 2020).

6. Radiomics and Radiogenomics: AI-based radiomics and radiogenomics leverage machine learning algorithms to extract quantitative features from medical images and correlate them with genomics data, clinical outcomes, and treatment response. This integration of imaging and genomic data holds promise for precision medicine, aiding in patient stratification, treatment selection, and monitoring of therapeutic response (Gillies et al., 2016; Lambin et al., 2017).

AI in Disease Diagnosis

Accurate and timely disease diagnosis is vital for effective treatment and patient care. The emergence of AI techniques in healthcare has revolutionized disease diagnosis by leveraging machine learning and deep learning algorithms to analyze vast amounts of medical data. This study explores the potential applications of AI in disease diagnosis, highlighting its impact across various medical specialties. AI for disease diagnosis include the following areas:

1. Radiology: AI algorithms have demonstrated remarkable capabilities in analyzing medical images, assisting radiologists in the diagnosis of various conditions. Machine learning and deep learning techniques enable automated detection and classification of abnormalities in X-rays (Rajpurkar et al., 2018) and improved accuracy in interpreting mammograms for breast cancer diagnosis (McKinney et al., 2020).

2. Pathology: AI aids pathologists in disease diagnosis by analyzing histopathological images. Deep learning models have shown promising results in detecting and classifying cancerous cells in tissue samples, such as in the

diagnosis of prostate cancer (Nir et al., 2018) and breast cancer (Ehteshami Bejnordi et al., 2017).

3. Dermatology: AI algorithms have been developed to analyze dermatological images and assist in the diagnosis of skin conditions. Deep learning models have shown comparable performance to dermatologists in the identification of melanoma (Esteva et al., 2017) and other skin diseases (Haenssle et al., 2018).

4. Cardiology: AI techniques are being utilized in cardiology for diagnosing cardiovascular diseases. Machine learning algorithms can analyze electrocardiograms (ECGs) for the detection of arrhythmias (Hannun et al., 2019), while deep learning models aid in the identification of cardiac abnormalities in echocardiograms (Zhang et al., 2021).

5. Genomics: AI-based approaches are used to analyze genomic data for disease diagnosis and personalized treatment. Machine learning algorithms can integrate genetic information, clinical data, and medical imaging to improve diagnosis and predict treatment response in diseases such as cancer (Gao et al., 2020).

6. Clinical Decision Support Systems: AI is employed to develop clinical decision support systems that assist healthcare providers in diagnosing diseases. These systems analyze patient data, medical records, and clinical guidelines to provide evidence-based recommendations for diagnosis and treatment planning (Wright et al., 2019).

AI in Drug Discovery and Development

Drug discovery and development is a complex and time-consuming process that requires significant resources. The integration of artificial intelligence (AI) techniques has emerged as a powerful tool in expediting the identification and development of novel therapeutic compounds. This study examines the applications of AI in various stages of drug discovery and development, aiming to showcase its potential in revolutionizing the pharmaceutical industry. Applications of AI in Drug Discovery and Development are as follows:

1. Target Identification: AI algorithms can analyze biological and molecular data to identify potential drug targets. Machine learning models can leverage omics data, such as genomics, proteomics, and metabolomics, to identify disease-associated targets and pathways (Aliper et al., 2016). Additionally, AI techniques aid in uncovering new drug indications by repurposing existing drugs through network-based approaches (Cheng et al., 2018).

2. Virtual Screening: AI-based virtual screening methods accelerate the identification of potential drug candidates by predicting their interactions with

target proteins. Machine learning algorithms, such as support vector machines and random forests, have been successfully employed to screen large chemical libraries and prioritize compounds for further experimental validation (Gong et al., 2019). Deep learning models, such as convolutional neural networks, have demonstrated remarkable accuracy in predicting ligand-protein binding affinities (Jiménez et al., 2018).

3. Lead Optimization: AI techniques facilitate lead optimization by generating and optimizing chemical structures with desirable drug-like properties. Generative models, such as generative adversarial networks and variational autoencoders, can generate novel compounds with specified properties (Segler et al., 2018). Reinforcement learning algorithms can optimize chemical synthesis routes, reducing time and cost in the production of lead compounds (Schwaller et al., 2019).

4. Toxicity Prediction: AI algorithms enable the prediction of drug toxicity and adverse effects, aiding in candidate selection and reducing the risk of costly late-stage failures. Machine learning models trained on large databases of toxicological data can accurately predict the toxicity profiles of new compounds (Fourches et al., 2010). Deep learning techniques, such as recurrent neural networks, can analyze chemical structures and identify potential toxic substructures (Zhang et al., 2019).

AI in Personalized Medicine

Personalized medicine has emerged as a paradigm shift in healthcare, aiming to provide individualized treatment strategies based on patient-specific characteristics. The integration of artificial intelligence (AI) techniques has the potential to transform personalized medicine by harnessing vast amounts of patient data and genomic information. This study examines the applications of AI in personalized medicine, emphasizing its impact on disease risk assessment, diagnosis, treatment selection, and monitoring. A few applications of AI in Personalized Medicine are given below:

1. Disease Risk Assessment: AI algorithms can analyze diverse patient data, including clinical records, lifestyle factors, and genomic profiles, to assess an individual's risk of developing certain diseases. Machine learning models can identify patterns and biomarkers associated with disease susceptibility, enabling early intervention and preventive measures (Topol, 2019).

2. Diagnosis: AI techniques aid in accurate and efficient disease diagnosis by integrating patient data, medical imaging, and genomic information. Machine learning algorithms can analyze complex datasets and assist healthcare

professionals in identifying patterns, making differential diagnoses, and predicting disease progression (Obermeyer & Emanuel, 2016).

3. Treatment Selection: AI-driven approaches help in selecting optimal treatment strategies based on individual patient characteristics. Machine learning models can analyze patient data, including clinical profiles and genetic information, to predict treatment response and identify personalized therapeutic approaches (Chen & Asch, 2017).

4. Monitoring and Prognosis: AI algorithms enable continuous monitoring of patient health status and provide real-time feedback. Wearable devices and sensors collect patient data, which can be analyzed using machine learning models to detect early signs of disease progression, monitor treatment effectiveness, and predict prognosis (Ravi et al., 2017).

AI in Medical Predictive Analytics

Medical predictive analytics has emerged as a powerful tool in healthcare, allowing for proactive decision-making based on data-driven insights. Artificial intelligence (AI) techniques play a crucial role in analyzing large and complex patient datasets, enabling accurate predictions for disease prognosis, early detection, treatment response, and patient monitoring. This study explores the applications of AI in medical predictive analytics, highlighting its potential to transform clinical practice. Applications of AI in Medical Predictive Analytics:

1. Disease Prognosis: AI algorithms can analyze patient data, including electronic health records, laboratory results, and genetic information, to predict disease progression and prognosis. Machine learning models can identify risk factors, biomarkers, and clinical patterns associated with different outcomes, facilitating personalized treatment plans and interventions (Obermeyer et al., 2016).

2. Early Detection: AI-driven approaches enable early detection of diseases by analyzing diverse data sources, such as medical imaging, wearable devices, and patient-reported data. Machine learning algorithms can detect subtle patterns and anomalies in data, aiding in the early identification of conditions and improving treatment outcomes (Chartrand et al., 2017).

3. Treatment Response Prediction: AI techniques assist in predicting patient responses to specific treatments, optimizing therapy selection and improving patient outcomes. Machine learning models can integrate patient characteristics, genetic profiles, and treatment data to forecast individual responses and guide personalized treatment strategies (Kourou et al., 2015).

4. Patient Monitoring: AI algorithms enable continuous monitoring of patient health, facilitating early intervention and proactive care. Wearable

devices and remote sensors collect real-time patient data, which can be analyzed using machine learning models to detect deviations from normal patterns, predict health deterioration, and trigger timely interventions (Ravi et al., 2017).

AI in Robotic Surgery

Robotic surgery has revolutionized the field of minimally invasive procedures, offering enhanced dexterity, visualization, and precision. The integration of artificial intelligence (AI) techniques in robotic surgery further augments its capabilities, enabling intelligent surgical planning, real-time intraoperative assistance, and postoperative monitoring. This study explores the applications of AI in robotic surgery, highlighting its potential to transform surgical practice and improve patient outcomes. A few applications of AI in Robotic Surgery are conducted below:

1. Surgical Planning: AI algorithms assist in preoperative planning by analyzing patient data, medical imaging, and clinical records. Machine learning models can identify anatomical structures, predict surgical outcomes, and aid in personalized surgical approaches, enhancing surgical precision and reducing complications (van der Meijden et al., 2021).

2. Intraoperative Assistance: AI-driven approaches provide real-time guidance and decision support during robotic surgeries. Computer vision techniques combined with machine learning algorithms enable surgical navigation, tissue recognition, and instrument tracking, facilitating precise and safe interventions (Kassahun et al., 2020).

3. Postoperative Monitoring: AI algorithms analyze postoperative data, including patient vitals, laboratory results, and imaging findings, to monitor surgical outcomes and detect complications. Machine learning models can predict recovery trajectories, identify early signs of complications, and assist in personalized postoperative care management (Gupta et al., 2018).

AI in Health Monitoring and Wearable Devices

Health monitoring and wearable devices have transformed the way individuals track and manage their health. The integration of artificial intelligence (AI) techniques in these devices empowers users with continuous remote monitoring, real-time analytics, and personalized insights. This section explores the applications of AI in health monitoring and wearable devices, highlighting their potential to revolutionize healthcare management and improve patient outcomes.

Applications of AI in Health Monitoring and Wearable Devices:

1. Disease Management: AI algorithms in wearable devices can analyze health data, such as heart rate, blood pressure, and glucose levels, to provide personalized disease management insights. Machine learning models can detect patterns, predict health fluctuations, and alert individuals and healthcare providers about potential risks or early signs of deterioration (Liu et al., 2020).

2. Fitness Tracking: AI-driven approaches enhance fitness tracking capabilities by interpreting data collected from wearable devices. Machine learning algorithms can analyze activity patterns, sleep quality, and exercise routines to provide personalized recommendations for achieving fitness goals, optimizing performance, and preventing injuries (Pierleoni et al., 2015).

3. Remote Patient Monitoring: AI-enabled wearable devices enable remote patient monitoring, facilitating proactive healthcare management outside traditional clinical settings. Machine learning models can continuously analyze physiological data and provide real-time feedback to healthcare professionals, allowing early intervention, personalized treatment adjustments, and improved patient outcomes (McManus et al., 2019).

CONCLUSION

Artificial Intelligence (AI) has emerged as a transformative force in medicine and healthcare, offering significant potential to revolutionize the field. Through advanced algorithms and machine learning techniques, AI enables the analysis of complex datasets, extraction of valuable insights, and development of predictive models. This subsection has explored various applications of AI in medicine, including diagnosis, treatment planning, drug discovery, patient care, and health monitoring. AI has demonstrated its ability to improve patient outcomes, enhance clinical decision-making, and streamline healthcare processes.

In the domain of diagnosis, AI algorithms have shown remarkable performance in detecting and classifying diseases, particularly in medical imaging and pathology. This has the potential to lead to earlier and more accurate diagnoses, ultimately improving patient outcomes. Additionally, AI-driven treatment planning and precision medicine approaches hold promise in tailoring therapies to individual patients based on their unique characteristics and medical histories.

AI is also revolutionizing the drug discovery and development process, accelerating the identification of potential drug candidates, optimizing their properties, and predicting their efficacy. This has the potential to reduce the

time and cost involved in bringing new drugs to market, benefiting patients worldwide.

Furthermore, AI-powered patient monitoring and predictive analytics enable continuous health monitoring, early detection of abnormalities, and personalized interventions. Wearable devices and remote monitoring systems equipped with AI algorithms empower individuals to actively participate in managing their health while providing healthcare providers with valuable real-time data for proactive decision-making.

However, the integration of AI in medicine and healthcare also poses challenges. Ethical considerations, such as algorithm transparency, privacy concerns, and biases, need to be addressed to ensure responsible AI implementation. Data quality, standardization, and interoperability remain critical factors for successful AI deployment. Collaborative efforts between clinicians, researchers, and technology experts are essential to harness the full potential of AI while maintaining patient safety, privacy, and trust.

In conclusion, AI holds immense promise to transform medicine and healthcare by leveraging the power of intelligent data analysis. The applications of AI discussed in this study demonstrate its ability to enhance diagnosis, treatment planning, drug discovery, patient care, and health monitoring. With continued research, innovation, and ethical considerations, AI has the potential to revolutionize healthcare delivery, improve patient outcomes, and pave the way for a future of personalized, data-driven medicine.

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Chapter 7

Toxicity Profile of Vitamin A: a Review of Current Situation

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ABSTRACT

Purpose This review aims to provide an up-to-date view on the toxicity profile, interactions and management of hypervitaminosis state of vitamin A, including its sources and metabolism. **Methods** While preparing this review, a comprehensive literature search was made by examining books, research/review articles, case control studies in detail, and the toxicity profile of vitamin A was shed light by bringing together the relevant information. **Results** Vitamin A is an important micronutrient mainly involved in the regulation of various cellular processes. Today, while vitamin A is used in the treatment of various diseases, it can also be supplied in the form of vitamin supplements without a prescription. Since it is a widely used vitamin, its toxicity is important. Acute toxicity may present with nonspecific findings such as headache, nausea, whereas chronic toxicity may present with specific findings such as gingivitis, intracranial hypertension. In addition, vitamin A has been shown to have teratogenic effects. **Conclusion** Considering the fact that vitamin A is available without a prescription, it can be argued that the risks of vitamin supplements should be well documented. Since vitamin A is a vitamin that causes harmful effects on the fetus, both deficiency and excess during pregnancy, more research is needed on both the dose and the duration of administration during this period. It is known that vitamin A interacts with various drugs and especially alcohol. Therefore, when using nutritional supplements containing vitamin A, the risk of interaction with concomitant medications and individual drinking habits should be considered.

Keywords: Vitamin A; retinol; retinoic acid; retinol binding protein; toxicity

1. INTRODUCTION

Vitamin A, which refers to a group of compounds, including retinol, retinaldehyde, and retinoic acid, is a fat-soluble vitamin [1, 2]. It was discovered by McCollum and Davis in 1915 and has the distinction of being the first discovered vitamin [2]. It is mainly involved in the regulation of cells and tissue growth and differentiation [3]. The effects of vitamin A on epithelial cells, including keratinocytes, have led them to be prescribed for the treatment of various skin diseases, particularly acne, pityriasus rubra pilaris, nonspecific skin rash, ichthyosis, and dry skin, as well as to investigate its chemopreventive potential [4, 5]. Vitamin A also has many different functions such as bone development, reproduction, strengthening the immune system [6, 7] and is particularly critical in ocular function: it plays a role in cell differentiation, maintenance of eye integrity and prevention of xerophthalmia [8]. Vitamin A deficiency is the leading cause of preventable blindness [9]. Vitamin A is an important vitamin with its deficiency and excess. Today, while vitamin A is used in the treatment of various diseases, it can also be supplied in the form of vitamin supplements without a prescription Since it is a widely used vitamin, its toxicity is important. This review aims to provide an up-to-date view on the toxicity profile, interactions and management of hypervitaminosis state of vitamin A, including its sources and metabolism.

2. SOURCES and METABOLISM of VITAMIN A

Vitamin A cannot be synthesized by the body and is obtained through the diet [9]. The main nutritional source is preformed vitamin A from animal sources and fortified foods, and carotenide compounds from fruits and vegetables, especially ß carotene (provitamin A) [10, 11]. It is accepted that the absorption of vitamin A from vegetable sources is weak, and it is thought that consumption of food of animal origin is more effective in order to reach sufficient levels in the body [12, 13]. Retinyl esters found in food of animal origin (milk, yogurt, and cheese, liver, fish oils, and human milk) [9] are metabolized to retinol [14]. Provitamin A obtained from plant sources (carrots, pumpkin, kale, spinach, sweet potato, papaya, mango, and red palm oil) is oxidized to retinol in the intestinal mucosa [9, 11]. Retinol is the active form of vitamin A [11]. Vitamin A exerts its effects through the oxidized metabolites of retinol; retinoic acid (RA) and retinaldehyde [15, 16]. Retinaldehyde is involved in the visual cycle by playing a role in rhodopsin biosynthesis [15, 16, 17] while retinoic acid functions as a critical regulator of cellular functions in all tissues [17]. It functions as a potent regulator of gene expression through receptor-mediated events [15, 17, 18, 19]. Since vitamin A has many forms as

mentioned above and they have variable activity, various units such as international units (IU), retinol equivalents (RE) and retinol activity equivalents (RAE) are used to measure the amount of vitamin [10]. Retinol absorbed from the intestines is transported to the liver, where it is stored as retinyl esters regardless of dietary source [11, 20] and then newly synthesized retinyl esters are packaged as chylomicrons and secreted into the lymphatic system [15, 20]. It is thought that nuclear hormone receptors such as peroxisome proliferator-activated receptors (PPAR α, β and γ) are involved in the esterification and storage of retinol [21]. After entering the circulation, chylomicron packets undergo lipolysis of triglycerides and are broken down into free fatty acids and chylomicron residues, and the majority of chylomicron residues are cleared by the liver [15, 22, 23, 24]. Hepatocytes uptake retinol esters from chylomicron residues, and after hydrolysis of retinol esters, retinol can be stored as esters of long-chain fatty acids in rich lipid droplets in hepatic stellate cells (HSC), also known as Ito cells, located in the disseminated cavity of the normal liver [1, 25, 26, 27] or forms a complex with a carrier protein called retinol binding protein (RBP) [28]. These proteins act as a mediator for the delivery of retinol to target tissues and regulation of plasma concentrations [14]. RBP, which is secreted by the liver bound to retinol, combines with another protein, trastiretin, in the plasma to form the main transport complex and delivers retinol to specific cell surface receptors at tissue sites of action [11, 29]. Excessive, chronic intake of vitamin A results in exceeding RBP carrying capacities. Vitamin A not bound by RBP circulates with serum lipoproteins. When presented to tissues in this form, it can cause both overstimulation of normal retinol activity and different toxic effects. Unbound retinol can not only penetrate the lipoprotein bilayers of cells and impair membrane integrity, but also cause increased release of lysosomal hydroxylases such as cathepsin, which disrupt the extracellular matrix of tissue. Considering this information, it appears that RBP helps to protect the tissues from the surfactant properties of the vitamin, as well as the delivery of retinol to the tissues [30].

3. TOXICITY of VITAMIN A

Since vitamin A is a fat-soluble vitamin, excess amounts are stored in various parts of the body, primarily the liver (50-90% of vitamin A stores [31], eyes and lungs [32, 33]. It can accumulate in the body, causing dangerous acute or chronic hypervitaminosis [3]. Toxicity usually occurs as a result of improper use of dietary supplements [34]. Acute hypervitaminosis A typically occurs days or weeks after ingestion of vitamin A at doses greater than 100 times the Recommended Dietary Allowance (RDA) [35]. The RDA for female and male

is 700 mcg RAE and 900 mcg RAE, respectively, while the RDA during pregnancy and lactation is 770 mcg RAE and 1300 mcg RAE, respectively. Upper Intake Level (UL) is 3000 mcg for all groups specified [36]. The specified values are shown in Table 1. Symptoms are usually headache, dizziness, nausea, blurred vision, and motor coordination problems secondary to intracranial hypertension [37]. Symptoms are usually reversible when overdose is discontinued [38]. Consumption of foods rich in preformed vitamin A is also known to cause toxicity. At the top of the food chain, the livers of carnivores such as polar bears and large marine fish are the only natural foodstuffs that contain enough vitamin A to cause toxicity in humans. Cases of acute toxicity have been reported by Arctic explorers and fishermen from consumption of livers containing up to 100,000 IU/g of vitamin A [11]. While the natives of the arctic regions are aware of this risk and pay attention to the amount of liver they eat, poisoning cases have been reported after consuming fish such as grouper and sea fish in some Asian societies [39, 40, 41]. There have also been reports of hypervitaminosis A in many species that feed on large amounts of liver, particularly in pets [42]. Toxic reactions have been reported in infants receiving synthetic doses of vitamin A as low as 75,000-300,000 IU. As a result of increased intracranial pressure in these children, anorexia, hyperirritability, vomiting, and bulging fontanelles occurred and peeling of the skin occurred within a few days, but all symptoms disappeared within a short time and no long-term effects were reported [11].

Table 1 Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake Level (UL) for Vitamin A

RDA		**UL (for preformed Vitamin A)**
Female	700 mcg RAE / 2333 IU	3000 mcg
Male	900 mcg RAE / 3000 IU	3000 mcg
Pregnacy	770 mcg RAE / 2566 IU	3000 mcg
Lactation	1300 mcg RAE / 4333 IU	3000 mcg

Chronic hypervitaminosis A may occur with regular intake of more than 10,000 IU daily, to which synthetic retinoids can contribute [37]. Dosage regimen, physical form of the vitamin, general health status of the individual, ethanol and protein intake, and interactions with vitamins C, D, E and K are among the factors affecting chronic hypervitaminosis A [38]. Acute toxicity may present with nonspecific findings, whereas chronic toxicity may present with skin dryness, gingivitis, hair loss, muscle and joint pain, fatigue,

depression, anorexia, abnormal liver test results, vision loss, severe intracranial hypertension, and neurological symptoms [37, 43, 44]. It is known that hypervitaminosis A rarely causes hypercalcemia [45]. Vitamin A is thought to cause this effect, possibly by stimulating osteoclastic resorption or inhibiting osteoblastic formation [46]. Hypercalcemia due to hypervitaminosis A has been observed to resolve with saline infusions and oral furosemide treatment [47]. Stellate cells can be activated following liver injury and express the alpha actin gene and produce a large amount of extracellular matrix, adopting a myofibroblast-like phenotype with high fibrogenic capacity [26, 48]. Hypervitaminosis A can cause liver damage ranging from portal hypertension due to hypertrophy and hyperplasia of HSCs to fibrosis and cirrhosis [48, 49]. Although rare, there is also a case of intrahepatic cholestasis [49]. The diagnosis of liver injury from chronic hypervitaminosis A is probably often overlooked because its symptoms are variable and the definitive diagnosis requires liver biopsy [43]. Clinical findings disappear after discontinuation of the drug, but there are cases in the literature requiring liver transplantation [43]. Carotenoids are not considered toxic because their conversion to retinol is relatively ineffective. As a result of high intake, excess carotene accumulates in the body and hypercarotenosis develops, which is manifested by yellow pigmentation on the skin. However, this slowly disappears when the pigment source is removed [11]. This condition differs from jaundice due to lack of scleral pigmentation [34, 50]. Karatonemia has also been rarely associated with nephrotic syndrome, liver diseases, and hypothyroidism [34, 51]. However, one study reported an increased risk of lung cancer and death in smoking men who took large amounts of ß carotene supplements [52]. While no noninvasive markers are available to assess hypervitaminosis A status, serum retinol levels may also not reflect plasma and tissue levels [32, 43, 53] and diagnosis is usually based on a history of vitamin A intake, the absence of other detectable causes of chronic liver disease and based on observation of stellate cell hyperplasia and hypertrophy on liver biopsy [54]. Patients who develop the hypervitaminosis state may have low, normal, or high serum vitamin A levels [32]. This is because in the early stages of chronic intake, the serum levels of vitamin A are low when stored in the liver, and the serum levels are high when the liver capacity is full [45]. Therefore, serum retinol levels are thought to be a better biomarker for identifying vitamin A deficiency rather than excess [43, 53].

3.1 Teratogenicity

Pregnancy is a period in which special attention should be paid to nutrition in order to protect the health of both the mother and the fetus. During this period, due to the important effects of both deficiency and excess of vitamin A on the fetus, its use is a vitamin that should be given special attention. Since vitamin A is necessary for morphological and functional development and ocular integrity, this vitamin is needed especially in the 3rd trimester, when fetal development accelerates [9, 55]. According to the World Health Organization (WHO), vitamin A deficiency during pregnancy is considered a public health problem at the population level, especially in some developing countries [8, 9]. On the other hand, it has been shown that excessive intake of vitamin A causes teratogenicity in both human and animal studies [56, 57]. Intake of more than 15,000 IU/day from combined sources of food and supplements and more than 10,000 IU/day from supplements alone has been associated with an increased incidence of malformations [58, 59]. Experts recommend that women who are pregnant, suspected of pregnancy, or breastfeeding mothers do not take more than 10,000 IU of vitamin A supplements per day [60, 61]. Teratogenic manifestations include craniofacial (cleft lip/palate), cardiovascular (transposition of great vessels), thymic and central nervous system (microcephaly, hydrocephalus) malformations [62]. Although the pattern of malformations observed in these birth defects is not always consistent with retinoic acid syndrome [63], it may also be called retinoic acid syndrome in some cases [64]. Teratogenicity is known to be the most worrisome side effect of systemic retinoid use, and isotretinoin is estimated to increase the risk of malformation by 25-fold [63]. The retinoids tretinoin and adapalene, a naphthoic acid derivative with potent retinoid activity, are categorized as pregnancy drug category C and the retinoid tazarotene X under the former Food and Drug Administration (FDA) classification [65]. It has been shown that the teratogenicity risk of topical retinoids is quite low [65, 66]. It is thought that vitamin A's metabolites such as trans-retinoic acid and 13-cis-retinoic acid cause teratogenicity by affecting gene functions during critical periods of organogenesis and embryogenesis [67, 68]. There are also implications that vitamin A causes teratogenicity by affecting the axial model arrangement in the embryo, possibly by affecting the expression of the homeobox gene Hoxb1, especially in neural crest cells [69]. ß carotene, unlike preformed vitamin A, is not known to be teratogenic [60].

4. MANAGEMENT of HYPERVITAMINOSIS A

Vitamin A toxicity usually occurs due to oral or topical exposure, the general treatment principle is based on cessation of vitamin intake [11, 34, 38]. Symptoms are usually reversible when overdose is discontinued [38]. In hypertriglycemia due to oral retinoid use, if the triglyceride level is above 800 mg/dL, there is a risk of pancreatitis and dose reduction or drug discontinuation may be required. If the triglyceride level is below the specified amount, the treatment can be continued by monitoring the triglyceride levels [63, 70]. For the treatment of hypercalcemia due to hypervitaminosis A, saline infusions and oral furosemide therapy are preferred [47]. Acetazolamide treatment is applied to reduce the increase in intracranial pressure due to hypervitaminosis A [34, 63]. In order to reduce skin irritations such as skin redness, skin dryness, skin peeling due to topical retinoid use, it is recommended to use skin softeners as well as reducing the volume and application frequency of the drug. Eye drops containing artificial tears and methylcellulose are preferred for dry eye following hypervitaminosis [34, 63].

5 INTERACTIONS of VITAMIN A

It is known that vitamin A interacts with some drugs and various drugs affect the level of vitamin A in the body [61]. Orlistat is one of these drugs. Orlistat (Alli®, Xenical®), one of the most commonly prescribed antiobesity drugs, prevents the uptake of approximately 30% of dietary fat by blocking the activity of gastric and pancreatic lipases [71]. Therefore, it reduces the absorption of fat-soluble vitamins such as vitamins A, D, E and K, and ß carotene. For this reason, it is recommended that patients using this drug take a multivitamin supplement containing fat-soluble vitamins [72, 73, 74]. It is also known that vitamin A may pose a risk of hypervitaminosis A when used together with synthetic retinoids derived from vitamin A and used orally (for example, acitrein (Soriatene) used for the treatment of psoriasis) [61, 75]. A well-known example of interaction for vitamin A is with ethyl alcohol (ethanol). It has been shown that hepatic vitamin A levels are low in alcoholics [76, 77]. Ethanol is metabolized in the human body primarily to acetaldehyde and then to acetic acid. In parallel with this pathway, dietary vitamin A is first metabolized to retinaldehyde and then to retinoic acid [78]. Therefore, it is an expected result that these two alcohols, which are metabolized in parallel ways, interact by competing for the same or similar enzymatic pathways [79]. To explain in more detail, ethanol inhibits vitamin A metabolism by competing with vitamin A for alcohol dehydrogenases and acetaldehyde dehydrogenases, which are involved in the oxidation of vitamin A to retinoic acid. Ethanol also interacts with

cytochrome P450 enzymes, especially CYP2E1, increasing vitamin A catabolism and causing hepatic vitamin A depletion [76, 77, 80]. It is also known that ethanol increases the mobilization of vitamin A from the liver to the extrahepatic tissues [77]. Based on this information, it can be said that alcohol consumption causes a decrease in hepatic levels by increasing the destruction of vitamin A and its mobilization to extrahepatic tissues. Considering these interactions, it is recommended to consider individual drinking habits when supplementing with vitamin A [79].

6. CONCLUSIONS and FUTURE PERSPECTIVES

Today, the most common cause of vitamin A toxicity is the misuse of high doses of vitamin A prescribed for dermatological diseases, especially acne vulgaris, and vitamin supplements available without a prescription. It is also known that excessive consumption of foods rich in vitamin A causes toxicity [11, 31, 34]. Hypervitaminosis A may cause symptoms such as headache, nausea, which are reversible after dose cessation, as well as toxicity severe enough to require liver transplantation [37, 43]. Therefore, the risks of vitamin supplements should be well documented. Since vitamin A is a vitamin that causes harmful effects on the fetus, both deficiency and excess during pregnancy, more research is needed on both the dose and the duration of administration during this period. It is known that vitamin A interacts with various drugs and especially alcohol [61, 77]. Therefore, when using nutritional supplements containing vitamin A, the risk of interaction with concomitant medications and individual drinking habits should be considered.

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Chapter 8

Genetic Causes of Glaucoma: An Overview of Current Knowledge and Future Directions

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Abstract

Glaucoma is a leading cause of irreversible blindness worldwide, characterized by progressive optic nerve damage and visual field loss. While elevated intraocular pressure (IOP) is a major risk factor for glaucoma, the disease has a complex etiology involving genetic and environmental factors. In recent years, significant progress has been made in identifying genetic variations associated with glaucoma, providing insights into the underlying mechanisms of disease pathogenesis. This review summarizes the current state of knowledge regarding the genetic causes of glaucoma, highlighting the major genes and pathways implicated in the disease. We also discuss the challenges and opportunities for further research in this field, including the development of personalized genetic testing and therapeutic strategies for glaucoma patients.

1- Introduction

Glaucoma is a heterogeneous group of optic neuropathies characterized by progressive loss of retinal ganglion cells (RGCs) and their axons, resulting in irreversible vision loss. The disease affects approximately 70 million people worldwide and is projected to increase in prevalence due to aging populations. (1) While elevated IOP is a major risk factor for glaucoma, up to 40% of patients have normal IOP levels, indicating the presence of other contributing factors, including genetic susceptibility. (1) In recent years, significant progress has been made in identifying genetic variations associated with glaucoma, providing insights into the underlying mechanisms of disease pathogenesis. This short review aims to provide an overview of the current state of knowledge regarding the genetic causes of glaucoma, highlighting the major genes and pathways implicated in the disease.

2- Genetic Causes of Glaucoma

2.1. Mendelian Inheritance Patterns

Glaucoma can be inherited in a variety of ways, including autosomal dominant, autosomal recessive, and X-linked modes of inheritance. (2) Several genes have been identified as causative or contributing factors in Mendelian forms of glaucoma. For example, mutations in MYOC, which encodes for myocilin, a protein involved in regulating aqueous humor outflow, have been associated with juvenile- and adult-onset open-angle glaucoma. (2, 3) Mutations in CYP1B1, which encodes for a cytochrome P450 enzyme, have been implicated in primary congenital glaucoma. (3, 4) Mutations in OPTN, which encodes for optineurin, a protein involved in autophagy and vesicle trafficking, have been associated with normal-tension glaucoma and some forms of primary open-angle glaucoma. (4, 5)

2.2 Genome-Wide Association Studies

In addition to Mendelian forms of glaucoma, genome-wide association studies (GWAS) have identified multiple common genetic variants associated with increased risk of glaucoma. For example, a common variant in the CDKN2B-AS1 locus, which regulates the expression of CDKN2B and CDKN2B-AS1, has been associated with primary open-angle glaucoma and normal-tension glaucoma. Other GWAS-identified genes include TMCO1, CAV1/CAV2, SIX1/SIX6, and AFAP1. (6, 7)

2.3 Gene-Environment Interactions

While genetic factors can contribute to glaucoma risk, they can also interact with environmental factors, such as IOP and aging, to influence disease onset and progression. For example, the risk of glaucoma associated with MYOC mutations is greater in individuals with higher IOP. Other gene-environment interactions have been identified, including between CYP1B1 mutations and consanguineous marriage. (7, 8, 9, 10)

Challenges and Opportunities

Despite significant progress in identifying genetic causes of glaucoma, several challenges remain. For example, many glaucoma patients do not have a known genetic cause, and the functional effects of many genetic variants remain unclear. Additionally, genetic testing for glaucoma is not yet widely available, and there is a lack of consensus regarding which genes should be tested and in which populations. Finally, while identifying genetic variants associated with glaucoma is a critical first step, understanding the underlying biological mechanisms and developing targeted therapies requires further research. However, there are also opportunities for further advances in this field. With the increasing availability of genetic testing and the development of more efficient and accurate sequencing technologies, it is now possible to identify rare genetic variants associated with glaucoma in large cohorts. This could lead to the discovery of novel genes and pathways involved in disease pathogenesis. Additionally, the use of genetic data in conjunction with other clinical and demographic factors could facilitate the development of personalized risk prediction models and tailored treatment strategies for glaucoma patients.

Conclusion

In conclusion, significant progress has been made in identifying genetic causes of glaucoma, providing insights into the underlying mechanisms of disease pathogenesis. While challenges remain, there are also opportunities for further advances in this field, including the development of personalized genetic testing and targeted therapies for glaucoma patients. Continued research in this area is critical for improving our understanding of glaucoma and ultimately preventing or treating this debilitating disease.

Several key areas of research have the potential to advance our understanding of the genetic causes of glaucoma. First, functional studies of known genetic variants can help elucidate the underlying biological mechanisms of disease pathogenesis. For example, studies using animal models or cellular systems can help identify the specific cell types or molecular pathways affected by these

variants. Second, genome-wide association studies (GWAS) can help identify common genetic variants associated with glaucoma risk. While most known genetic variants have been identified through targeted sequencing of candidate genes, GWAS have the potential to identify novel genes and pathways not previously implicated in disease pathogenesis. However, large sample sizes and careful control for population structure are needed to achieve sufficient statistical power. Third, studies of rare genetic variants can provide insights into the genetic architecture of glaucoma. Rare variants are often more penetrant than common variants, and identifying these variants can help identify novel genes and pathways involved in disease pathogenesis. Additionally, studies of families with multiple affected individuals can help identify rare genetic variants that segregate with disease. Finally, studies of gene-environment interactions can help elucidate the complex interplay between genetic and environmental factors in glaucoma pathogenesis. For example, identifying genetic variants that modify the effects of environmental risk factors (such as elevated intraocular pressure) could lead to the development of more personalized risk prediction models and targeted therapies. In summary, significant progress has been made in identifying genetic causes of glaucoma, but challenges remain in understanding the functional effects of genetic variants and developing targeted therapies. Advances in functional studies, GWAS, rare variant analysis, and gene-environment interactions have the potential to further our understanding of glaucoma and improve patient outcomes.

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Chapter 9

Exploring the Lunar Cycle's Influence on Sleep Patterns

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ABSTRACT

The moon has captured the imagination and curiosity of people throughout history. Its celestial presence and ever-changing appearance have fuelled numerous cultural beliefs and legends, including the idea that the lunar cycle can influence various aspects of human life. One of these areas of interest is the possible effects of the lunar environment on sleep patterns.

Viewed from Earth, the lunar cycle shows changes in the Moon's image. It covers a period of approximately This cycle corresponds to approximately 29.5 days and during this period the Moon; The New Moon goes through eight distinct periods, namely the First Quarter, the Widening Hump, the Narrowing Hump, the Full Moon, the Third Quarter, the Declining Crescent, and the Growing Crescent.Various cultures around the world have attributed mystical or significant qualities to different phases of the moon.

Folklore has associated the Full Moon with insomnia, insanity (from which the term "mad" derives) and other psychological disorders. Although these beliefs persist in modern society, scientific evidence to support them is limited.

Different results have been obtained from scientific studies on the effects of the lunar cycle on sleep patterns. While the sun is the primary source of light and the synchronizer of circadian rhythms for many, moonlight is the regulator for many nocturnal activities. Moonlight appears very bright to the human eye and can affect both sleep and nighttime activity in the absence of another light source. Many studies contradict the effect of the lunar cycle on sleep; but recently, research under tightly controlled laboratory conditions has revealed the existence of the effects of the lunar cycle on sleep.Human studies show that there is a disruption in sleep with the full moon. It is important for such studies to consider the confounding effects of factors known to affect human sleep, such as obstructive sleep apnoea and insomnia.

Although conclusive evidence has not been presented, researchers have put forward several theories to explain what the current mechanisms are behind the effects of the lunar cycle on sleep. One hypothesis suggests that the Moon's gravity may affect sleep by influencing human circadian rhythms that regulate our sleep-wake cycles.

Another hypothesis suggests that the brightness of the moon during certain lunar phases may influence sleep quality.

The relationship between the lunar cycle and sleep patterns continues to intrigue researchers and arouse public curiosity. While historical beliefs and cultural anecdotes persist, scientific studies to date have not provided convincing evidence to support a direct causal link between the lunar cycle and sleep disorders. However, it is important to recognise that individual experiences may

differ and that some people may perceive sleep disturbances during certain lunar phases.

Whatever the influence of the moon, maintaining healthy sleep habits and seeking professional advice for persistent sleep problems remains the key to a good night's sleep.

Key words: Lunar cycle's, Moon cycle's, Moon, Sun, Sleep, Sleep quality

Introduction:

Throughout history, the moon has captivated human imagination and curiosity. Its celestial presence and ever-changing appearance have sparked numerous cultural beliefs and myths, including the notion that the lunar cycle can impact various aspects of human life. One such area of interest is the potential effects of the lunar cycle on sleep patterns. While scientific research on this topic is ongoing and inconclusive, it remains a subject of fascination and exploration. In this article, we delve into the connection between the lunar cycle and sleep, shedding light on existing theories and studies (Bevington 2015; Simon 2018) .

Understanding the Lunar Cycle:

The lunar cycle refers to the continuous changes in the moon's appearance as seen from Earth. It spans approximately 29.5 days, during which the moon transitions through eight distinct phases, including New Moon, Waxing Crescent, First Quarter, Waxing Gibbous, Full Moon, Waning Gibbous, Third Quarter, and Waning Crescent. These phases occur due to the moon's orbit around the Earth and its illumination by the sun (Chashiragy 2021).

Historical and Cultural Beliefs:

Various cultures across the globe have attributed mystical or significant qualities to different phases of the moon. Some ancient civilizations believed that the moon's energy directly influenced human behavior and health. Folklore associated the Full Moon with insomnia, lunacy (from which the term "lunatic" derives), and other psychological disturbances. While these beliefs persist in modern society, scientific evidence supporting them is limited (Bevington 2015; Simon 2018; Cashiraghy 2021).

Scientific Studies:

Scientific investigation into the potential effects of the lunar cycle on sleep patterns has produced mixed results. Several studies have attempted to establish a relationship between lunar phases and sleep quality or duration, but their findings have been inconclusive. While the moonlight simultaneously organizes

many night activities; The sun is the primary source of light and the replicator of circadian rhythms for most species. Moonlight is strikingly bright and can affect sleep and nighttime activity in the absence of any other light source. However, its real impact on humans remains a matter of debate. Many studies contradict the effect of the lunar cycle on sleep, but recent studies under tightly controlled laboratory conditions have determined the effects of the lunar cycle on sleep as a result of the study (Casiraghi 2021).

In the current world, the influence of the Moon on biological rhythms such as tree-wide variation, reproductive patterns of crabs, spawning events in the Great Barrier Reef, and nocturnal activity of monkeys has been documented. Human studies prove a deterioration in sleep quality and duration during the lunar full moon. One sleep study concluded, using several measurements, that the full moon worsens sleep quality and duration. During this cyclical phase of the moon, participants took five minutes longer to fall asleep, slept 20 minutes less, took longer to reach REM sleep, experienced a 30% reduction in deep sleep duration, and reported reduced sleep quality. Another study is; He concluded that during the full moon cycle of the moon, difficulty falling asleep and waking up increased, while the duration of sleep decreased by 25 minutes. One analysis looked at data from 319 people who underwent a one-night sleep study. In this review; He found that they had lower sleep efficiency, lighter sleep, and a delay in reaching REM sleep during the full moon. One of the most comprehensive studies on this topic was conducted with 464 American university students in one of Argentina's three indigenous communities and major cities. He concluded that all groups had difficulty falling asleep and slept less during the full moon cycle compared to previous weeks, regardless of exposure time and location to artificial light.A study involving 31 people in a suburban area of Switzerland found that self-reported short sleep duration was more common on full moon nights (Röösli et al., 2006). Another study involving 20 male subjects in Tunisia found that perceived sleep levels were of lower quality on full moon days than on other cycles (Dergaa et al., 2019).

In a study conducted in a laboratory in Switzerland, it was reported that the total sleep time before the full moon decreased and then increased, and the onset of sleep was delayed during the full moon period (Cajochen et al., 2013; Smith et al., 2014). In these polysomnography sleep studies, the effect of the Moon phase on total sleep duration and sleep onset latency was greater.

Considering the confusing effects of factors known to affect human sleep, such as obstructive sleep apnea and insomnia, is important for such studies (Sateia, 2014). According to the results of the research conducted in Sweden, which is one of the largest data group studies to date investigating the relationship

of the moon cycle with sleep, it has been stated that the moon cycle can affect sleep in humans and that there are more significant sleep changes in men than in women. Also, the strength of this study was that when investigating the lunar cycle and sleep, sleep disorders common in the general population (eg, insomnia and obstructive sleep apnea) were not taken into account (Benedıct 2022)

Roösli et al. (2006) studied the effect of the moon phase on sleep. A feasibility study was also conducted for mobile phone base stations, which are thought to affect sleep. It was concluded that the sleep duration was less and of poorer quality in the full moon. The fatigue levels of the individuals participating in the study were also found to be high during the full moon.

In a study by Cajochen et al., during the full moon, electroencephalogram (EEG) delta activity changed and it was found that NREM sleep, which is an indicator of deep sleep, decreased by 30%, time to fall asleep increased by 5 minutes, and total sleep time decreased by 20 minutes. These changes were associated with decreased subjective sleep quality and decreased endogenous melatonin levels (Cajochen 2013).

It has been reported that the full moon is associated with sleep, creating discomfort and higher cortical reactivity in adults (Smith et al. 2014). One study in three large samples of 470, 757, and 870 sleep recordings found no effect of the lunar cycle on human sleep (Cordi et al., 2014). Similarly, it did not find any significant differences between moon phases regarding subjective sleep quality. (Haba-Rubio et al. 2015; Depner et al. 2014; Haack et al. 2004; Scheer et al. 2009; Spiegel et al. 1999; Wright et al. 2006).

One notable study analyzed the sleep patterns of participants in a controlled laboratory environment and found no significant correlation between lunar phases and sleep quality. The study concluded that while moonlight exposure could impact sleep timing and duration in some individuals, the lunar cycle itself did not exert a direct influence (Komado 2021).

Another study, investigated sleep quality and lunar phases among a large group of participants over an extended period. The researchers also found no significant association between the lunar cycle and sleep disturbances (Romdhani et al. 2019).

Potential Mechanisms:

Despite the lack of conclusive evidence, researchers have proposed various theories to explain the potential mechanisms behind lunar cycle effects on sleep, if any. One hypothesis suggests that the moon's gravitational pull might influence sleep by affecting human circadian rhythms, which regulate our sleep-wake cycles. However, empirical data supporting this theory are limited, and the

moon's gravitational force on Earth is considerably weaker than the Earth's own gravity.

Another hypothesis posits that the brightness of the moon during specific lunar phases might affect sleep quality. Excessive moonlight could potentially disrupt sleep patterns, but this effect would likely vary depending on factors such as geographic location, bedroom light conditions, and individual sensitivity to light (Cajochen 2013; Smith et al., 2014).

Conclusion:

The relationship between the lunar cycle and sleep patterns continues to intrigue researchers and capture public fascination. While historical beliefs and cultural anecdotes persist, scientific studies conducted so far have not provided compelling evidence to support a direct causal link between the lunar cycle and sleep disturbances. However, it remains essential to acknowledge that individual experiences may vary, and some people might perceive sleep disturbances during certain lunar phases.

As science progresses and new studies emerge, our understanding of the moon's influence on sleep patterns may evolve. For now, it seems that other factors, such as daily routines, stress levels, and sleep environment, play more significant roles in determining sleep quality and duration. Regardless of the moon's influence, maintaining healthy sleep habits and seeking professional advice for persistent sleep problems remains the key to a good night's rest.

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Chapter 10

Overview Of Anesthetic Agent

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ABSTRACT

Anesthesia is necessary for procedures on experimental animals to alleviate pain and restrain them. To ensure successful anesthesia, factors like drug type, dosage, and administration method are crucial. It is also important to minimize stress and manage pain post-anesthesia. However, selecting and using anesthetic drugs in experimental animals poses challenges. Cost considerations play a role in anesthesia applications, and the small size of laboratory animals limits processes. In rodents, isoflurane is commonly used due to its safety and quick induction and recovery. However, its use may be limited by equipment requirements or anatomical issues. Combining anesthetic agents can overcome the drawbacks of using them individually. Balanced anesthesia, achieved through combinations, aims to minimize the impact on animal physiology and facilitate a quick, painless awakening. Administering sedatives before inhaled agents reduces stress, while adding inhalants to injectables increases anesthetic concentration for longer procedures.

Key words: anesthesia, combination techniques, newer agents

Most procedures on experimental animals require anesthesia because of the pain and the need to restrain the animal. To manage a successful anesthesia process, not only the type of drug, dose and route of administration are important. However, it is necessary to reduce stress and control pain after anesthesia. There are problems with the selection and use of anesthetic drugs in experimental animals. In today's anesthesia applications, cost is kept in the foreground. In addition, the small size of laboratory animals limits the processes [1]. In rodents, inhalants such as isoflurane are generally the anesthetic of choice because of their safety and ability to induce rapid induction and recovery. However, there are limitations in its use due to equipment need or anatomical incompatibility. Therefore, the use of injectable agents has been included in the routine. Most injectable anesthetic drugs are administered as an intraperitoneal bolus, although intravenous administration is possible in mice [2]. At this point, it is important to weigh the weights. However, muscle relaxation and analgesia, which are necessary for the implementation of the procedures, should also be included in the anesthesia process. Issues such as the release of cytokines from the damaged tissues during the operation, the sensitization of the nervous system to stimuli due to cytokines, and the delay of wound healing require the use of analgesics in addition to anesthetic drugs. The use of drugs in combination in this way in order to perform a correct anesthesia procedure is called “balanced anesthesia”. An anesthetic regimen should be chosen that has as little impact as possible on the experimental protocol and does not alter the experimental results. The techniques used to achieve the mentioned results should be constantly improved in the light of new developments. Different anesthetic regimens should be applied for different experimental protocols. Therefore, it is necessary to know different anesthetic drugs and their combinations. However, the contribution of these applications to animal physiology should be investigated [1]. Therefore, it is important to know the pharmacological and toxicological effects of the drugs used and to conduct additional studies to clarify the missing points. In this chapter, anesthetics are classified as general anesthetics intravenous (ketamine, propofol, barbiturates, etomidate, etc.) and local general anesthetics (nitrous oxide, halothane, sevoflurane, isoflurane, benzodiazepine, etc.) and their pharmacokinetic properties and their place in combination therapy are mentioned.

1. Intravenous General Anesthetics

1.1. Ketamine

In use for 50 years, ketamine produces an anesthetic with a different sensation than volatile anesthetics. Hypnosis - low concentrations produce a psychotomimetic effect, higher doses produce a dissociative state of anesthesia

with increased sedation and loss of consciousness, intense analgesia, increased sympathetic activity and maintenance of airway tone and respiration [3].

Ketamine, one of the dissociative anesthetic group drugs, is a non-competitive N-methyl-D-aspartate (NDMA)-receptor antagonist. In addition, it acts on nicotinic acetyl-choline ion channels, delta and mu-opioid agonism and opioid potentiation, nitric oxide, non-NMDA glutamate receptors and metabotropic glutamate receptors, and decreased cholinergic neuromodulation [3].

The difference of ketamine from other intravenous agents is that it has analgesic, hypnotic and amnesic effects. Ketamine, which has a strong analgesic effect, has a mild narcotic effect and also has the lowest hallucinogenic potential among the phencyclidine derivatives [4].

The drug, which has intravenous and intramuscular uses, can also be administered by intraperitoneal route in laboratory animals. It has been determined that the half-life for intraperitoneal use in mice is 13 minutes [5]. In anesthetized animals, the corneal reflex is lost very early, so if the operation will take a long time, it is necessary to use ophthalmic pomades to prevent complications. Causes severe respiratory depression when used alone to induce surgical anesthesia in rodents. Therefore, it is usually used in combination with drugs such as xylazine, medetomidine, diazepam, midazolam. This application also prevents side effects such as muscle tremors [1].

1.2.Propofol

Potent intravenous sedative-hypnotic drugs such as propofol (2,6-diisopropylphenol), the main class of inhibitory ligand-gated ion channels in the mammalian brain, act through γ-aminobutyric acid type A ($GABA_A$) receptors [6]. Although propofol has been shown to positively modulate and directly activate the $GABA_AR$ channel complex, the intracellular response to the interaction between $GABA_AR$ and propofol is still unclear. Studies have shown that propofol causes a sudden increase in intracellular calcium concentration and subsequently reorganizes the cytoskeletal protein actin [7].

It is prepared in a lipid emulsion called propofol amnesia milk, which contains soybean oil, glycerol, egg lecithin and a small amount of preservative EDTA and is milky white in color [8]. Propofol used via intravenous administration is dose dependent and has a rapid onset effect in less than 1 minute, but its anxiolytic effect has been found to be dose independent [9]. The induction dose will show a clinical duration of action of 10 minutes, while prolonged and repeated administration will prolong the effect by accumulating in the surrounding tissues. Propofol, which has a large volume of distribution, shows 97-99% binding to proteins. It is metabolized by hepatic oxidation and conjugation to sulfate and

glucuronide conjugates. 60% of the drug undergoes hepatic clearance, 40% extrahepatic clearance by the kidneys and the drug is usually eliminated by the kidneys. The half-life of propofol is biphasic, with an initial half-life of approximately 40 minutes and a terminal half-life of usually 4 to 7 hours. The contextsensitive half-life can be up to 1 to 3 days after 10 days of infusion. However, the duration of clinical effect is much shorter [8].

The ideal drug for sedation, i.e. ease of use, rapid onset, rapid offset and minimal residual sedation make propofol the ideal drug for sedation. Additional benefits include cardioprotective effects in patients undergoing coronary bypass surgery thanks to its antioxidant properties, and cerebroprotective effects in injuries and tumors by reducing cerebral blood flow and decreasing metabolic rate.

However, propofol has side effects such as pain during injection, hypotension, hypoventilation, hypoventilation, bradycardia and hyperlipidemia, and besides its clinical use, it is widely used in basic neuroscience research to better understand consciousness, memory and learning [10].

Transient local pain at the injection site is the most common side effect. To reduce this effect, iv lidocaine may be given before propofol bolus. Apart from these side effects, hypotension, myoclonus, rarely ECG changes (QT interval prolongation), which is an important clinical finding, and very rarely discolored urine (a green tint) may be observed [9]. It is safe in pregnancy but crosses the placenta and may cause neonatal CNS and respiratory depression. It is the safest of all agents for induction in stable obstetric patients [11]. Propofol infusion syndrome (PIRS) is a very rare but serious side effect. It usually occurs as a result of propofol infusion > 4 mg/kg/hour for more than 24 hours. It occurs within 4 days of propofol treatment. The syndrome manifests as metabolic acidosis, hyperkalemia, hyperlipidemia and rhabdomyolysis and may progress to renal and cardiac failure and ultimately death [12]. Treatment consists of discontinuation of propofol infusion and supportive therapy. It may potentiate the effect of other drugs that cause CNS or respiratory depression and a drop in blood pressure, and caution should be exercised with other agents that may prolong the QT interval.

1.3. Barbiturates

Barbiturates are a group of sedative-hypnotic drugs used in the treatment of seizures, insomnia, preoperative anxiety, and induction of coma for increased intracranial pressure, in addition to their utility for inducing anesthesia.[13]. Thiopental was the predominant intravenous anesthetic induction agent until it was replaced by propofol [14].

Enhancement of inhibition by barbiturates occurs primarily at synapses where neurotransmission is activated by GABA at GABAA receptors. In addition, although barbiturates facilitate chlorine-dependent and picrotoxin-sensitive binding of GABA to GABAA, they also facilitate the binding of benzodiazepines. It differs from benzodiazepines in that it potentiates GABA-induced chlorine currents by prolonging the period of intense channel opening [15].

The sodium salts of barbiturates are absorbed more rapidly and satiety delays the onset of action of orally administered barbiturates. The intravenous route is usually reserved for induction or maintenance of general anesthesia (e.g. methohexital or thiopental). Thiopental, at doses as low as 5 mg/kg bolus, shows first-order kinetics (development of the pharmacokinetic process at a rate directly proportional to the current drug concentration), whereas phenobarbital is rapidly absorbed with a peak concentration in 2-4 hours [16, 17]. Barbiturates are highly fat soluble and undergo redistribution from the central nervous system to peripheral tissues [9]. They are biotransformed by oxidation and approximately 25% of phenobarbital is excreted unchanged in the urine.

Barbiturates can be administered orally and parenterally (intramuscularly and intravenously). If intramuscular injections of solutions of sodium salts such as phenobarbital or amobarbital are to be made, large muscle masses should be preferred, otherwise potential necrosis may occur in superficial areas.

Barbiturates are classified according to the duration of their effects. Short- and medium-acting barbiturates have an effect lasting 2 to 6 hours, while long-acting barbiturates have an effect lasting longer than 6 hours.

Table 1. Barbiturate classification according to the duration of their action

Ultra Short-Acting	Short-Acting	Intermediate-acting	Long-acting
Methohexital	Pentobarbital	Amobarbital	Phenobarbital
Thiopental	Secobarbital	Butalbital	Primidone

I.v. administration may cause hypotension and tachycardia. In addition, respiratory depression and apnea may occur. Leakage of thiopental out of the tissue may cause severe tissue necrosis, which can be treated with hyaluronidase and phentolamine [18]. Case reports have shown that topical Emla and topical lidocaine also work. Barbiturates such as butalbital can cause withdrawal symptoms and should be tapered gradually under supervision to reduce the risk [19]. They may cause allergic reactions associated with mild hepatotoxicity. Phenobarbital is the member of the group with the most serious side effects. DRESS (Drug reaction with eosinophilia and systemic symptoms), Stevens-

Johnson syndrome, and toxic epidermal necrolysis may occur due to phenobarbital [20].

Phenobarbital is an inducer of CYP1A2, 2B6, 2C9 and 3A4/5 isozymes. It reduces the efficacy of warfarin, steroids, psychoactive drugs and immunosuppressants metabolized by these enzymes. In addition, it reduces plasma levels of antiepileptic drugs such as lamotrigine, oxcarbazepine, phenytoin, thiagabine and valproate. [21]. The combination of phenobarbital with hepatitis C drugs such as paritaprevir/ritonavir, ombitasvir and dasabuvir should be avoided [22]. Avoid the combination of pyrimidone with apremilast, a phosphodiesterase 4 inhibitor (PDE4) [23]. The combination of barbiturates with other CNS depressants such as benzodiazepines and opioids can cause excessive sedation and severe respiratory depression [24].

Absolute contraindications include status asthmaticus and acute and intermittent variegated porphyria. Hypersensitivity reactions, including anaphylaxis, to barbiturates or excipients have been reported and this is a contraindication to their use [17].

1.4. Etomidate

Etomidate is a potent imidazole-based general anesthetic developed as part of an antifungal development program, coincidentally discovered to have strong hypnotic activity and a high therapeutic index [25]. Cardiovascular stability and minimal respiratory depression compared to other induction agents make it a useful and safe agent for general anesthesia. It is suggested to produce sedation and hypnosis by increasing the function of $GABA_A$ receptors in the brain [26]. Etomidate enhances the potency of GABA to induce $GABA_A$ receptors and directly activates $GABA_A$ receptors in the absence of GABA. GABA potentiating and direct activating effects occur at different concentrations of etomidate [27].

For etomidate, only the i.v. route is available. Therefore, it has become necessary to look for other routes for sedative and/or anxiolytic use. Some studies have reported good absorption after oral transmucosal administration [28]. In a study conducted in dogs, it was determined that the drug reached peak blood concentration 10 minutes after etomidate administration to the oral mucosa. [29]. It binds only to albumin in plasma and 75% of its distribution is realized by binding to proteins. It is highly fat soluble and the volume of distribution is proportional to body weight. There is limited data on placental passage, but a study in pregnant sheep showed that etomidate can cross the placenta rapidly, but an unknown factor limits its passage across the placental barrier [30]. It is metabolized by hepatic esterases and most of its metabolites are excreted in the

urine and a few in bile. Less than 2% of etomidate is excreted unchanged. Its effect starts in 30-60 seconds and reaches peak blood concentration in 1 minute.

It inhibits 11-beta-hydroxylase responsible for the conversion of 11-deoxycortisol to cortisol for 6-12 hours. As a result, transient inhibition of the synthesis of adrenal steroids is one of the most important side effects of etomidate. Since it will suppress endogenous cortisol and aldosterone synthesis, it should not be administered as a continuous bolus.[31]. The most common adverse reaction is intravenous pain during injection. This pain can be alleviated by giving i.v. lidocaine before injection. Transient skeletal muscle movements and myoclonus may be observed. Studies have shown that pretreatment with midazolam, dexmedetomidine, narcotics, propofol, dezocine or ketamine before a bolus dose of etomidate reduces the incidence of myoclonus.[32, 33].

It is a general anesthetic agent commonly used in rapid sequence intubation but has an increased risk of adrenal suppression associated with increased mortality in septic patients [34]. Preclinical studies have shown that it may cause increased neuronal apoptosis and cognitive deficits [35].

2. Inhaled Anesthetic Agents

2.1. Nitrous Oxide

Nitrous oxide produces only analgesic and anxiolytic effects without loss of consciousness at subanesthetic concentrations [36]. In a study with 7 volunteers who were subjected to harmful tetanic stimuli in hyperbaric conditions, the minimum alveolar concentration (MAC) was found to be 104 % [37]. Therefore, it is considered the least effective inhaled anesthetic. For the use of lower doses of N_2O, it is used in combination with various volatile or non-gaseous agents such as isoflurane and ketamine [38]. The main current mechanism is non-competitive inhibition of NMDA, a subtype of glutamate receptors. The function of non-NMDA glutamate receptors has not been fully elucidated, but these receptors have been reported to have inhibitory effects [39]. For these reasons, inhibition of excitatory glutamatergic neurotransmission is central to the thesis of anesthetic effect. Another mechanism said to contribute to the mechanism is that K^+ channels such as TREK-1, when activated, increase potassium conductance and hyperpolarize neurons. The effects on $GABA_A$ receptors are not as pronounced as intravenous and halogenated volatile anesthetics [40].

It passes into the blood as free gas, does not combine with hemoglobin and is not biotransformed. Quickly absorbed by the alveoli, the effect of N_2O starts within 2-5 minutes [41]. Since nitrous oxide diffuses through the alveolar basement membranes faster than other gases, it can produce a second gas effect, resulting in the rapid release of N_20, which causes the remaining alveolar gases

to concentrate. Very little of it is metabolized by anaerobic bacteria in the intestine by reduction. The main route of elimination is the lungs.

The main route of administration is inhalation. Application is defined by the European Society of Anaesthesiology Task Force on Nitrous Oxide; 30% or 50% oxygen combination for surgical procedural sedation and dental procedures, 50% or 70% oxygen combination for general anesthesia [42, 43].

When used alone, it has very few respiratory side effects, but when used in combination with sedatives, hypnotics or opioids, it may cause a respiratory depressant effect due to its potentiating effect. One of the most prominent side effects is diffusion hypoxia. After the cessation of N_2O, rapid oxygen dilution and subsequent hypoxia occur in the alveoli. To prevent this from occurring, 100% oxygen should be administered following the cessation of nitrous oxide. Other side effects include postoperative nausea and vomiting, fever, pulmonary atelectasis and infectious complications, hyperhomocysteinemia, subacute myeloneuropathy [44, 45].

Nitrous oxide inactivates methionine synthase through oxidation of cobalt in vitamin B12 and can cause megaloblastic anemia, leading to neurological and hematological problems in critically ill patients. Therefore, its use should be avoided. Methionine synthase is also required for the conversion of homocysteine to methionine, and elevated homocysteine levels can lead to adverse coronary events and severe heart disease. It should not be used in the first trimester due to its effects on B12 and folate metabolism. Nitrous oxide should be avoided in pneumothorax, small bowel obstruction, middle ear surgery and retinal surgery involving the formation of intraocular gas bubbles because of its rapid dissemination and its ability to increase gas volume and pressure in confined spaces. Although N_2O is not flammable, it is contraindicated in interventions where a catheter will be used because it promotes combustion. In addition, it should not be used in patients with pulmonary hypertension and impaired consciousness [42].

2.2.Halotan

When it was introduced in 1956, it quickly replaced ether and chloroform [46]. Although it had many disadvantages, its non-flammability and fluidity in application led to rapid and widespread use, but it was replaced by sevoflurane in the 1990s with the increasing popularity of sevoflurane [47]. Although it has been replaced by isoflurane, sevoflurane, halothane is the last common non-ether anesthetic used in the operating room and is the most soluble of the current anesthetics and the most potent of the inhalation anesthetics [46]. Halothane exerts its general effects through ion channels, it may bind to potassium channels

in cholinergic neurons and produce an immobilizing effect, in addition, hyperpolarization of NMDA and calcium channels may occur. Halothane can induce microsomal enzymes and may cause hepatotoxicity. Type 1 toxicity may occur with or without prior exposure to halothane, is asymptomatic (vomiting, nausea, fever, etc.) and occurs hours after surgery and is self-limiting within 1-2 weeks. Type 2 toxicity results in necrosis, liver failure and death within 2-14 days with hepatomegaly, fever, anorexia, myalgia, nausea, diffuse rash and encephalopathy.

2.3. Sevoflurane, Isoflurane

The current hypothesis is that inhaled anesthetics increase inhibitory postsynaptic channel activity (gamma-aminobutyric acid (GABA) and glycine) and inhibit excitatory synaptic channel activity (N-methyl-D-aspartate (NMDA), nicotinic acetylcholine, serotonin and glutamate) in the central nervous system [48]. Sevoflurane exerts its effect by passing into the pulmonary capillary blood when inhaled as a gas and then reaching the central nervous system. Four factors such as inhaled concentration, partition coefficient, ventilation per minute and pulmonary blood flow determine the effect of sevoflurane and the rate of induction. Sevoflurane undergoes little hepatic metabolism and has little urinary excretion. Its clearance is also related to these 4 factors [49]. It decreases blood pressure by decreasing systemic vascular resistance and causes a dose-dependent decrease in cardiac output. Unlike other inhalation anesthetics, it has fewer side effects on the respiratory tract. These side effects are seen in patients with pre-existing lung diseases such as asthma and cystic fibrosis. Sevoflurane decreases cerebral metabolic rate, increases cerebral blood flow and intracranial pressure. There is no data on its use in pregnancy. Its use should not be avoided in very necessary cases. It is contraindicated in patients with sensitivity to other halogenated anesthetics and in patients with suspected malignant hyperthermia. The fluoroacetic acid metabolite has been shown to be responsible for nephrotoxicity and hepatotoxicity. No cases of neurotoxicity that should be avoided have been reported so far [50].

Isoflurane is a non-flammable, volatile anesthetic with a pungent odor. This pungent odor makes induction of general anesthesia difficult [51]. Although it has little effect on left ventricular function, it causes a dose-dependent decrease in systemic vascular resistance due to mild beta-adrenergic stimulation. Thus, it decreases cardiac output by reducing preload, which is balanced by an increase in heart rate. Its induction of coronary dilatation may lead to the phenomenon of coronary steal. Although isoflurane has no special side effects, it should be adjusted according to the patient's hemodynamics as it may cause sudden blood

drops. The risk of malignant hyperthermia should be considered in susceptible patients or in patients with a family history of malignant hyperthermia. May cause renal failure by metabolizing to trifluoroacetic acid [52]. Recent animal studies indicate that learning disorders and behavioral changes occur in the post-operative period [53].

2.4. Benzodiazepin

In clinical anesthesia, benzodiazepines are frequently used as anxiolytics, sedatives, hypnotics and anticonvulsants. Classical benzodiazepines such as diazepam are positive allosteric modulators of GABA, have sedating effects at low doses and anesthesia inducing effects at high doses. Flumazenil, a competitive antagonist for the α1-γ2 high-affinity benzodiazepine site, is used to reverse general anesthesia [54]. Chlordiazepoxide was the first benzodiazepine synthesized, its hypnotic and sedative effects were discovered incidentally 2 years later, and diazepam was introduced for induction of anesthesia 10 years later. Although the distribution properties of these agents commonly used in anesthesia are similar, there are differences in their metabolism and excretion [55]. The four benzodiazepines used in anesthesia are classified as short-acting (midazolam), intermediate-acting (lorazepam, temazepam) and long-acting (diazepam) based on their metabolism and plasma clearance. Major factors affecting pharmacokinetics include age, weight, gender, race, hepatic or renal impairment. As the drug diffuses from the plasma into the adipose tissue, the volume of distribution increases and the return of the drug to the plasma is delayed and the increased volume of distribution results in a prolonged elimination half-life.

Midazolam reaches peak plasma concentrations within 30-80 minutes following oral intake. It undergoes presystemic elimination during oral ingestion and binds 94-98% to plasma proteins. Diazepam has a bioavailability of 94% after oral ingestion and reaches peak plasma concentration in 60 minutes. The pharmacokinetics of diazepam are affected by obesity, liver damage and age. Advanced age decreases the clearance of diazepam. It is metabolized in the liver by the enzymes CYP2C19 and CYP3A4. Lorazepam also has a bioavailability of around 90%, reaching peak plasma concentrations 2 hours after ingestion. It shows >90% binding to plasma proteins. It is conjugated with inactive glucronide in the liver and 70% is excreted in the urine. Pharmacokinetics do not change with age, weight, gender and renal disease, but clearance of lorazepam decreases in case of liver disease. In addition, lorazepam clearance decreases with probenecid and valproic acid. Remimazolam, a new short-acting GABAA receptor agonist with high affinity for the GABA receptor, showed a faster onset

of action, deeper sedation and faster recovery than midazolam in a study in sheep. In addition, it did not show dose-dependent depth like propofol. Midazolam is the benzodiazepine of choice for induction of anesthesia. When combined with other anesthetics, midazolam produces a synergistic effect similar to propofol [56]. In anesthetic practice, opioids are combined with benzodiazepines and this combination produces a synergistic effect. The interaction between midazolam and ketamn is additive, while the interaction between thiopental-midazolam and propofol-midazolam is synergistic. Benzodiazepines do not have analgesic effects and should therefore be used in combination with analgesic drugs during anesthesia. Studies have shown that especially midazolam prevents postoperative nausea and vomiting. Among the side effects, they do not have adrenal gland suppression properties and may have very limited allergenic effects. The major side effect of midazolam is respiratory depression. Lorazepam and diazepam may cause venous irritation and thrombophlebitis in addition to respiratory depression.

Newer GABA-Minergic Agents

3. Fospropofol

Fospropofol, also known as GPI15715 or Aquavan, is a novel sedative and hypnotic agent, it is also the water-soluble prodrug of propofol and is metabolized to produce free propofol, formaldehyde, and phosphate [57]. Its water solubility is superior to propofol in the form of oil-water emulsion. Although its mechanism of action is not known, it is assumed to act via GABA and Glycine in the Central Nervous System. Hydrolysis half-life is 8 minutes, volume of distribution is small, terminal half-life is around 46 minutes.

Fospropofol disodium injection has received Food and Drug Administration (FDA) approval for use as an intravenous sedative-hypnotic agent for monitored anesthesia care. Its side effects are similar to propofol, but it has a lower incidence of hypotension, respiratory depression, apnea and loss of airway patency due to its slow onset of action. Paresthesia and pruritus in the perineal and perianal areas occur 5 minutes after administration. Information that propofol infusion syndrome may occur with the use of fospropofol is not clear [58].

4. Etomidate Analogs

The first designed soft analog of etomidate was methoxycarbonyl-ethomidate (MOC etomidate). This soft analog was derived from the parent compound and designed to be metabolized predictably and rapidly to its inactive metabolites [59]. By adding different groups to etomidate in this way, binding to the hydrophobic catalytic site of the 11β-hydroxylase enzyme is prevented and adrenal suppression is reduced [60]. Studies have found that MOC-etomidate is

a rapid hypnotic and shows ultra-fast hypnotic recovery. A 30-minute single bolus of etomidate significantly decreased adrenocorticotropic hormone-induced serum corticosterone levels in rats, whereas MOC-etomidate did not show this effect [61]. After the production of MOC-ethomidate, 12 soft analogues of etomidate were produced, called Generation 2, to slow metabolism and reduce dosing. Of these, Cyclopropyl-Methoxycarbonyl Methomidate (CPMM) had the most similar properties to etomidate. Although not as fast as MOC-ethomidate, it was found to be rapidly hydrolyzed by esterases in the blood. Studies also showed that its dosing was 1-2 times lower than that of MOC-etomidate. Although continuous infusion of CPMM induced adrenal suppression, the recovery period was reported to be faster than etomidate. In line with these results obtained in animals, it has been an agent advanced to clinical phase 1 human studies [62].

The main aim of the strategies developed outside of these is to reduce the adrenal suppression caused by etomidate and at the same time to produce a short and content insensitive hypnotic effect. For this purpose, carboethomidate has been studied on the basis of decreasing 11β-hydroxylase affinity and preserving hypnotic activity with GABAα. Another approach has focused on enantiomer selectivity. Studies show that the $GABA_A$ receptor and hypnotic potentials of S-etomidate are much lower than those of the R enantiomer [62].

5. Alphaxalone

Alfaksalon, which is mostly used in cats and dogs, has also been studied in laboratory animals in recent years. This drug acts as a positive allosteric modulator of GABA receptors. It is also a neuroactive steroid that causes hyperpolarization of the neuron and produces a strong anesthetic effect [63]. When used at low doses, it keeps the GABA receptor channel open, similar to the effect of benzodiazepines, while when applied at high doses, it acts directly on the receptor, similar to the effect of propofol and barbiturates [64]. Although licensed for intravenous use, its formulation allows for intramuscular use [63]. It is considered advantageous because it does not cause tissue damage when it goes out of the vein [65]. In a study comparing intravenous and intramuscular use, was reported a higher bioavailability with intramuscular administration. In the same study, it was also reported that the drug given intramuscularly has a 2.5 times higher half-life [66]. Huynh et al. reported that a single intramuscular dose can provide sedation and recommended doses of 4-6 mg/kg with oxygen support [63]. In dose studies, lower doses such as 1, 2.5 and 5 mg/kg have also been investigated and it has been reported that 2.5 mg/kg provides deep sedation and 5 mg/kg produces a longer deep sedation. However, as a result, it has been

reported that the optimal dose of 2.5 mg/kg alfaxalone in a single application to induce sedation in rabbits [67].

Alfaxalone has been investigated by various routes of administration in laboratory animals such as rats, ferrets and guinea pigs [68-71]. Although there are many studies investigating intravenous use in rabbits [72], the number of studies on intramuscular administration is very few [71]. Studies to date have shown that alfaxalone can be used safely in rabbits and has significant potential as both a sedative and anesthetic. Bradley et al. investigated the sedative, anesthetic, and cardiovascular effects of alfaxalone in rabbits in 2019, both alone and in combination with other commonly used tranquilizers. It has been reported to provide a longer and more reliable sedation when combined with other sedative agents. The duration of sedation was prolonged by 25 minutes when combined with midazolam, and by an average of 117 minutes when combined with dexmedetomidine. They concluded that the prolongation of the sedation period was due to the fact that midazolam and alphaxalone were GABA receptor agonists, thus creating an additive effect. It has been reported that sedation and induction are smooth and fast, and at the same time, rabbits survive this period without any problems in the wake-up phase after anesthesia. It was stated that there was no problem in intramuscular administration, the rabbits continued to gain weight throughout the study and easily tolerated repeated applications [73]. In a study in mice, it was reported that the combination of alfaxalone with xylazine produces anesthesia and can be used intraperitoneally. In fact, researchers have reported that it produces a longer duration of anesthesia than the xylazine-ketamine combination [74]. In another study, it was reported that alfaxalone combined with medetomidine and interestingly administered subcutaneously was more effective than the dose administered intraperitoneally [75]. In another study with surprising findings, while the intraperitoneal alphaxalone-xylazine combination was found to be successful in the orthopedic surgery model in mice, it caused high mortality in laparotomy procedures and therefore subcutaneous use was recommended [74, 76].

Combination techniques

Combining different anesthetic agents allows to overcome the disadvantages that arise when drugs are used separately. The aim of balanced anesthesia, which occurs with the use of combinations, is to reduce the effects of drugs on the physiology of animals and to perform the awakening period quickly, painlessly and without any problems [1].

Administration of sedatives prior to inhaled agents simply reduces the resulting stress. The addition of inhalants to injectable agents will increase the anesthetic concentration, allowing prolonged procedures to be performed [1].

The combination of medetomidine and isoflurane has been used more and more in studies where MR imaging is used to investigate brain functions in rodents. In these studies, medetomidine was first reported in 2002 [77], and low-dose isoflurane with medetomidine was first reported in 2012 [78]. It is a potent alpha 2 agonist producing sedation, analgesia and brdycardia. As it causes intense urination, the animal can get wet, causing hypothermia and dehydration. In addition to having a synergistic effect with ketamine and fentanyl, it also significantly reduces the dose of inhaled agents [1]. In combination with isoflurane (>0.1% isoflurane), the epileptic activity induced by medetomidine is suppressed [79]. In addition, this combination allows anesthesia to be maintained for more than 4 hours [78]. In a study in male Sprague-Dawley rats, it was shown that stable serum concentrations of medetomidine would be achieved if the first subcutaneous dose of medetomidine, 0.12 mg/kg, was administered continuously in combination with 0.5% isoflurane followed by a repeat subcutaneous dose of 0.08 mg/kg/h [80]. Animals may react to sound when medetomidine is administered sedatively. Therefore, it is necessary to provide a quiet environment for 10-15 minutes after the injection in order for the maximum effect to occur. The biggest advantage is that its effect can be eliminated with atipamezole. It can also be used in combination with neuroleptanalgesics [1]. The use of opioid analgesics with sedatives is called neuroleptanalgesia. In this case, although the animals do not react to the environment, their consciousness is clear. It causes weak muscle relaxation with mild respiratory depression. For this reason, fentanyl/fluanisone combinations are used with midazolam or diazepam within the scope of anesthesia protocol in laboratory animals. Since midazolam is water soluble, it can be given as a cocktail in the same syringe as fentanyl/fluanisone. Calculation can be made by diluting with sterile water for the appropriate dose. Benzodiazepines, such as midazolam and diazepam, provide good relaxation of skeletal muscles in rodents, resulting in significant sedation. They have a minimal effect on the cardiovascular system and strengthen the effect of the anesthetic.

Another group of drugs that produce dose-dependent sedation are alpha 2 agonists. Initially, bradycardia is observed resulting from sino-atrial and atrio-ventricular heart block that develops in response to drug-induced hypertension. Afterwards, moderate hypotension is observed. Hyperglycemia and polyuria occur in the administration of all drugs belonging to this group [1].

Xylazine HCL is the most well-known of the alpha 2 agonists and produces a strong depression of the central nervous system. It is used for sedation or

premedication as it can significantly reduce the amount of anesthetic substance required with its use. Used mainly in combination with Ketamine HCL [1].

In anesthesia for laboratory animals, the combination of ketamine from the dissociative anesthetic group and the 2-agonists medetomidine and dexmedetomidine or xylazine are frequently used. However, rodents anesthetized with ketamine combined with 2-agonists have previously been shown to have elevated AST, ALT, and creatine kinase levels [81]. At the same time, the xylazine-ketamine combination activates glycogenolysis and inhibits insulin expression, causing the development of hyperglycemia [82]. This effect disappears when tiletamine is combined with xylazine instead of ketamine, one of the dissociative anesthetics, and zolezepam is added to this mixture [63]. It has been reported that IL-1ß and interleukin 6 (IL-6) expression increased and TNF-α expression decreased in rats with xylazine-ketamine combinations [83]. Similar effects were investigated in new combinations, as these findings may alter study results. Biochemical parameters were evaluated in male/female rats and mice administered with the combination of Tiletamine-Zolezepam-Xylazine and it was reported that this combination is ideal for the collection of terminal blood samples [84].

Additional side effects of anesthezics

The circadian clock may shift as a side effect of some anesthetic drugs. This situation changes the experimental results by affecting both behavioral and physiological parameters [85]. There are many studies showing the effects of general anesthetic drugs on the circadian clock in many species. In these studies, it was found that 1% isoflurane prolonged the next resting period of the active phase of the circadian clock during a 6-hour general anesthesia period in mice (Xia 2016), 2% isoflurane caused a delay in the expression of peripheral mononuclear blood cells [86], and 1.3% isoflurane for 5 hours. It has been reported that it delays the active phase with its application [87] and that it delays the active phase with the application of 2.5% sevoflurane for 4 hours [88]. These effects can occur in both sexes, even at different ages and weights. Isoflurane, sevoflurane, propofol, and ketamine anesthetic agents have been shown to cause time-dependent changes in circadian rhythms in rodents and other animals. For example, when ketamine is administered to rats during the resting phase, it causes a phase prolongation of 60-150 minutes. When applied during the active phase, it causes the phase to be delayed by 40-200 minutes [89]. GABA agonists such as sevoflurane, propofol and isoflurane exert differential effects on circadian behavior. While sevoflurane causes phase delays in rats [88], propofol causes phase shifts in rats when administered during the transition to wakefulness

(Dispersyn, 2009). If isoflurane anesthesia of rats is administered during the active phase, it shifts their resting cycles by two hours [90]. Mansouri et al. investigated the awakening behavior of rats anesthetized with sevoflurane and propofol in their study. Differences between the behaviors indicating awakening were determined in both anesthetic protocols. It has been reported that these differences are due to the pharmacokinetics of the drugs and even this effect is more than gender. In addition, it has been reported that gender is not effective at the time of awakening, but the restoration of normal cognitive behaviors the day after anesthesia may be related to gender [91].

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Chapter 11

Breaking the Cycle: Understanding Workplace Stress and Musculoskeletal Problems

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ABSTRACT

Workplace stress and musculoskeletal problems have become pervasive issues in today's fast-paced work environments, creating a detrimental cycle that impacts both employees and organizations. The interplay between these two factors highlights the urgency and importance of addressing these challenges comprehensively. Workplace stress is the result of demanding work conditions, excessive workloads, conflicts, and a lack of support or control. It affects employees across industries and occupations, leading to decreased job satisfaction, increased absenteeism, burnout, and compromised mental health. In turn, workplace stress contributes to the development of musculoskeletal problems. Musculoskeletal problems encompass a range of conditions affecting the muscles, bones, tendons, ligaments, and other support structures. Prolonged exposure to workplace stressors can lead to disorders such as back pain, neck strain, repetitive strain injuries, and carpal tunnel syndrome. These conditions cause physical discomfort, reduced mobility, and pose significant healthcare costs for organizations. Understanding and addressing workplace stress and musculoskeletal problems are crucial for the well-being of individuals and the success of organizations. A supportive work environment that prioritizes employee well-being fosters a positive organizational culture, enhances employee engagement, productivity, and retention. Moreover, organizations can mitigate the financial burden associated with healthcare expenses, absenteeism, and reduced productivity through proactive measures. This article aims to shed light on the intricate relationship between workplace stress and musculoskeletal problems. By exploring the underlying causes, identifying their impact, and presenting effective solutions, individuals and organizations can break free from this distressing cycle. Increased awareness and actionable strategies pave the way for healthier, more productive work environments, benefiting employees and organizations alike.

Keywords: Exercise, Musculoskeletal problems, Workplace stress.

INTRODUCTION

The global service sector is expanding rapidly, leading to an increase in the number of office workers worldwide. A comfortable and safe working environment is essential for maintaining the well-being and productivity of these workers (Davis, 2018). However, while most service-sector work is conducted in offices, failing to consider ergonomic principles when designing workspaces can lead to serious health problems for employees. This not only affects the workforce, but also reduces productivity levels. It is therefore important to raise awareness among office workers and implement measures to prevent these often-overlooked health issues. Specifically, following ergonomic rules and adhering to occupational health and safety standards is critical for preventing musculoskeletal system diseases (Van Eerd et al., 2022).

Based on data from the International Labor Organization (ILO), it has been estimated that approximately 250 million workers worldwide are exposed to occupational accidents annually, with an additional 160 million workers exposed to losses resulting from occupational diseases. Despite the growing reliance on machine-intensive production, labor-intensive production and service industries continue to occupy a significant place in the global economy (Sepadi & Nkosi, 2021). Unfortunately, workers in both modes of production are susceptible to various diseases. Ergonomics represents a vital component of occupational safety and health, as it encompasses a wide range of factors that influence the well-being of workers in diverse workplace environments. These factors include lighting, noise, thermal comfort, vibration, workspace and equipment design, seating, footwear, as well as work schedules such as working hours, overtime hours, shifts, breaks, and night work.

The workplace poses many risks and hazards that can lead to employee illness or disability. Hazards refer to potential sources of harm or damage in the workplace or external environments that can impact employees or the workplace itself. Risks, on the other hand, denote the possibility of loss, injury, or other adverse outcomes arising from such hazards. Work-related risk factors can be categorized into three primary types: physical and ergonomic factors, psychosocial factors, and personal risk factors (Joseph et al., 2023).

Physical and ergonomic factors are characterized by repetitive movements, forced movements, and incorrect postures, such as bending forward without bending the knees, lifting heavy objects, and prolonged wrist bending when using a computer, which are significant contributors to the development of carpal tunnel syndrome. In addition, remaining in one position for extended periods and exposure to vibrations are other notable physical effects (Shezi et al., 2021).

Psychosocial risk factors comprise of increased work stress, limited decision-making opportunities due to uniformity in occupational tasks, fluctuating workload, a lack of managerial responsibility, limited social circles within the workplace, multi-headed management structures, and suboptimal power transfers in family companies. Other stressors may include short or infrequent breaks, insufficient peer support, demanding workloads that surpass individual capacity, and inadequate supervisor support (Panigrahi, 2017).

Personal risk factors include aging, poor physical fitness, smoking, and obesity (Hamer et al., 2020). Although there are risk factors for Occupational Musculoskeletal System diseases in every job and workplace, some jobs carry a higher risk for some diseases. For low back pain, primarily those who work in the industry with physical activity and heavy work; For neck and upper extremity (neck, shoulder, elbow, hand and wrist) diseases, those who work with repetitive movements in the industry and those who use computers are at high risk (Odebiyi & Okafor, 2023). The first thing to be done in order to offer people a suggestion about their physical activity level will be to determine their physical activity level (Bisson & Lachman, 2017).

Several studies have investigated the relationship between cardiovascular risk factors and body composition among office workers. For instance, a study conducted in Japanese male office workers using bioelectrical impedance analysis found a significant association between the LDL-C/HDL-C ratio and body mass index and body fat percentage. Furthermore, Nakanishi et al. reported that excess body fat, as measured by waist circumference, waist-to-hip ratio, and body mass index, was associated with an increased risk of coronary artery disease (Nakanishi et al., 2000). Another study conducted in adolescent women by Miranda et al. found that overweight and obese individuals with high BMI exhibited higher levels of anthropometric indicators, blood pressure, uric acid, and hs-CRP concentrations, and lower HDL concentrations (Miranda et al., 2020). These individuals also had a sedentary lifestyle, insulin resistance, and higher concentrations of proinflammatory markers, which were associated with the higher risk of developing cardiovascular diseases.

Moreover, physical activity has been found to affect body composition and reduce the risk of developing cardiovascular diseases. Some endocrine basis for this has been demonstrated. Accordingly, recent studies have shown that irisin, also known as exercise hormone, released from skeletal muscles with increased physical activity, has cardiovascular and respiratory benefits (Demirel et al., 2021a; Demirel et al., 2021b; Demirel et al., 2022a; Demirel et al., 2022b; Demirel et al., 2022c; Demirel & Ozyener, 2022). Besides, in a study conducted in Turkey, to evaluate the relationship between physical activity levels and

quality of life among desk workers, it was found that increasing physical activity levels led to significant improvements in physical and mental health scores. These findings suggest that promoting physical activity can positively impact the health and well-being of desk workers (Arslan et al., 2019).

Moreover, it has been established that desk workers who use computers for prolonged periods are susceptible to experiencing discomfort and pain in the back, waist, neck, and shoulder regions. These musculoskeletal disorders not only affect the health and well-being of workers but also have an impact on work efficiency and productivity (Ardahan & Simsek, 2016). The physiological responses of the body to such changes are complex and multifactorial, involving various mechanisms including muscular, nervous, and circulatory systems.

In this section, we will focus on work-related health problems in the workplace and discuss the concepts of physical activity and exercise interventions.

Factors Affecting the Occurrence of Work-Related Disorders

Today's environment poses a danger to health with many physical, psychological, emotional, and mental stimuli. In recent years, musculoskeletal injuries caused by work-related overload are quite common. Repetitive movements, long-term static working positions, work that requires effort, and non-ergonomic working conditions create physical stress. In addition, many factors such as lack of time brought by work life, uncontrollable factors, distractions in the background, general uncertainties, interpersonal superior-subordinate relationships create psychological stress (von Onciul, 1996).

Table 1: Sources of Stress in the Workplace: Physical and Emotional/Mental Factors

Physical Stress	Emotional and Mental Stress
Sound	Fear of punishment or sanctions
Chemicals	Worry about promotion
Temperature Changes	Anger at injustice
Physical Trauma	Adapting to a new position
Radiation	Tedium
Bad Posture	Competing with a colleague
Going Under a Heavy Load	Not getting along with subordinates
Monotonous Jobs	Conflicting instructions
Night Shifts	Negative thoughts
Overtime	Time pressure
Structural changes	
Monotonous work	
Low Salary	

This series of physical and psychological stresses begins to create a cascade of changes in the individual, and this process continues in stages. Researchers refer to this as the "General Adaptation Syndrome." As the person activates their adaptation mechanisms to cope with workplace stressors, they initially exhibit an alarm reaction. Then, the resistance phase begins, which ultimately leads to exhaustion (Cunanan et al., 2018).

Alarm reaction: When encountering difficulties, the person creates an immediate response. A person's autonomic nervous system fires in response to stress, displaying a "fight or flight" warning. Many body systems work together to act, and the person's mood, cardiovascular system, respiration, muscle tension, and fine motor activities change (Chu et al., 2022).

Table 2: Common Physical and Psychological Symptoms of Stress

Symptoms
Irregular heartbeat
Palpitations
Superficial breathing
Frequent breathing
Muscle pains
Dryness in the throat
Nausea
Anxiety
Numbness in the hands/feet
Dizziness
Sweating

Resistance Phase : Alarm reactions end somewhere and individuals reach the resistance phase. In this stage, people develop a "survival" strategy and develop a method of fighting against existing stress. People develop adequate or inadequate coping methods. They create short and long-term solutions or try to get out of a difficult situation with short escapes. Unfortunately, short-term relief is insufficient and in the long run causes secondary problems that result in reduced work performance. People often need help to create long-term solutions (Panigrahi, 2017).

Exhaustion Phase : The stress response is actually a healthy response and is necessary to keep the person motivated and adaptable. When the demands of the body and mind are too high and cannot be properly met, it begins to cause distress to the person. Prolonged stress can cause chronic problems and ultimately lead to depletion of all reserves and energy. This can cause general fatigue, weakness, blurred vision, dizziness, chest tightness, respiratory problems, and gastrointestinal symptoms. In addition, sleep and eating disorders, excessive weight gain or weight loss are also quite common (Panigrahi, 2017).

Table 3: Common Physical Symptoms of Stress and Examples

Physical Symptoms	Examples
Tension headache	pounding or pressure-like headache
Migraine	moderate to severe headache with additional symptoms such as nausea, vomiting, and light sensitivity
Irritable bowel syndrome	abdominal pain, bloating, and changes in bowel movements
Impaired resistance (against cold or viral infections)	frequent colds or infections
Asthma, skin disorders	wheezing, shortness of breath, and skin rashes
Backache	pain in the lower, middle or upper back
Gastritis and stomach problems	stomach pain, bloating, and indigestion
High blood pressure	hypertension or elevated blood pressure readings
Neck-shoulder-arm and hand pain	pain and discomfort in the neck, shoulders, arms, or hands

The burnout phase of stress is associated with a range of emotional symptoms, including frustration and depression. These symptoms can manifest as uncontrollable crying, reduced interest in social relationships, hobbies, and personal activities such as exercise and self-care. In more severe cases, individuals may exhibit self-harm or suicidal tendencies. Emotional distress can also result in irritability, coldness, and rude behavior towards others. Additionally, individuals may experience panic attacks and difficulty sleeping, which can exacerbate work-related stress (Bocheliuk et al., 2020).

During this phase, individuals may also experience deficits in concentration and coordination, leading to impaired performance and judgment, negative attitudes towards work, and indecisiveness. Interpersonal relationships with colleagues may also deteriorate, further contributing to the cycle of burnout. Ultimately, these factors can lead to a loss of self-confidence and further reduced performance. The use of substances such as alcohol, cigarettes, and tranquilizers may also be observed as individuals attempt to cope with their symptoms (Golonka & Gulla, 2021).

Table 4: Sensory Symptoms of Exhaustion Phase in Workplace Burnout

Sensory Symptoms of Exhaustion Phase
- Accidents
- Loss of thinking ability
- Decreased performance
- Inadequate concentration
- Absence from work
- Increase in errors and apologies
- Constant delay to the effort of making it on time
- Increase in misunderstandings
- Loss of short-term memory

Burnout Syndrome: This term describes the sensory and psychological consequences of prolonged, sustained stress. Idealistic willingness, opposing roles, and excessive responsibility are the starting points for the development of this condition. Emotional and mental exhaustion causes aversion, apathy, and disgust towards everyone and everything (Roy, 2018).

Table 5: Factors Affecting Coping with Stress in the Workplace

Factors Affecting Coping with Stress
Personal structure
Life and work style
Coping mechanisms
Sensory stability
Previous experience
Expectations
Trust in oneself

Stress management typically requires two primary types of changes: external and internal. External changes refer to modifications made to an individual's lifestyle and working conditions, which can be done to reduce stress levels. These changes may include adjustments to working hours, workload, or work environment, as well as lifestyle changes such as diet, exercise, and leisure activities (Bianchi et al., 2015).

Internal changes, on the other hand, involve modifications to an individual's behavioral, perceptual, and biological responses to stress. These changes may include the adoption of new coping mechanisms, such as relaxation techniques,

mindfulness, or cognitive-behavioral therapy, as well as addressing any underlying mental or physical health issues that may be contributing to stress levels. Internal changes also involve developing a greater awareness of one's thoughts and emotions and learning to manage these in a more constructive way. Overall, both external and internal changes are essential for effective stress management (Weber & Jaekel-Reinhard, 2000).

Table 6: Suggestions for Managing Stress in the Workplace

Suggestions
Regular exercise
Correct posture
Relaxation techniques (e.g. Yoga)
Regular nutrition
Engage in relaxing hobbies
Quitting alcohol and smoking habits
Developing communication skills

Physical Activity and Regular Exercise

Physical activity and exercise are the sum of actions that improve the health of an individual as a tool of the preventive health approach, maintain the improved state, and increase the resistance against fatigue and diseases. Lack of exercise and a low level of physical fitness are very important risk factors for morbidity and premature death. It has been revealed that regular physical activity prevents hypokinetic diseases and premature deaths due to these diseases, and provides a high quality of life in terms of health (Yilmaz, 2023).

Table 7: Relationship between Physical Activity and Health Problems

Situation	Risk Reduction	Symptom Reduction	Result Improvement	Activity Type
Alzheimer's	+			M
Anxiety	++	++	+++	M
Asthma	+	+		M
Chronic Heart Disease	+++	+++	++	M, E
Heart attack	+	++	++	S, M
Cancer				
Chest	++	+	++	M
Colon	+++	++	++	M
Endometrium	+			M
Lung	+			M
Prostate	+	+	++	M
Depression	++	++	++	M
Type 2 Diabetes	+++	+++	+++	M, SEE
Hypertension	++		+++	M, SEE
Longevity		+++	+++	M
Obesity	++	++	+++	SEE, M
Osteoarthritis		+	+	S, M
Osteoporosis	++			S(W), M
Peripheral Vascular Disease		+		M
Pregnancy		+	++	M
Cigarette	+	++	++	M
Stress	++	++	++	M
Ulcer	+++			M

(Low impact: +, Medium impact: ++, High impact: +++; M= Moderate activity, SEE= Significant energy expenditure, S= Strength exercises, W= Weightlifting exercise)

Regular physical activity has been shown to have a multitude of positive effects on various bodily systems. In both young and elderly individuals, regular physical activity has been associated with improvements of 10-30% in cardiovascular system function, which is dependent on the intensity of the activity (Janiszewski & Ross, 2009). Additionally, regular physical activity has been found to contribute to the reduction of cardiovascular risk factors. It also

causes positive improvements in insulin sensitivity without causing a change in body composition and helps reduce blood pressure in hypertensive individuals. Moreover, it contributes to an increase in HDL and HDL2 cholesterol, a decrease in triglyceride cholesterol/HDL ratio, and a decrease in body fat ratio, which leads to improvements in blood lipid profile (Muscella et al., 2020).

Regular physical activity has also been found to increase work capacity, and both resting and exercise have been associated with reduced heart rate, diastolic and systolic blood pressure, and myocardial oxygen requirement at submaximal workload. With exercise, decreases in muscle strength and mass decrease, while the strength of bones, muscles, ligaments, and tendons, and joint cartilage density increase. Muscles become hypertrophied and the capillary density in the muscle increases. Regular physical activity also helps strengthen the movement system, which can prevent the development of painful diseases of the neck, back, lumbar region, and joints, and increases the freedom of movement of individuals, their capacity to perform daily work and duties, and reduces the risk of osteoporosis (Lewis & Hennekens, 2016).

Furthermore, regular physical activity can help replace the responsibility of work with exercise for fun and health, especially after retirement. This can make individuals feel more productive and reduce anxiety and depression, facilitate positive thinking, and help cope with stress. Regular physical activity also contributes to the decrease in mortality and morbidity, increases the quality of life, promotes more efficient socioeconomic work, and helps preserve and improve cognitive functions (Carta et al., 2021).

Stress and Exercise

The concept of stress or tension has become ubiquitous in our daily lives and has been a subject of extensive research across many fields of study. Stress refers to a collection of situations that elicit mental or physical tension, resulting in stress, anxiety, or other forms of distress, which arise from either the individual or their environment. Stress is an individual's attempt to maintain a sense of balance within themselves (Goldstein & Kopin, 2007). The features that cause stress may vary, as the factors that trigger tension differ. The primary cause of stress is the daily physiological changes that individuals experience throughout their lives (Diehl et al., 2012). However, the critical aspect lies in the psychological meanings and descriptions that individuals attribute to the elements of stress, either internal or external. The level of tension experienced determines the meaning that individuals assign to the stress factor and how they interpret the events. The negative symptoms associated with stress can cause

detrimental effects on an individual's overall health and work status (Baum et al., 1999).

Stress is a highly complex concept that is challenging to define and is commonly used. Researchers have developed various definitions of stress. Stress or tension is considered a physiological state, whereas worry, anxiety, depression, or inhibition are not viewed as stress. These mental events may be the initial determinants of physiological responses, but they are not regarded as stress (Afek et al., 2020).

Stress has become an element of modern lifestyle. Stress, which has become a term used in our daily life, is a factor that can affect all stages of human life. Living with tension for a long time also leads to the emergence of different health issues in people. It negatively affects the normal work of a person, making their life stressful and reducing their quality of life. Stress has negative effects on human health (Zawadzki et al., 2019). These negative effects emerge with the nervous and biological consequences it causes and are determined by the nervous state and social influences of the person. Various studies have stated that tension interacts with psychological aspects of people such as perception of control and social support. These studies have shown that the hormones adrenaline and cortisol, which spread through the adrenal medulla and pituitary-adrenal states, affect the human body (Padgett & Glaser, 2003). According to studies, stress has a causal role in the occurrence of cardiovascular diseases, which are among the main causes of death in our century. For example, in a study by Everson-Rose and Lewis (2005), negative emotions such as depression, hostility, anger, and anxiety are seen as creators of psychological tension. These authors revealed that the deterioration in interpersonal relations and negative cardiovascular diseases increase the rate of getting and dying from these diseases (Everson-Rose & Lewis, 2005).

Some studies on stress show that tension is also strongly associated with mental health. In one of his articles, Hammen states that scientific evidence reveals a strong causal link between exposure to stressful events and depression (Hammen, 2005). The diagnosis of post-traumatic stress disorder, which is considered in the anxiety disorders group among the disorders related to the psychological state, acknowledges that the person is caught in stressful events. It is suggested that human-induced trauma-related situations are more effective than others in the occurrence of post-traumatic stress disorder (Barskova & Oesterreich, 2009). Stress has a causal role in the formation of behaviors that result in death, such as exposure to life events and ending one's life. For example, in a study conducted with outpatients with psychological conditions, they found that thoughts and attempts to end their life were more common

among those who were exposed to stressful life events than those who did not experience such events. These researchers stated that ending their life behaviors are more common among people who have experienced a stressful event and who are inadequate in terms of their ability to eliminate negativity, which is an indicator of resilience (Kleiman & Beaver, 2013).

Taylor and Dorn found that stress increases the rate of traffic accidents occurring on highways. It also affects the learning and memory of a person's nervous system (Taylor & Dorn, 2006). It is well-known that exposure to stressful events causes the learning of two responses, fear and startle, through classical conditioning. Studies suggest that exposure to stressful events negatively affects the retention and recall of learned information. It is also important to measure stress, which affects human life in many ways, and to develop tools that can be used for this measurement. Two widely used methods for measuring stress are currently available (Lupien et al., 2007).

Stress is a word that has been frequently used in recent years and has been interpreted in different ways. It is something that everyone experiences at some point in their life. Stress is often viewed as a situation where a person feels uncomfortable, restless, and incompatible in their work and private life. Today, the development of technology, industrialization, scientific advancements, fast and easy communication, and the expectation of being the most successful in our daily life and business situations have all contributed to an increase in stress levels. In our private lives, the rapid deterioration of love and respect in human relationships, the inability of people to stand up for themselves, and the pushing of all kinds of limits have also contributed to stress levels. And as a result of not being able to cope with these situations, negativity arises. The negative mental state experienced can be explained as stress/tension. In ancient times, stress could also be used to mean trouble, calamity, disaster, anxiety, sadness, sorrow, grief, etc. Today, however, the definition of stress has evolved to encompass situations involving force, pressure, and difficulty, whether physical, mental, or emotional (McEwen & Akil, 2020).

The first studies on stress were made by a person named Hans Selye. According to him, stress is actually a normal wear and tear in the body over time. Stress can bring about thoughts of harming one's body or undesirable and negatively accepted mental states such as anxiety, frustration, and fatigue. With this explanation, it is stated that stress is a concept that only harms the body (Lu et al., 2021). The most general and understandable explanation of stress is that it harms the human body in the face of distressing situations. It may occur as an emotional, mental, and physical response. Stress is an internal response of a person to various environmental stimuli. It is known as the reaction given to the

physical or psychological strain that a person experiences in the face of any event or circumstance that they are caught or affected by (Panigrahi, 2017). It is a physiological result that works for the continuity of human life and is "given" by nature. This physiological response in the face of any perceived or real danger is defined as the individual's encounter with situations that require new adaptation, feeling oneself under threat, and experiencing a deterioration in balance (Lu et al., 2021). One of the important negativities that prevent people's energy, quality of life, and living a productive life at the desired level is tension. Stress negatively affects well-being. When a person does not feel well, it is seen that this negativity is reflected in the whole life process.

Types of Stress

Stress can be explained as two types:

• Stress that is considered good: Stress that is considered good creates a motivating force for people. It encourages one to use how to get the most. This type of stress can be called a stimulant in the more technical case.

• Stress that is considered bad: Stress that is considered bad occurs when people are exposed to environmental situations, the pressures on them increase, and they get a situation that they cannot cope with. In such situations, people use the phrase "we are stressed" a lot. If people cannot cope with stress and cannot resolve negativities, they are faced with many undesirable situations ranging from psychological negativities to physical diseases (Herman et al., 2015).

When a person is under stress, his body gives many psychological and mental reactions. The most visible bodily reactions include pain and stiffness in the neck and back, tension and contractions in the lumbar region muscles, joint pains, heart palpitations, stomach and intestinal system disorders, and digestive system malfunctions. In addition, breathing difficulties, headaches, tremors in the hands at varying levels, hypersensitivity to noise and sound, cold or heat strokes or flushes, excessive sleeping or an irregular sleep, overtraining, and stomach cramps may also occur. Examples of psychological responses include being emotional, anxious, feeling dejected, sluggish, exhausted, restless, irritable, nervous, aggressive, or apathetic, feeling depleted of energy. Mental reactions of people when they are stressed can be counted as a decrease in thinking power, focusing on negativities, indecision, inability to organize, confusion of the mind, decrease in interest and love, forgetful memory, mental stagnation, spending social life in solitude (Padgett & Glaser, 2003).

If people, and women in particular, are in a state of stress, they must first accept the situation they are in to get rid of this negative situation. They should

identify the problems that create this tension situation and seek solutions to them. In order for people not to lose their health, one of the first things they should do if they are stressed is to control their situation.

Categories of Stress

• The alarm or warning phase: In the alarm phase, the body responds to fight or flight against the current situation. As a result of the changes that occur in the body during the response, which is defined as fight or flight, the person becomes ready to face the source of tension or to escape. Headache, chest pains, indigestion, muscle tension, heart palpitations, high blood pressure, sleep disturbance, heartburn, intestinal disorders, and high-temperature bad blood fats are examples.

• Resistance stage: The stress situation is continuous at this stage. At this stage, the human body is in a kind of adaptation to the stressful environment. The human body gives the impression of functioning under normal conditions. In this case, the human body actually lost its current resistance.

• Exhaustion phase: The human body is now losing its resistance to stress. Exhaustion and extreme tiredness have emerged. The body's adaptation or adaptation energy is weakened. The body, which has exhausted all its energy stores, has entered the state of exhaustion and has become more open to all kinds of diseases. In that case, women who do not do sports can give very different negative reactions physically, psychologically, and mentally when they are in tense situations (Kranner et al., 2010).

Exercise Prescription for Health

Although physical activity and exercise are often used interchangeably, these terms are not synonymous. Physical activity refers to bodily movements performed by contraction of skeletal muscles that require energy expenditure above the basal level. It is a term that includes all kinds of muscle movements and covers a wide area from daily life activities to various sports activities such as gardening, load-bearing, and sports activities, etc. Exercise, on the other hand, refers to planned, structured, voluntary, continuous activities aimed at improving one or more elements of physical fitness (cardiovascular fitness, muscle strength and endurance, flexibility, and body composition). Exercise is programmed physical activity for purposes such as fitness, physical performance, weight control, or overall health (Stults-Kolehmainen & Sinha, 2014).

An "Exercise Prescription" specially prepared for the individual should be written. The following features are included in the exercise prescription according to the FITT principle:

•Frequency: How many sessions per week?

•Intensity (intensity of the exercise): What intensity?

•Type (Type, type of exercise): What method will be applied?

•Time (The duration of the exercise): How long?

•Exercise progression: How to gradually increase the workload? (Katsukawa, 2016).

A training session consists of four consecutive components (Thompson et al., 2013):

•Warm-up (5'-10')

•Conditioning (20'-60') a. Aerobic exercise b. Muscle strengthening and endurance

•Cooldown (5'-10')

•Stretching

The warm-up phase includes low-to-moderate cardiovascular endurance activities. It is the transitional phase that aims to increase body temperature, reduce muscle stiffness, and prevent muscle fatigue after exercise. Warm-up exercises are necessary to avoid injury and cardiovascular complications. Generally, walking at a low speed or cycling without resistance is sufficient. The cooldown phase, after the conditioning phase, includes 5-10 minutes of low-to-moderate cardiovascular endurance activities to gradually reduce heart rate and blood pressure and remove waste metabolic products produced in the muscles. Stretching exercises can be performed separately from and after the warm-up and cooldown phases (Ardic, 2014).

Issues to Consider During Exercise

In order to prevent injuries, the following should be considered:

-History of injury

-Adequate warm-up and cool-down exercises

-Gradually increasing the intensity and duration.

For Heat Regulation;

-Exercises performed in natural environments should be preferred.

-Cold times of the day should be preferred for exercise.

-Plenty of fluids should be consumed.

-Appropriate clothing for the temperature should be chosen (Yu, 2012).

Work-Related Musculoskeletal Problems, Back-Neck-Shoulder Pain

The spine, which starts from the neck and forms the back, waist and coccyx, is the most important bony framework that forms the structure of the human being. A problem at any level of this structure will affect the others and cause deterioration of the person's functions.

The problems generally start with pain, tension, and stiffness, and progress to a deterioration of posture called postural imbalance, limitation of normal body movements, and degeneration in bone and muscle structure. This, in turn, reduces the person's quality of life and lowers labor productivity.

Physiologically and biomechanically, good posture is a posture that provides maximum efficiency in the body with minimum effort. In clinical studies, the posture in which the head slides forward brings problems related to the neck, back, and waist. This abnormal position places excessive stress on the chewing muscles, teeth, and all supporting structures, causing remodeling of the jaw. Additionally, this disorder paves the way for the formation of a rounded shoulder. This postural imbalance continues along the entire spine, causing extreme changes in the normal curvatures of the body, such as the hump on the back, or the excess or disappearance of the dimples in the waist (McBride & Harcombe, 2012).

Neck pain is one of the problems we all face every day. Pain in the neck may be accompanied by symptoms such as pins and needles and tingling in the hands and/or fingers. Sometimes, severe muscle contractions and headaches appear as a reflection of neck problems. In that case, the most effective method for protection is to prevent this incorrect posture and to regularly perform exercises that promote straightness and stretching, especially for desk workers (Yang et al., 2015).

Table 8: Workplace Ergonomics: Suggestions for Optimal Workstation Setup and Body Posture

Workplace Ergonomics	Suggestion
Chair height	Feet flat on the floor, thighs fully supported
Chair width	Up to 10 cm in front of the chair, fully supported lower and upper back, behind the knee
Arm support	Full elbow and forearm support
Back support	Back support should be thickened at waist level
Table height	Level with the arm resting position during keyboard use, 5 cm above the resting position while reading
Computer screen position	Straight in front of the worker, screen rotation less than 10°. Screen eye distance: about 50 cm
Computer screen height	Top of screen at eye level
Keyboard	On the table, flat to the screen
Mouse (pad)	Close to keyboard
Body Posture	
Back	Straight and full back support
Neck	Less than 20° of head rotation. Minimal neck bend to the front or back
Shoulders	In the relaxed position
Arms	Close to the body
Hand-Wrist-Forearm	In a straight line, rotated 5°-20° in the direction of the little finger
General	Constant change of posture throughout the day

CONCLUSION

In conclusion, work-related health problems are a significant issue in the workplace, with many employees experiencing physical and emotional stress on a daily basis. Physical activity and exercise interventions can be effective ways to manage these stressors and promote overall health and well-being among workers. By encouraging regular exercise, proper posture, and ergonomic workstation setups, employers can help their employees reduce the risk of work-related injuries and illnesses, improve their productivity, and increase their job satisfaction. Additionally, providing stress-management training, support groups, and flexible work arrangements can help workers better manage the emotional and mental stress that can arise in the workplace. Ultimately, creating a healthy work environment that promotes physical activity, stress reduction, and overall well-being is crucial for both employees and employers in maintaining a productive and positive workplace culture.

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Chapter 12

Impact of Yeast Species and Strains on Volatile Flavor Compounds in Fermented Foods and Beverages

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ABSTRACT

The impact of yeast species and strains on volatile flavor compounds in fermented foods and beverages goes beyond sensory attributes, encompassing potential health implications. Volatile flavor compounds not only contribute to the taste and aroma of fermented products but may also possess bioactive properties with health-promoting effects. Yeast metabolism during fermentation gives rise to a wide range of volatile flavor compounds, some of which have shown potential health benefits. Certain esters produced by yeast exhibit antioxidant and anti-inflammatory properties, linked to the prevention of chronic diseases such as cardiovascular ailments and certain cancers. Additionally, specific yeast strains generate volatile flavor compounds like terpenes and phenolic compounds, which possess antimicrobial properties that can aid in combatting harmful microorganisms in the gut. Moreover, yeast-mediated fermentation enhances the bioavailability of nutrients in food. Fermentation processes driven by yeast have been found to increase the content of bioactive compounds, including vitamins and minerals, in fermented products. This improved nutrient accessibility promotes better absorption and utilization by the human body. The modulation of volatile flavor compounds by yeast species and strains also impacts the sensory aspects of fermented foods and beverages, potentially influencing dietary choices and satiety. By creating appealing flavors and aromas, yeast enhances the palatability of these products, encouraging consumption and potentially influencing dietary diversity and overall nutrient intake. The bioactive properties of these compounds, coupled with yeast-mediated fermentation, have implications for human health and nutrition. Understanding how specific yeast species and strains influence the formation of volatile flavor compounds provides insights into the potential health benefits associated with consuming fermented products.

Keywords: Fermentation, Volatile flavor compounds, Yeast strains.

INTRODUCTION

Fermented foods and beverages, such as beer, wine, and bread, represent an essential component of human diets that have been consumed for centuries. The sensory attributes of these products are largely attributed to the volatile aroma compounds that are produced during fermentation. Yeast is a crucial microorganism in this process, as it metabolizes sugars and produces various volatile compounds, including esters, alcohols, and acids, which significantly contribute to the final flavor and aroma profile of the product. The effect of yeast species and strains on the volatile flavor compounds of fermented foods and beverages has been the subject of extensive research due to its fundamental importance in product quality and consumer acceptance. As such, a comprehensive understanding of how different yeast species and strains affect these volatile compounds is crucial in the optimization of product development and production processes. The review will synthesize and evaluate the latest research findings in this area, highlighting the strengths and weaknesses of different approaches and identifying key areas for future research. Ultimately, this review will contribute to a better understanding of the role of yeast in the production of fermented products and will provide valuable insights for the optimization of product quality and sensory characteristics.

Fermentation: The Key to Food Production

The breakdown process of carbohydrates through the action of enzymes synthesized by bacteria, yeast, and molds is commonly referred to as fermentation (Patel et al., 2023). Howell et al. (2005) have defined fermentation as the reduction of organic compounds through catabolism, which results in the production of biochemical energy (Howell et al., 2005). Through the use of complex metabolic pathways, fermentation enables cells to convert ammonium and sugar-like molecules into the desired end product (Tadege et al., 1999). Fermentation is one of the oldest known methods of food production and preservation, and its cost-effectiveness is one of its most significant advantages (Ledormand et al., 2021).

The term "fermentation" is derived from the Latin word "fermentare" meaning "to leaven, ferment," which in turn comes from "fermentum" or "substance causing fermentation" and "fervere" or "to boil." Fermentation is a non-oxygenic metabolic pathway carried out by various microorganisms and cells, leading to the breakdown of organic substrates and the production of unique properties in the desired end products (Attri & Goel, 2023). Traditional fermented foods, such as dairy-based products (kefir, koumiss, kurut), cereal-based products (tarhana, boza, idli), fish and meat-based products (sucuk,

pastırma, fish sauce), soy-based products (natto, soy sauce, tempeh), and vegetable and fruit-based products (kimchi, cucumbers, cabbages, wine, vinegar), are produced worldwide, including in Turkiye (Chilton et al., 2015; Kabak & Dobson, 2011). Fermented products can be classified into three categories based on their state: solid-state, liquid-state, and semi-solid-state fermentations. Tempeh and fermented soybeans are examples of solid-state fermentation products, soy sauce is an example of liquid-state fermentation, and some antibiotic production processes are examples of semi-solid-state fermentation products (Xu et al., 2010).

According to the use of microorganisms, there are two types of fermentations, natural fermentation, which spontaneously occurs due to the natural flora of raw materials used, and controlled fermentation, which uses a defined starter culture. Controlled fermentations are also divided into two types: monoculture fermentation, which uses one type of microorganism, and multicultural fermentation using two or more cultures (Skrzypczak et al., 2020; Tangyu et al., 2019).

Fermented foods and beverages are the nutritional and cultural heritage of every society in the world, making a significant contribution to the diet of millions of people and the economy. Biochemical changes by microorganisms during fermentation contribute directly to the release or change of bioactive compounds in the food matrix and affect their functional, rheological, and sensory properties (Ruiz Rodríguez et al., 2021; Septembre-Malaterre et al., 2018). For example, Tarhana is one of the traditional fermented products produced by fermenting wheat flour-yogurt and a mixture of vegetables and spices through yeast and lactic acid fermentations. Due to the low pH (3.8 - 4.2) and humidity (6-9%) values in the final product, which create an unfavorable environment for pathogenic microorganisms, tarhana can be stored for 1-2 years without spoiling (Cankurtaran Kömürcü & Bilgiçli, 2022). Fermentation also favors the formation of ascorbic acid, riboflavin, niacin, pantothenic acid, and folate compounds in several products (Ansari & Pourjafar, 2022).

Table 1: Some Microorganisms Commonly Used in Fermented Foods and Beverages

Group	Species	Product	References
Bacteria	Lactobacillus acidophilus	Fermented dairy products (milk, cheese, yogurt, kefir), fermented vegetable and fruits	(Marco et al., 2017)
	Lactobacillus casei	Cheeses, fermented olives, fermented milk and vegetables, sausages, kefir	(Millette et al., 2007)
	Lactobacillus sakei	Fermented meat, cereals, and vegetables	(Zagorec & Champomier-Vergès, 2017)
	Lactobacillus delbrueckii subsp. bulgaricus	Yogurt, cheese, kefir, fermented milk, kimchi, fermented tea	(Dan et al., 2019)
	Lactobacillus rhamnosus	Fermented fruit drinks, cucumber, kimchi, sausages, cheese	(Salva et al., 2011)
	Lactobacillus helveticus	Kefir, fermented milk, soy beverages, vegetables, cheeses	(Begunova et al., 2020)
	Bacillus subtilis	Fermented soybeans (natto chestnut, hawaijar, kinema)	(Sini et al., 2007)
	Bacillus licheniformis	Fermented soybeans (Chunghookjang), food additives; protease activity	(Muras et al., 2021)
	Acetobacter aceti	Vinegar, ciders	(Gullo et al., 2014)
	Gluconacetobacter entanii	Vinegar	(Akasaka et al., 2013)
	Komagataeibacter europaeus	Vinegar	(Yetiman & Kesmen, 2015)
Yeast	Kluyveromyces marxianus	Cheese ripening, enzyme production, fermented tomato and red pepper pomace, cocoa beans, kefir	(Fonseca et al., 2008)
	Debaryomyces hansenii	Cheese ripening, enzyme production, fermented salami, beverages, probiotics	(Breuer & Harms, 2006)
	Saccharomyces cerevisiae	Cheese ripening, enzyme production, alcoholic beverages, sourdough, probiotics, meat, kefir, peas	(Saerens et al., 2010)
Filamentous fungi	Aspergillus niger	Beverages, citric acid production, enzyme production (food additives)	(Costa et al., 2016)
	Aspergillus oryzae	Beverages, soy sauces	(Son et al., 2018)

Lactic acid bacteria (LAB) Fermentations

Lactic acid bacteria (LAB) are primarily responsible for the fermentation of vegetables, olives, wine, milk, and meat products. Many LAB are commonly used in food preparations for their preservative effects. They are a group of gram-positive, non-sporing, non-aerobic but aerotolerant, acid-tolerant cocci or

rods, which can be classified as heterofermentative or homofermentative based on their sugar fermentation pathways (Figure 1). Lactic acid bacteria obtain the energy they need for their metabolism chemotropically by oxidizing chemical compounds. They also obtain energy by degrading organic sugars under anaerobic conditions and produce various metabolites based on their metabolic pathways (Gänzle, 2015). Figure 1 shows the representation of heterofermentative LAB, Lactobacillus brevis, and Lactobacillus delbrueckii subsp. bulgaricus.

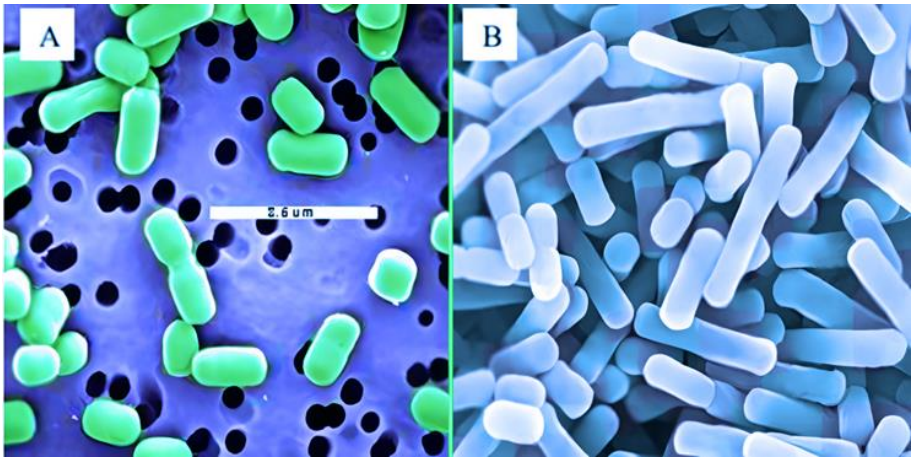

Figure 1: Heterofermentative Lactic Acid Bacteria: (A) Lactobacillus brevis, and (B) Lactobacillus delbrueckii subsp. bulgaricus.

Homofermentative and heterofermentative LAB produce different end products due to their genetic and physiological differences when utilizing glucose. Heterofermentative LAB are referred to as lactic acid bacteria that produce lactic acid, ethanol, and CO2 from hexoses via the pentose phosphate pathway (Z. Liu et al., 2022). On the other hand, homofermentative LAB are lactic acid bacteria that produce mainly lactic acid via the fermentation of hexoses through the Embden-Meyerhof pathway (glycolysis). However, heterofermentative lactic acid bacteria are much more significant in the production of flavor and aroma compounds such as acetaldehyde and diacetyl (Raj et al., 2022).

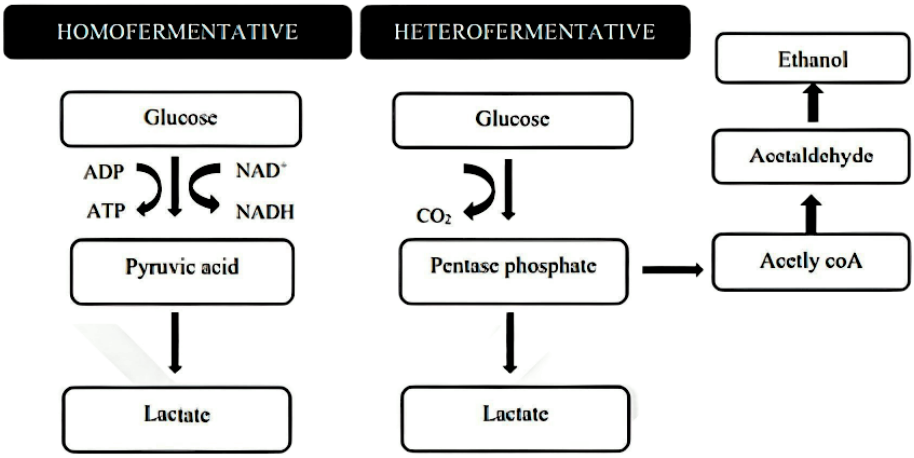

Figure 2: The Metabolic Pathways of Both Homofermentative and Heterofermentative Lactic Acid Bacteria

Lactic acid bacteria (LAB) are a ubiquitous group of microorganisms found in various traditional fermented foods, including dairy products, fermented sourdough, meats, vegetables, and beverages, as well as in the gastrointestinal and respiratory systems of humans and other mammals (Gänzle, 2015).LAB are generally recognized as safe (GRAS) microorganisms and are commonly used in the development of functional foods, as they are known for their probiotic properties and associated health benefits. LAB also play a significant role in improving the sensory properties and biochemical composition of foods and beverages. The shift in consumer dietary habits due to health-related concerns, such as heart diseases, high cholesterol, and weight control, has led to an increased demand for low-calorie milk and fermented dairy products (J. M. Liu et al., 2022)

The functional properties of LAB contribute to the development of functional food products and food additives. A previous study has demonstrated that protease-facilitated fermentation of milk by two lactic acid bacteria, Streptococcus thermophiles and Lactobacillus bulgaricus, can release bioactive peptides with antihypertensive effects (Salva et al., 2011; Solanki et al., 2022). The researchers found that lactic acid fermentation accelerated the production of bioactive peptides in fermented milk, which inhibited Angiotensin Converting Enzyme (ACE) activity in vitro (Solanki et al., 2022).

Especially, lactic acid bacteria with probiotic properties are widely used as starter cultures in the production of dairy products. These bacteria increase the shelf life of foods by producing metabolites such as bacteriocin during

fermentation, while also playing a role in regulating the human intestinal microbiota and preventing the occurrence of inflammatory diseases (Saez-Lara et al., 2015). For example, kenaf seeds are a favorable source of natural preservatives for food applications owing to their high antibacterial properties. Fermentation of kenaf seed proteins with Lactobacillus casei has been shown to result in high antibacterial activity (98%–100%), making it a potential source of natural preservatives for food applications (Arulrajah et al., 2020).

Non-lactic Acid Bacteria Fermentations

Bacillus is a group of Gram-positive bacteria that can form catalase-positive endospores and have a rod-shaped structure. They are facultatively anaerobic and aerobic organisms, mostly mesophilic, but there are also psychotropic and thermophilic species. These microorganisms are generally isolated from processed or unprocessed foods with an alkaline environment. The most important features of Bacillus species are their ability to survive under adverse environmental conditions and form spores (Millette et al., 2007; Muras et al., 2021). For instance, some Bacillus species such as B. cereus and B. licheniformis are commonly found in raw milk and often represent secondary contamination during milk processing. Bacillus species are difficult to kill due to their long life cycle, which makes them useful in various applications, but also presents challenges in controlling spore-forming diseases (S Yacoub et al., 2017). Figure 3 shows the demonstration of Bacillus subtilis and Bacillus licheniformis species.

One of the most important features of Bacillus species is the production of thermostable extracellular enzymes such as lipase, amylase, protease, and laccase, which cause proteolytic and lipolytic activity during fermentations. In a study by Sanjukta et al. (2015), soybean was fermented by B. subtilis MTCC 5480 and B. subtilis MTCC 1747. As a result of the proteolytic activity of Bacillus strains, the degree of hydrolysis percentage of soybean proteins was reported to be around 38.75-44.8% (Sanjukta et al., 2015). Bacillus bacteria play a more effective role than lactic acid bacteria and other microorganisms in the development of alkali-fermented products as well as they play an effective role in the production of high-value-added desired fermented products. B. subtilis, B. licheniformis, B. amyloliquefaciens, B. pumilus, B. sphaericus, B. cereus, and B. xylanilyticus, with B. subtilis, have been reported as the most commonly used Bacillus species in fermentation studies (Vouidibio Mbozo et al., 2017). For instance, Dong et al. (2020) stated that the fibrinolytic activity of natto chestnut achieved by 6479 IU/g under the optimized fermentation conditions with Bacillus subtilis at a temperature of 38°C, fermentation time 56

h, and 5% inoculum rate. At the same time, it has been reported that fermented natto chestnut, which has been enhanced with the amount of antioxidant and total phenolic compounds, will be a healthier food product for human consumption (Dong et al., 2020).

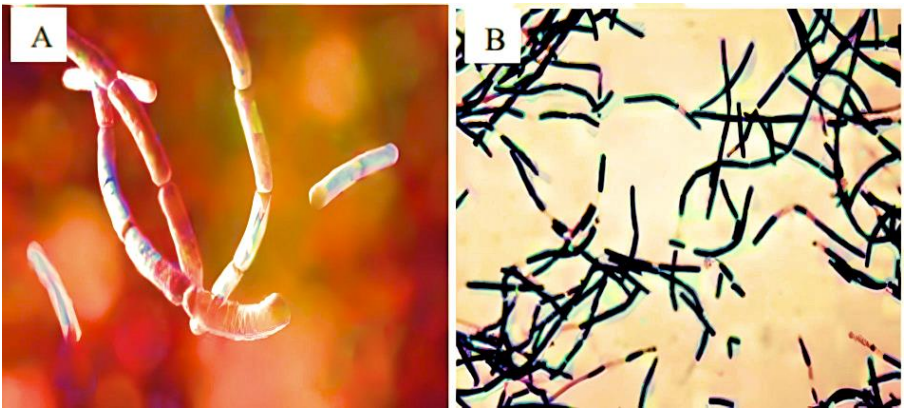

Figure 3: Demonstration of Bacillus subtilis (A) and Bacillus licheniformis (B) species

Acetic acid bacteria constitute a significant group of microorganisms involved in the production of fermented products, including kombucha and vinegar. These gram-negative bacteria are capable of producing acetic acid from ethanol via several biochemical reactions under aerobic conditions. Among the most important genera of acetic acid bacteria are Gluconobacter and Acetobacter. These bacteria can be found in media containing substrates such as sugar or alcohol, such as juice, wine, vinegar, and beer. The complete oxidation of these substrates in bacterial metabolism leads to the accumulation of organic acids in the environment. Most acetic acid bacteria produce acetic acid from ethanol, but they are also employed in the production of high-value metabolic products, such as bacterial cellulose, gluconic acid, and L-sorbose (Saichana et al., 2015).

Acetic acid bacteria are widely utilized in the production of various fermented products such as vinegar, wine, beer, and other beverages owing to their unique taste and nutritional benefits. Vinegar, for instance, which comprises acetic acid and other organic compounds, is used as a food preservative and flavor enhancer. The fermentation process of vinegar occurs in two stages, anaerobic and aerobic. In the first stage, sugar is converted to ethanol through yeast fermentation under anaerobic conditions. In the second stage, acetic acid fermentation is carried out by the oxidation of ethanol under

aerobic conditions (Solieri & Giudici, 2009) (Figure 4). Studies have shown that vinegar has beneficial effects on human health, such as its potential to act as an antidiabetic, antihypertensive, and to reduce the risk of cardiovascular diseases (Ali et al., 2016; Solieri & Giudici, 2009).

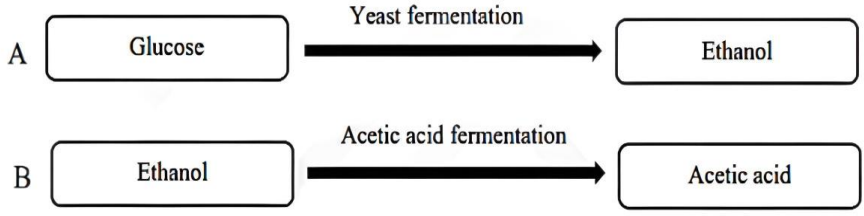

Figure 4: Two stages of Vinegar Fermentation: (A) Anaerobic and (B) Aerobic Fermentations

The Role of Yeasts as Functional Starter Cultures in Food Fermentations

Yeasts are a major group of microorganisms that are commonly used in the production of traditional fermented foods. They are single-celled eukaryotic microorganisms belonging to the phylum Ascomycetes and Basidiomycetes (Breuer & Harms, 2006; Fonseca et al., 2008). Yeasts offer several advantages in industrial fermentation processes, such as their ability to ferment a variety of carbon sources, to compartmentalize reactions in organelles, to carry out many post-translational modifications, and to show several enzymatic activities, such as lipolytic, proteolytic, pectinolytic, glycosidasic, and urease activities (Fleet, 2006).

In yeast fermentations, apart from alcohol, glycerol, and CO2, various other by-products are also formed depending on the yeast species, substrate type, and fermentation conditions. These by-products include organic acids, such as formic, malic, acetic, fumaric, succinic, oxalic, and citric acids, as well as volatile organic compounds such as esters, alcohols, and carbonyl compounds, which can alter the aroma and flavor properties of the food products. For example, Saccharomyces cerevisiae, which is commonly used as a starter culture in the production of alcoholic beverages, enhances the flavor and aroma of the beverage by producing secondary metabolites, including alcohols, carbonyls, esters, and sulfur compounds, during the fermentation process (Howell et al., 2005; Saerens et al., 2010).

Table 2: Yeast Species and Their Applications in Food and Beverages

Yeast Species	Products
Saccharomyces cerevisiae	Beer, wine, bread, mead, fermented vegetables, kefir, probiotics, nutritional yeast
Kluyveromyces lactis	Cheese, kefir, fermented milk, whey
Debaryomyces hansenii	Ripening of cheese, fermented meat, probiotics
Candida albicans	Kombucha, fermented vegetables, sourdough
Pichia fermentans	Wine, beer, kefir, fermented vegetables
Saccharomyces boulardii	Probiotic, diarrheal relief, immune support
Zygosaccharomyces bailii	Wine, cider, vinegar, kefir
Kazachstania exigua	Beer, wine, kefir
Torulaspora delbrueckii	Wine, cider, fermented milk, kefir
Hanseniaspora uvarum	Wine, beer, sake, fermented vegetables, kefir

Traditionally, yeasts have been favored in industrial bioreactors due to their high secretion capacity, ability to utilize a wide range of carbon sources, rapid growth rate, and versatility in many metabolic reactions. For example, the yeast species K. marxianus has significant importance in bioethanol production due to its unique characteristics such as thermotolerance, ability to metabolize sugars including lactose and inulin, activity of lytic enzymes, and faster growth rate compared to other eukaryotic microorganisms. Moreover, K. marxianus has shown promising functional properties that have led to its application in various industries such as food, energy, cosmetics, and pharmaceuticals (Varela et al., 2017).

Yeasts have the capability to secrete both extracellular and intracellular enzymes, including proteases, xylanase, amylase, cellulases, lipases, galactosidases, pectinases, invertase, and phytases. The aminopeptidase and carboxypeptidase enzymes of yeast, in particular, play an important role in the hydrolysis of milk proteins and the release of bioactive peptides. Additionally, the lipolytic and proteolytic activity of yeasts improves the aroma and flavor properties of cheese and contributes to its maturation. Various yeast species, such as S. cerevisiae, D. hansenii, Kluyveromyces marxianous, and Yarrowia lipolytica, found in cheese, not only favor the cheese's low pH, low moisture value, and high salt concentration but also affect its maturation and shelf life through lipolysis, proteolysis, fermentation of carbon sources, and autolysis of their biomass (Sahin et al., 2022).

Recently, yeasts have attracted attention in several studies due to their health-promoting activities and their use as probiotic agents. Yeast species, including Kluyveromyces marxianous, Saccharomyces cerevisiae, Debaryomyces hansenii, Candida krusei, Torulaspora delbrueckii, Yarrowia lipolytica, and Pichia kudriavzevii, have potential probiotic properties. Yeasts isolated from dairy products accelerate the formation of bioactive compounds with various bioactivities due to their proteolytic activities during the fermentation process. Various yeast species have been reported to successfully produce milk protein-derived antihypertensive peptides (Manzoor et al., 2022).

In a study conducted by Chaves-López and colleagues in 2012, various yeast species were isolated from Colombian Kumis to investigate their potential Angiotensin converting enzyme (ACE) inhibitory activity and their ability to eliminate bitter taste from fermented milk products. The study found that 18 out of 93 yeast species were able to increase the ACE inhibitory activity of fermented milk products by values ranging from approximately 8.69% to 88.19%. Notably, the yeast species P. kudriavzevii KL84A and K. marxianous KL26A exhibited the highest ACE inhibitory activity. Additionally, the study found that the bitter taste was eliminated at the end of fermentation. Yeast fermentation was also found to contribute to the development of flavor properties and to accelerate the formation of bioactive peptides through the hydrolysis of milk proteins (Chaves-López et al., 2012).

Furthermore, various strains of K. marxianus have been found to have important probiotic effects on human intestinal microbiota and cellular immunity, as well as antioxidant, anti-inflammatory, and hypocholesterolemic functions. These strains, when isolated from dairy products, have not only demonstrated anti-pathogenic activity but also anti-cancer activity in gastric cancer cells through the release of certain metabolites. In addition, K. marxianus strains isolated from kefir have been shown to have a greater cholesterol-lowering activity than other probiotic yeasts such as S. cerevisiae and K. lactis (Youn et al., 2023). Recently, probiotic yeast and yeast extracts have been utilized as a natural source of antioxidants due to their possession of antioxidant enzymes such as catalase, superoxide dismutase, and oxygenated carotenoids and resveratrol (Mounir et al., 2022). For example, the antioxidant activity of fermented sausage with a probiotic yeast D. hansenii was produced by the degradation of hydrogen peroxide due to the catalase activity of D. Hansenii (Ramos-Moreno et al., 2019).

Moreover, yeast fermentation enables the hydrolysis of organic compounds, thereby increasing the digestibility of products and improving the nutritional composition of products. During sourdough and plant-based food fermentations,

the enzymes of yeast exhibit hydrolysis activity, leading to an increase in the amount of vitamins, minerals, phenolic compounds, and sterols in the media (Tlais et al., 2020). Various staple crops such as barley, maize, millet, oats, rice, rye, sorghum, and wheat have been used as substrates for fermentations. For example, in a study, rice and rice bran fermented by yeasts such as Saccharomyces bouldardii and Monascus purpureus were found to contain high amounts of phytochemicals such as steroids, unsaturated fatty acids, vitamins, and phenolics, and were effective for the management of diseases such as cancer, cholesterol, diabetes, and Alzheimer's (Baciu et al., 2023; Ren et al., 2022). Similarly, fermentation of soybean residues with S. cerevisiae increased both the antioxidant activity and the total phenolic content of soybean residue (Liu et al., 2023).

Fermentations carried out by yeasts lead to the occurrence of desirable organoleptic profiles in foods, which in turn increases their acceptance by consumers. The production of such flavor compounds is facilitated by flavor precursors such as amino acids and peptides that are formed as a result of the proteolytic activity of yeast starter cultures (Chaves-López et al., 2012). In a study conducted by Hu et al. (2019), the beany odor of soybean residues was eliminated by K. marxianus fermentation. Nine substances, such as hexanol, n-hexanal, and 1-octen-3-ol, have been reported to cause beany odor in soybean residue. A decrease in the amount of substances that cause beany odor was observed due to the secretion of the β-galactosidase enzyme from K. marxianus and the production of amino acids such as valine and serine. It was also reported that creamy and sweet odor was obtained due to the production of various aroma compounds in fermented soybean residues by K. Marxianus (Hu et al., 2019). In another study, D. hansenii was used to eliminate the boar taint odor in dry fermented sausages made with entire male back fat. Sausages with a fruity odor and a less oxidized form were obtained as a result of the reduction of lipid oxidation by D. hansenii fermentation, and boar taint odor was eliminated with the release of new aroma compounds, as revealed by sensory evaluations (Corral et al., 2017).

Furthermore, S. cerevisiae is one of the most important starter cultures used to improve the aroma and flavor properties in the beverage industry. Various secondary metabolites such as esters, higher alcohols, carbonyl compounds, and organic acids favor the improvement of aroma and flavor characteristics of beverages. For example, glycerol, one of the aroma active compounds produced during fermentation, gives a "mouthfeel" sensation to beverages, while esters provide fruity/floral aroma and flavor to beverages. Esters such as isoamyl

acetate, which has fruity properties that impact banana flavor, can be produced from sugar beet molasses by yeast fermentation (Satora & Pater, 2023).

Volatile flavor compounds, also known as aroma compounds, play a crucial role in our sensory experience of food and beverages. These compounds are responsible for the characteristic smells and tastes that we perceive. However, in addition to enhancing the sensory appeal of our meals, volatile flavor compounds also offer several health benefits such as antimicrobial, antihypertensive, and bronchodilatory effects (Demirel, 2022a; Demirel, 2022b; Demirel, 2022c; Demirel 2022d; Demirel, 2022e; Ribeiro-Santos et al., 2017). The production of volatile flavor compounds during fermentation is a fundamental aspect of the flavor and aroma profile of fermented products such as beer, wine, and bread. Yeast species and strains play a significant role in determining the type and amount of these compounds produced. In beer production, for instance, certain strains of Saccharomyces cerevisiae have been identified as the source of fruity esters like isoamyl acetate, while other strains produce phenolic compounds that contribute to spicy or clove-like flavors. Wine production involves the use of different yeast strains that produce diverse aromatic compounds, such as terpenes and thiols, that play a crucial role in the wine's aroma and flavor profile. However, some strains of Saccharomyces cerevisiae may produce acetic acid, which can lead to vinegary flavors in wine (Ali et al., 2016; Yetiman & Kesmen, 2015).

Studies have also explored the effect of yeast species and strains on the flavor and aroma of bread products (Timmermans et al., 2023). Sourdough bread, for example, is produced using a mixture of yeast and lactic acid bacteria that produce lactic and acetic acids, which contribute to the bread's sour taste and aroma. Therefore, the selection of the appropriate yeast species and strain for fermentation is a critical factor in producing fermented products with desirable sensory characteristics. Understanding how yeast species and strains influence the flavor and aroma profile of these products can facilitate the production of high-quality and consistent products with unique and desirable sensory properties (Liu et al., 2023).

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Chapter 13

Monoclonal Antibodies for Targeted Cancer Therapy

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ABSTRACT

The identification of key molecules regulating cellular immune events paves the way for the development of new immunotherapeutic approaches for cancer therapy. Monoclonal antibodies and antibody-drug conjugates have been widely used in oncology and in many clinical studies in recent years. Monoclonal antibodies have become irreplaceable in cancer in terms of both diagnosis and treatment. Their high selectivity and favorable toxicity profiles have allowed these agents to be included in standard treatments, despite their high cost. Such agents will provide a unique opportunity for the treatment of the patient population based on the expression of specific tumor targets. The aim of this book chapter is to explore the use of monoclonal antibodies in targeted cancer therapy.

Keywords: Monoclonal antibody, antibody-drug conjugates, targeted cancer therapy, Rituximab, Trastuzumab

INTRODUCTION

Cancer is a disease that occurs when body cells multiply uncontrollably and invade neighboring tissues (Kayaalp, 2009). The main treatment methods are surgery, chemotherapy and radiotherapy. Chemotherapy involves eliminating tumor cells or at least limiting their proliferation with the use of low molecular weight drugs. The disadvantages of many cytotoxic agents are bone marrow suppression, gastrointestinal tract lesions, hair loss, nausea, and development of clinical resistance. These side effects occur because cytotoxic agents act on both tumor and healthy cells (Shewach & Kuchta, 2009; Thurston, 2007). Cancer immunotherapy essentially has several advantages over conventional therapies, it is expected to be less toxic to patients' healthy organs and more specific to diseased tissues (Friedmann-Morvinski & Eshhar, 2006). Cancer cells can be detected through the immune system, and under certain conditions, the immune system can control or even eliminate tumors (Kyi & Postow, 2014). Both innate and adoptive immunity contribute to the recognition and rejection of malignant cells (de Visser, Eichten & Coussens, 2006; Caligiuri, 2008). The next finding covers tumor control of the immune system, and the identification of key molecules that regulate cellular immune events leads to the development of new immunotherapeutic approaches for cancer therapy (Finn, 2012; Melero et al., 2007). In the treatment of cancer, the interest in the binding of different forms of pharmaceutical carrier systems to the active substance directly or in capturing the active substance and targeting the drugs in this way is increasing day by day. By targeting, conventional, biotechnological and gene-derived drugs can be selectively transported to specific parts of the body such as organs, tissues and cells. With this selective targeting, undesirable side effects are reduced, the most appropriate therapeutic response is obtained, and substances with toxic effects at high doses can be used safely (Çevik, Aydın & Gürsoy, 2012). Treatment with monoclonal antibodies against some types of tumors and antiidiotype antibodies against some others is being attempted. There are studies on selective aggregation of the drug in tumor tissue by binding tumor cell-specific monoclonal antibodies to drug molecules (Kayaalp, 2009).

Targeting in Cancer

It is a treatment developed directly against the molecular abnormality or target that causes the development of cancer. Targeting in cancer;

By blocking the cell growth signal

- By inhibiting new vessel formation
- By inducing apoptosis
- By stimulating the immune system

• It can be achieved by delivering the molecule that will show toxicity to the cancer cell (Kayaalp, 2009; Shewach & Kuchta, 2009).

In cancer treatment, it is aimed to selectively target and destroy cancer cells without affecting healthy cells. Ideal features for targeting to the tumor;

It can be listed as increasing the localization of the drug in the tumor by active or passive targeting, reducing its localization in non-targeted cells, minimizing the drug leakage from the transitional regions, protecting the drug from degradation, keeping the drug in the targeted area for the desired time, facilitating its uptake into the cell, and biocompatible and biodegradable delivery system components.

The interest in the targeting of drugs in different forms of pharmaceutical carrier systems (such as particulate systems, liposomes, emulsion systems) directly binding to or trapping the active substance and thus targeting drugs is increasing day by day. Some of the new drug delivery systems used for targeting are liposomes, micelles, nanoparticles, dendrimers and nanocapsules. Systems such as nanoparticles, microcapsules, microspheres and liposomes prepared using macromolecules such as dextran, albumin, DNA, polyamino acids and other polymers from non-specific carriers accumulate in the tumor due to environmental features such as increased retention effect due to low lymphatic drainage around the tumor and tumor size. These systems can be directed to tumors by passive targeting, or they can be combined with molecules such as antibodies, ligands, and peptides for active targeting. By targeting, conventional, biotechnological and gene-derived drugs can be selectively transported to certain parts of the body.

With this selective targeting, undesirable side effects are reduced, the most appropriate therapeutic response is obtained, and substances with toxic effects at high doses can be used safely (Çevik, Aydın & Gürsoy, 2012).

Recombinant DNA Technology

The term recombinant DNA refers to the formation of a new combination by combining DNA molecules that cannot naturally coexist, that is, combining DNA molecules obtained from different biological sources. It uses genetic techniques and nucleic acid biochemistry methods developed in studies with bacteria and viruses together. The first steps in this regard were taken in the 70s. In 1973, professor of genetics at Stanford University, Dr. Stanley N. Cohen, a biochemist, genetic engineer, and educator at UC-San Francisco. Herb Boyer showed that the genes in a cell's DNA can be cut with enzymes and linked/transferred to the desired place in the DNA of another cell with enzymes. This technique, which provides the opportunity to cut genes and transfer them to other living things, in

a sense means programming cells to produce desired proteins. This creature, which has taken the new gene into its body, now becomes a factory that can produce as much of the desired protein as desired. In 1974, Cesar Milstein and Georges Kohler succeeded in producing monoclonal antibodies. Thus, as the cells proliferate, antibodies with the desired properties can be produced in large quantities (Başağa & Çetindamar, 2006). Many of the therapeutic proteins are produced using recombinant DNA technology. A human protein intended to be used as a medicine is produced in high amounts in mammalian animal cells by cell culture method or in yeast or bacterial cells by fermentation method, and these proteins are then purified from the production medium. Normal cells either do not synthesize such proteins at all, or they are not suitable for production in the amount that can be used as medicine, since the amount of synthesis is very low. For this reason, it is necessary to transfer the human gene that produces the human protein to animals, yeast or bacteria with the help of recombinant DNA technology and to ensure that the transferred gene can synthesize a high amount of active protein. Therapeutic proteins in the monoclonal antibody group can only be produced in mammalian animal cells. Monoclonal antibodies used for therapeutic purposes are generally obtained by first administering the antigen that the antibody will target to mice, and then transforming the lymphocyte cells of the mouse into hybridoma cells. In many cases, the mouse antibody is not used directly for humans, some changes are made in the genes encoding the mouse antibody with the help of recombinant DNA technology, thus making the synthesized antibody similar to that of the human (Khan et al., 2016). For this purpose, all regions of mouse monoclonals except the antibody binding region are replaced with human fragments. In this way, chimeric antibodies are obtained. Later, the mice with the gene exchange started to produce humanoid antibodies in their bodies after the human antibody genes were made to be carried. Apart from this, serial monoclonal production is being studied without using mice, which is called the phage display technique. In this technique, a virus called a phage, which has the ability to infect bacteria, is used (Candaş, 2002).

Monoclonal Antibodies

Monoclonal antibodies that produced by a single B-cell clone and used in therapy are generally antibodies developed in mice. Mouse monoclonal antibodies have been able to show adequate effect on target molecules in clinical trials, but their use has been limited as patients subjected to treatment have improved immunity to these mouse proteins. This issue was overcome by "humanization" of mouse monoclonal antibodies. Recombinant DNA technology is used to acquire humanized monoclonal antibodies. Since antibodies modified

in this way cause less immune stimulation in patients, continued use is possible. There is a large amount of monoclonal antibody drugs currently in use and their quantity is expected to increase quickly. Exclusively, it is predicted that the number of monoclonal antibodies directed to antigens abundantly on the surfaces of cancer cells will increase quickly (Khan et al., 2016). Theoretically, monoclonal antibodies designed for a specific antigen in cancer cells can initiate an immune response by destroying cancer cells without harming normal cells (Akbuğa, 2002).

Monoclonal antibodies that can be produced *in vitro* can be successfully used in in vitro and in vivo diagnostic systems, prophylaxis and treatment by binding to antigens that promote their production without destroying their structures. Their ability to bind to their specific structures is exploited in different research areas including immunoblot, immunohistochemistry, immunochemistry and cellular analysis. It can be used as a prophylactic and therapeutic in different applications and in forms that have been redesigned with monoclonal, polyclonal, recombinant or engineering applications, as well as for diagnosis and treatment, as well as for prevention from different disease agents. They are used in the diagnosis of disease agents, cancer cells, tumor markers and metastases (Yücel, 2010).

Anti-BF or anti-BF receptor monoclonal antibodies produced by biotechnology methods by using growth factors or their receptors as antigens, when applied as drugs, bind with them in the body and block them. Thus, they create an antitumoral effect. From a pharmacological point of view, they are considered immunological antagonists (Kayaalp, 2009). Monoclonal antibodies exert their tumoricidal effects via signaling through the receptor to which they bind, antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Monoclonal antibodies have the ability to activate the receptors on the cell surface by linking them with each other. The signaling generated in this way can be of different nature; For example, anti-CD 20 monoclonal antibody induces apoptosis, while EGFR (epidermal growth factor receptor) binding antibody prevents binding of natural ligands, causing receptor blockade (Fadıloğlu, Tokem & Özçelik, 2008). They are given by intravenous infusion. They are high-priced drugs (Kayaalp, 2009).

Monoclonal antibodies (mAbs) associated with cancer therapy are classified according to their targets.

There are mAbs that inhibit the cellular autonomic life steps of the tumor. (Cetuximab and panitumumab, which inhibit the EGFR and are approved for the treatment of colorectal cancer) (Galluzzi et al., 2012).

Panitumumab and Cetuximab

Panitumumab; epidermal growth factor receptor is a tyrosine kinase receptor that is overexpressed in many cancer types such as breast, ovarian, colorectal and head and neck cancer. It is involved in cancer cell proliferation, tumor growth, angiogenesis and metastasis. It is a monoclonal antibody containing fully human sequences that binds and blocks the epidermal growth factor receptor (Şakalar, İzgi & Canatan, 2013). It is used in the treatment of metastasized colorectal cancer that expresses EGFR and has metastasized without the KRAS gene mutation, where previous combined chemotherapy has failed. It may cause a hypersensitivity reaction during its infusion (Kayaalp, 2009).

Cetuximab; is a chimeric monoclonal antibody against EGFR that contains both human and mouse protein sequences (Şakalar, İzgi & Canatan, 2013). It is used in combination with irinotecan in metastatic colorectal cancer that expresses EGFR, refractory to other drugs, and in combination with radiotherapy in locally advanced squamous cell head and neck cancer. Since it can cause acute anaphylactic reaction, it should be used in places where resuscitation is possible. Before the infusion, the patient should be given an antihistamine (Kayaalp, 2009).

There are mAbs that inhibit tumor-stroma reactions (Bevacizumab, which blocks vascular endothelial growth factor, VEGF and is used in lung, breast, kidney, colorectal cancer treatments) (Galluzzi et al., 2012).

Bevacizumab

It is a monoclonal antibody that binds and inhibits vascular endothelial growth factor. It inhibits angiogenesis in tumor tissue. It is used as indicated in metastatic breast cancer, recurrent ovarian cancer, metastatic colorectal cancer, non-small cell lung cancer in which squamous cells are not dominant, and off-label in metastatic renal cell cancer and high-grade glial tumors. It can cause serious gastrointestinal disorders including bowel perforation and obstruction, cardiovascular disorders including hypertension and pulmonary hypertension, neuropathy, and skin dryness (Kayaalp, 2009). When bevacizumab is combined with chemotherapy in patients with metastatic colorectal cancer, it significantly increased the survival rate (Şakalar, İzgi & Canatan, 2013; Dubois, Rissmann, & Cohen, 2011).

It bind expressed antigens to the surface of tumor cells and function selectively by immune effector mechanisms such as CDC, ADCC, ADCP (antibody-dependent cellular phagocytosis). (Anti-CD20: Rituximab and Ofatumumab used in the treatment of lymphoma, anti-CD52: alemtuzumab, HER2 (human epidermal growth factor) blocker used in the treatment of breast cancer:

Trastuzumab and Pertuzumab (Galluzzi et al., 2012; Şakalar, İzgi & Canatan, 2013).

Rituximab and Ofatumumab

Rituximab; It is a monoclonal antibody specific to B cells that binds to the CD20 antigen of B-type lymphocytes and destroys these cells by lysis (Kayaalp, 2009). CD20 protein is a molecule found on the surface of B lymphocytes. CD20 is not released, modified, or taken up into the cell. The CD20 molecule is expressed by cancer cells in chronic lymphocytic leukemia (CLL) and lymphomas originating from B lymphocytes. Rituximab coats the surface of cancer cells with antibodies. In this way, antibody-coated cells activate NK cells. NK cells cause antibody-dependent destruction. This destruction develops due to antibody-dependent cellular cytotoxicity. In addition, rituximab causes a number of changes in cancer cells and causes programmed cell death, which is also called apoptosis. In addition, rituximab increases the sensitivity of cancer cells to chemotherapy (Cerny et al., 2002). It may cause tumor lysis syndrome in patients with high tumor burden. It is used alone or in combination with radiotherapy or chemotherapy in follicular non-Hodgkin's lymphoma. It is also effective in combination with other chemotherapeutics in diffuse large B-cell non-Hodgkin's lymphoma. Especially during its first infusion, it may cause cytokine release syndrome (febrile, chills-chills, allergic rash on the skin, itching, angioedema, bronchospasm, shortness of breath, nausea, vomiting, facial flushing accompanied by symptoms). It should be used in hospitals with resuscitation facilities (Kayaalp, 2009).

Ofatumumab; It is a fully human monoclonal antibody that binds to the CD20 molecule expressed specifically on normal B lymphocytes and on chronic lymphocytic leukemia B lymphocytes and causes B cell lysis and death. The epitope to which Ofatumumab binds is different from the binding sites targeted by rituximab. Also, compared to rituximab, ofatumumab has a slower dissociation rate and more stable binding to CD20. The slower dissociation rate and more stable binding properties may be responsible for the efficacy of ofatumumab against cells with low CD20 antigen density and high complement inhibitory molecule expression. The US Food and Drug Administration (FDA) approved ofatumumab in January 2009, following an accelerated approval process for the treatment of fludarabine and alemtuzumab-resistant CLL patients. Ofatumumab was approved by the European Medicines Agency (EMEA) on April 19, 2010, under the trade name Arzerra® in accordance with the central registration procedure (Herter et al., 2013).

Alemtuzumab

CD52 is a differentiation antigen found on all lymphocytes and has been related with some lymphomas. Therefore, targeting this antigen has been shown to be effective against certain cancers. Alemtuzumab is a humanized monoclonal antibody in the IgG1 isotype that targets CD52 and received clinical approval for use in B-cell leukemia in 2001 (Buggins et al., 2002). The exact mechanism of action is unknown; however, the effect of the drug is explained by the fact that it causes antibody-dependent lysis in leukemic cells after binding to the cell surface. As with unconjugated monoclonal antibodies, the action of alemtuzumab is dependent on the ability of the monoclonal antibody itself to directly destroy the cell or activate effector mechanisms such as complement or T-cells to attack target cells. Conjugated antibodies use their toxic parts to destroy target cells, while monoclonal antibodies act as vectors. Only a certain fraction of cell surface antigens allow cell lysis via complement or cellular cytotoxicity mechanisms; CD52 exerts cell lysis through both cellular cytotoxicity and complement-mediated cytotoxicity. Alemtuzumab was also associated with the release of tumor necrosis factor (TNF), interleukin-6 and interferon gamma. The sensitivity of lymphoid malignancies to anti-CD52 antibodies varies over 200-fold. T-cell prolymphocytic leukemia is very sensitive *in vitro* and *in vivo.* However, despite the similar amount of antigen expression *in vivo,* monocytes and monocytic leukemias are resistant to Alemtuzumab (Herter et al., 2013). It is indicated in patients with B-cell chronic lymphocytic leukemia who have used alkylating agents and are resistant to fludarabine. Hematological side effects such as pancytopenia, neutropenia, thrombocytopenia, anemia, lymphopenia associated with the use of alemtuzumab; infectious side effects such as sepsis, pneumonia; Infusion-related side effects such as rigor, fever, nausea, vomiting, and hypotension may occur (Buggins et al., 2002; Herter et al., 2013).

Trastuzumab and Pertuzumab

Trastuzumab; It binds HER2 and blocks it. It overexpresses HER2 (Kayaalp, 2009). Various mechanisms have been suggested for the emergence of trastuzumab's efficacy; HER2 receptor blockade, inhibition of angiogenesis, and HER2 receptor down-regulation are these mechanisms. Its main area of use is in cases with HER2 receptor positive breast cancer. Trastuzumab developed against the HER2 surface receptor, which is overexpressed in approximately 30% of breast cancer patients, has ADCC or CDC after binding to the receptor, as well as inhibition of HER2 dimerization, reduction of receptor level, induction of p27 protein and formation of new neoplastic vessels. It has an antitumor effect through various mechanisms such as inhibition of Trastuzumab is also being

tested for adjuvant purposes in the treatment of early-stage breast cancer in patients with HER2 overexpression (Alvarez et al., 2011; Cerny et al., 2002). Among the side effects of Trastuzumab, cardiac toxicity is the most important. Especially in anthracycline users, this toxicity rate is much more pronounced. The interaction of Paclitaxel and trastuzumab is interesting. The HER2 gene inhibits the apoptotic effect of paclitaxel by activating a protein called p21. In this case, when the HER2 gene is decreased by down-regulation in patients given Trastuzumab, the development of paclitaxel-induced apoptosis is restored. While cardiotoxicity was 16% in combinations with anthracyclines, this rate was 2% in combinations with Paclitaxel. Therefore, it is suggested that combinations without anthracyclines would be more appropriate (Cerny et al., 2002). It is used alone or in combination with a taxane or an aromatase inhibitor in metastatic breast cancer with certain conditions. It is given by intravenous infusion. It should be applied in places where resuscitation is possible (Kayaalp, 2009).

Pertuzumab; It is the first molecule in the HER2 dimerization inhibitor class. By binding to the dimerizing part of the HER2 receptor and preventing dimerization, it stops HER signaling pathways that mediate cancer cell proliferation and survival (Lambert & Chari, 2014).

There are mAbs called radioimmunoconjugates (Y-ibritumomab tiuxetan and I-Tositumomab, anti-CD20 mAb radionuclide pairs are used in the treatment of lymphoma) (Galluzzi et al., 2012).

Ibritumomab and Tositumomab

They are radioisotope-labeled monoclonal antibodies. They ensure that the radioisotope that will destroy the tumor cells is bound by these cells, that is, the radioisotope is directed to the target. Ibritumomab is an anti-CD20 monoclonal antibody labeled with yttrium 90. Tositumomab is an anti-CD20 monoclonal antibody labeled with iodine 131. It is particularly effective in the treatment of low-grade non-Hodgkin lymphoma (Kayaalp, 2009).

Antibody-Drug Conjugates

Another application of monoclonal antibodies is the use as a drug carrier system in the form of antibody-drug conjugate (ADC). The monoclonal antibody binds to the cancer cell. The antibody-cytotoxic drug three conjugate passes through the intracellular membrane of the tumor cell , whereby the cell death is incorporated. This technology provides a wide therapeutic range by targeting only cancer cells, thus minimizing the potential side effects of the cytotoxic drug (Li et al., 2013; Stefanini et al., 2014, Joo, Visintin & Mor, 2013). On the other hand, there are a number of factors that limit targeted antibody therapy. The large

size of the antibodies, low penetration in the target region, non-specific uptake by the liver and RES (Reticuloendothelial System) are related to these types of molecules. Adult is the most important inconvenience. As a result, toxicity can be seen in the liver and bone marrow with poor penetration to the entire tumor, leading to dose-limiting. These limiting factors may be effective in the use of antibodies for the transport of radionuclide, cytotoxic drugs and toxins to the tumor site. In order to reduce the problems in antibody targeting , peptides are considered as suitable alternatives for tumor targeting. Peptides have advantages such as being small in size compared to antibodies, being chemically stable and suitable for derivatization, and generally not being caught in RES (10).

Ado-Trastuzumab Emtansine

Known as the antibody -drug conjugate, Ado-Trastuzumab Emtansine (T-DM1) binds to two existing cancer drugs to specifically distribute into cancer cells. These are the monoclonal antibody trastuzumab, which targets cells that produce excess HER2 protein, and the chemotherapy drug DM1, which targets microtubules. The binding of the monoclonal antibody trastuzumab with a thioether linker to emtansine (DM1), an extremely potent antimicrobial agent, resulted in an antibody-drug combination that affects HER-2 positive tumor cells. T-DM1 is a drug that has not yet been approved and licensed in our country (Lambert & Chari, 2014).

Inotuzumab Ozogamicin

Inotuzumab Ozogamicin (CMC-544) is a CD-22-targeting immunoconjugate currently being evaluated for phase 3 clinical trials in patients with non-Hodgkin B-cell lymphoma. It shows significant clinical activity in follicular and diffuse large B-cell lymphoma where multiple therapies have failed. Its stability was confirmed by monitoring in both human plasma and serum over a period of 4 days, which included extracting the unconjugated calichDMH from the antibody. It remains partially stable by being hydrolyzed at a rate of 1.5-2% per day in plasma or serum (DiJoseph, Khandke & Dougher, 2008). The most common adverse reactions in patients with relapsed/refractory NHL (non-Hodgkin lymphoma) when used alone are thrombocytopenia, neutropenia, elevated aspartate aminotransferase, leukopenia, nausea, fatigue, decreased appetite, and elevated alkaline phosphatase (Ricart, 2011).

Gemtuzumab Ozogamicin

Gemtuzumab Ozogamicin is an intravenously administered antibody-drug conjugate developed for the treatment of myeloid leukemia. Its composition is a

bacterium Micromonospora echinospora sp. It consists of recombinant humanized IgG4 kappa antibody conjugated with calicheamicin, a cytotoxic antitumor antibiotic obtained by fermentation from Calichensis. The antibody is generated by culture of mammalian cell suspension using a myeloma cell line. It is purified to remove or inactivate viruses. The complement determining regions are derived from the mouse antibody, while the constant region and framework regions have the human sequence. It binds specifically to the CD33 antigen expressed on the surface of leukemic myeloblasts and immature normal myelomonocytic cells. The CD33 antigen is an antigen expressed in more than 80% of patients with acute myeloid leukemia (AML). The effect of Gemtuzumab Ozogamicin is assessed on the overall response rate. There are no controlled studies demonstrating clinical benefit, such as improvement in disease symptoms or prolongation of life span, compared with other treatments (Herter et al., 2013).

Brentuximab Vedotin

Brentuximab Vedotin is an antibody-drug conjugate that targets CD30, a protein expressed on the surface of certain cancer cells, such as Hodgkin's Reed-Sternberg (HRS) cells and anaplastic large cell lymphoma (ALCL) cells. It has a chimeric structure formed by combining a monoclonal antibody called Brentuximab with the antimitotic agent Vedotin. It is targeted to CD30 receptors, which can be found on Hodgkin lymphoma cells as well as other cancer cell types such as anaplastic large cell lymphoma. It is thought to show its anticancer activity by the following mechanisms:

i. The antibody-drug conjugate binds to cells expressing CD30.
ii. The antibody-drug conjugate CD30 complex is internalized and transported to lysosomes.
iii. The microtubule disrupting agent monomethyl auristatin E (MMAE) is separated by selective proteolysis.
iv. It binds to the MMAE tubulin. It stops the cell cycle by disrupting the microtubule network inside the cell.
v. It induces apoptotic death of cells.

Peripheral neuropathy, weakness, nausea, neutropenia, diarrhea and fever are common side effects of the drug. It is used to treat patients with Hodgkin's disease who are deemed ineligible for transplantation after autologous hematopoietic stem cell transplantation has failed or after the failure of at least two combined chemotherapy regimens. It is also used in the treatment of patients with systemic anaplastic large cell lymphoma after failure of at least one combination chemotherapy regimen (Herter et al., 2013).

DISCUSSION AND CONCLUSION

Studies of monoclonal antibodies/antibody-drug conjugates and efficacy and safety studies for those who receive approval after phase studies have been conducted for years. A few studies are shown here as examples.

Emile G. et al., in their study published in 2013, aimed to report the efficacy and safety of using bevacizumab as a single agent in recurrent ovarian cancer. For Bevacizumab to be given, patients must have had a disease recurrence after at least the previous stage of chemotherapy . They stipulated that the patient should have a condition associated with gastrointestinal perforation and that the patients did not have a condition associated with gastrointestinal perforation. 37 previously treated patients (33 patients with platinum-resistant disease) were included in this study and they applied the doses as 2.5 mg per kilogram per week. The most common side effect was hypertension. This study, Emile G. et al. had previously been treated with bevacizumab as a single agent with an appropriate therapeutic index and it is a reasonable option for patients with ovarian cancer who do not wanting to experience the side effects of chemotherapy such as intestinal perforation (Emile et al., 2013).

The overall response rate to Gemtuzumab Ozogamicin is 30% in CD33-positive AML patients who experience the first relapse of the disease. The overall response rate is expressed as complete remission (16%) and the secondary response category (13%). The reason for adding a secondary category is that it delays the healing of platelets. A phase IV randomized trial is considered, as Gemtuzumab Ozogamicin response rates become comparable to standard therapy only when the secondary response category is considered. In this study, daunorubicin and cytarabine, which are standard AML treatments, will be administered together with Gemtuzumab Ozogamicin and compared in terms of benefit in patients who only received standard treatment. In addition , phase I studies are considered to evaluate the toxicity risk and significant drug-drug interactions of this combination therapy.

In clinical trials of Hodgkin lymphoma, the response rate to Brentuximab vedotin was 75%. Tumor shrinkage was noted in 96% of patients. Complete remission was reached in 34% of the patients. In some studies, on ALCL, the response rate after treatment was reported as 86% and the rate of complete remission as approximately 54% (Herter et al., 2013).

As a result, the development of hybridoma and recombinant DNA technologies in the last quarter of the twentieth century allowed monoclonal antibodies to be used first in in-vitro and ex-vivo for diagnostic purposes, and then in clinical use for therapeutic purposes. With the beginning of a better understanding of tumor immunology, monoclonal antibodies and antibody-drug

conjugates, which have been developed in the last 20 years, have been approved and have become a part of standard practices in the treatment of various cancer types. Monoclonal antibodies allow more effective treatment by targeting only cancer cells and minimizing the potential side effects of cytotoxic drugs. In some cases where chemotherapy support is required, treatment addition of monoclonal antibodies for cancer therapy is promising.

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Chapter 14

Animal Welfare and Ecological Sustainability in the Perspective of Food Ethics

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ABSTRACT

With this study, it was aimed to deal with animal welfare and ecological sustainability, which are important factors in consumption preferences, from the perspective of food ethics. When the concepts and approaches related to food ethics are evaluated, important interrelated topics such as consumer concerns, conscious food selection, ethical traceability, sustainability, animal welfare, and integrity emerge. Problems such as the increase in deaths during transportation, deformations in carcasses, losses in live weight and meat quality, which animals especially raised for consumption are faced with due to ignoring animal welfare standards, reveal the importance of animal welfare and sustainability. The growth of the world population, increasing household income and urbanization increase the global demand for protein sources and cause the dietary habits of consumers to change. The increasing importance of animal welfare and the concerns about the treatment of farm animals, especially in developed countries, cause individuals to question their consumption preferences and lay the groundwork for more sensitive approaches. Individuals with high ethical sensitivity turn to alternative foodstuffs labeled with the concepts of "*vegetarian*", "*ecological*", "*fair trade*", "*free-range*", and "*sustainability*" with health, safety, animal welfare, animal rights, ecological, religious, and socio-political concerns. As a result, food ethics, animal welfare, and ecological sustainability are "*universal*" concepts, and these concepts have their own ethical values. To solve the problems of animal welfare and sustainability, which are considered within the scope of food ethics, it is also important to reveal attitudes and behaviors suitable for the ethical decision-making process while reaching the solution to practical problems.

Keywords: Animal welfare, consumption preferences, ecological sustainability, food ethics.

INTRODUCTION

Animal products, which are among the most valuable nutrients in terms of nutritional components, have great importance due to their contribution to human health (Altun et al., 2004:11). For this reason, food choices are important in terms of health because they lead to better environmental and social outcomes (Thompson, 2015:192).

There are strong connections between food choice and the concepts of food ethics and ecological sustainability. Food ethics means researching, finding, and systematizing values that will be accepted as correct until the consumption stages in food science and management, and presenting them to the service of consumers in a way that will not cause health problems (Vural, 2015:196). It can also be considered as an ethical area to make better food choices for many people (Thompson, 2015:5) and to protect the interests of all sensitive creatures (Çelik and Yaşar, 2021:134).

The advances in science and technology, along with the Age of Enlightenment in Western civilization, have been influential in the reorganization of the economic and social structuring brought about by industrial production, along with traditional production being replaced by industrial production. The modernism movement, which developed in line with industrialization, has moved the new social structure and habits away from the traditional (Kocatepe and Tırıl, 2015:56). As human intervention in the environment increased, imbalances occurred in the relationship between humans and nature, and all living things continued to be adversely affected by this situation. In addition, the rapid increase in the world population makes it difficult to obtain safe food. Safe food is food that has not lost its nutritional value and is free from physical, chemical, and biological hazards (Erkmen, 2010:221; Erciş and Türk, 2016:6).

The subject of food ethics overlaps with other ethical and applied philosophy systems. Where food production, environment, or wilderness are viewed as human destruction, viable food production ethics are unlikely (which is the view of most environmentalists), while an independent discipline of food ethics is viable if food production is recognized as an important human-nature relationship. Therefore, there is a relationship that needs to be carefully managed between food ethics and environmental ethics. When the history of food ethics is evaluated, certain interrelated concepts and approaches such as consumer concerns, conscious food selection, ethical traceability, sustainability, animal welfare, and integrity emerge (Korthals, 2015:242).

Relatively, the concept of animal welfare; changed from a happy, healthy life to everything needed to keep animals at the highest level of productivity

needed for the success of traditional production. With technology, animal productivity has been sharply separated from animal happiness (Rollin, 1994:7). The shift to industrialized production had multiple effects, such as ignoring animal welfare, endangered meat quality, the inability of small farms to compete in markets, and as a result, endangered the sustainability of traditional knowledge and agricultural diversity (Bühler and Hamilton, 2007:61).

According to Frankic and Hershner (2003:522) quoted from FAO, sustainability is the direction of technological and institutional change to ensure that the needs of current and future generations are constantly met by managing and protecting the natural resource base. This type of sustainable development is technically feasible, economically viable, and socially acceptable while conserving soil, water, plant, and animal resources without causing environmental degradation. Sustainability includes meeting the current needs of future generations, predicting global population growth, and forcing current investments to produce enough food to feed more individuals (Thompson, 2015:163).

Ecological sustainability is the ability of one or more assets to exist and develop collectively or individually over long periods of time (Starik and Rands, 1995:909). Altieri (2009:102) describes sustainable production as an intersection of "*economic*", "*ecological*" and "*social*" aspects.

With this study, it was aimed to deal with animal welfare and ecological sustainability, which are important factors in consumption preferences, from the perspective of food ethics.

1. Animal Welfare and Ecological Sustainability

In a broad sense, "*Animal welfare is the providing health, happiness, and well-being free from ache, pain, and suffering during the care, feeding, housing, breeding, transportation, slaughter, treatment or use in scientific research of farm, pet, companion, exotic, laboratory and wild animals.*" (Yaşar, 2005a:37; 2005b:53; 2017:7). An animal's well-being refers to its physical and mental state, while good animal welfare refers to both a sense of health and well-being. It is one of the important issues that any animal raised by humans should at least be protected from unnecessary suffering. An animal's well-being, whether on the farm, transport, market, or slaughterhouse, needs to be considered as the "*five freedoms*". These freedoms define ideal situations for animals rather than acceptable welfare standards. These are freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury, or illness, freedom from an expression of normal behavior, and freedom from fear and distress (Rollin, 2013:11).

While philosophical theories guide us on our journey to find the right action, there are various ethical frameworks that can help us ask important questions about ethical dilemmas. While a combination of this philosophical approach and ethical frameworks must be used in practice, it is of great importance to question who should apply these ideas and who should decide which actions are ultimately good, bad, acceptable, or unacceptable (Morton, 2004:245).

In recent years, due to the increasing importance of animal health in breeding systems around the world, certain standards have been developed within the scope of the legislation to protect not only animal health but also welfare. The increase in deaths during the transport of animals in import and export, deformations in carcasses, and losses in live weight and meat quality which are problems that occur due to ignoring animal welfare standards, have revealed the importance of animal welfare and sustainability (Karslıoğlu Kara and Koyuncu, 2011:516). DeGrazia draws attention to the importance of animal welfare by stating that every reasonable effort should be made not to provide financial support to institutions that cause extensive and unnecessary harm (DeGrazia, 2002:71).

Global demand for animal products is expected to increase through 2050, based on forecasts for world population growth, increased incomes, and further urbanization (Scholten et al., 2013:3). The increase in living standards and the continuous development in the global economy are causing the depletion of resources and undermining ecological sustainability (Zhang et al., 2022:1). Biotechnological applications are preferred in many cases such as environmental and sudden climate changes, combating pests, improving the quality of animal products, and developing animal products according to demand (Kurar et al., 2013:166). According to Khazaal and Almiron (2016:388), the perception of new technologies and production systems that "*animals are happy, and food is healthy*" should not be just a cover art and camouflaged speciesism. It is important to reveal the intrinsic value of these creatures, which serve humanity, rather than their instrumental value, in terms of not serving speciesism, so the media has great duties.

Foods developed with biotechnology have to be safe, but there is disagreement about what food safety means and whether the use of biotechnology poses an unacceptable risk. Although there is technical and political disagreement about acceptable levels of risk in many areas, the standard in food safety is accepted as the lowest possible level of risk (Thompson, 2007:91). Genetically modified organisms (GMOs), obtained through genetic engineering, are among the most emphasized topics among biotechnological applications (Çelik and Turgut Balık, 2007:13). Organisms

produced and modified by genetic engineering techniques are defined in the literature with names such as GMOs, genetically modified products (GMPs), and bio-engineering organisms (Uzogara, 2000:180).

In the process ranging from classical biotechnology to modern biotechnological methods, these technologies continue to be used at different levels according to the development level of the countries, with the increasing level of research costs and complexity (Çetiner, 2005:3). The subject of genetic engineering, which allows changing the genetic structure of organisms, is still a topic of discussion on scientific platforms. While the rapidly advancing gene technology continues to exist in our daily lives in many different areas, it has also brought some positive and negative opinions (Pamuk, 2010:92).

There are positive opinions that biotechnology will increase plant and animal productivity, facilitate food supply by reducing cost, increase the nutritional value of foods, solve the problem of hunger and malnutrition, improve the shelf life and organoleptic quality of foods, be used for the treatment of diseases and organ transplantation, and have many ecological benefits. Negative views on this subject are the changes that may occur in the quality of food, the safety of the obtained food, the increase in allergic reactions, the toxic effects of the products, the development of microorganisms resistant to antibiotics in a short time, the decrease in ecological genetic diversity, the increase in foreign dependency in terms of economy, and therefore small-scale producers may suffer from this situation, and the increasing problems related to the issue in cultural and ethical (Çelik and Turgut Balık, 2007:21; Pamuk, 2010:92). In terms of its disadvantages and ethical dimension, GMP technology opponents use the term "*eco-terrorist*" for those who use this technology (Walters, 2010:110).

Today, it is reported that GMOs, which are used for high-yield purposes, especially in the agricultural field, carry a risk in terms of human health and natural resources in the long term, although they provide an increase in yield in the short term (Vural, 2015:194). When the risk perceptions and ethical beliefs of consumers towards GMP groups are evaluated, it is reported that age is a more effective variable than gender (Özgen et al., 2013:218).

Although transgene technology and transgenic animals have important and critical importance in applications, it also brings with them a number of problems and concerns such as ethical, ecological, economic, and welfare. Transgenic studies are given importance, especially in order to meet the increasing food demand in developing countries (Kurar et al., 2013:168). Considering the increasing population and consumption habits, the data that classical breeding practices will not be sufficient in the future brings the

necessity of GMOs to the agenda. Providing a product variety with high adaptation to stress, toxicity, and bacterial and viral factors created by harsh environmental conditions requires a long process, more expense, and labor (Hakkı et al., 2013:174). Evaluation processes for the presentation of products obtained from transgenic animals for human consumption are still ongoing (Kurar et al., 2013:168).

Ecological justice focuses on the relationship or interaction between humans and the natural environment. When people improve the environment for material needs (housing, agriculture, business, consumption), ecological justice emphasizes the evaluation of such actions in the context of damage or harm to other living things. This situation is often referred to as an ecocentric understanding of human and nature interaction (Walters, 2010:114).

Today, research on different production systems continues. In particular, studies with cloned animals were emphasized and possible disadvantages were revealed (Carter et al., 2002:131; Duram, 2010:85; Sinha et al., 2019:132). It has been reported that the narrowing in the genetic diversity of animals specific to the cloning process causes them to be more susceptible to opportunistic diseases, parasites, and changing environmental conditions, can act as vectors for the transmission of pathogens to humans and animals or compromise their immune systems, with an increased health risk compared to non-cloned animals. It has been determined that clone-induced pregnancies cause an increase in the risk of late pregnancy complications in cows and sheep used as surrogate mothers, and an increased risk of mortality and morbidity in young calf and lamb clones compared to calves and lambs produced without cloning and also, in cattle and sheep, the increased risk has been primarily associated with large calf syndrome (Duram, 2010:85).

Based on a review of relevant information on the health of animal clones, the composition of meat and milk from these animals, and the clone lineage, no hazards have been identified that could indicate food consumption risks of edible products from healthy clones of healthy adult and young cattle, pigs or goats compared to products from the conventional system. Other than relying on underlying biological assumptions and inferences from other species, there is insufficient information on the health status of sheep clones to draw conclusions about potential risks from the consumption of food products. Edible products derived from the clone line are reported to pose no additional food consumption risk compared to corresponding products from other animals. It is stated that edible products from healthy clones that meet current requirements for meat and milk in the trade will not cause an increased risk of food consumption compared to comparable products from normally derived animals (FDA, 2008:15). While

providing information on the safety and animal health of products derived from cloned animals, economic, environmental, social, and ethical issues related to this technology are not adequately addressed (Riddle, 2008:118).

Another issue that jeopardizes animal welfare and ecological sustainability is antibiotics. In animals consumed for food purposes, antibiotics are mostly preferred in the treatment of respiratory and enteric infections in intensively fed animal groups, especially in the early life of animals (broilers, weaning pigs, and calves), in the treatment of infections caused by various bacterial pathogens, and in the treatment of mastitis infections, which are especially common in cows with high milk yield (Addis, 2015:32).

Dairy cows are among the animal groups whose welfare is adversely affected due to intensive production. Welfare problems in dairy cows are generally caused by leg diseases, reproductive disorders, mastitis, and behavioral restriction, which is less important than these diseases, leg discomfort at a rate of 40% every year in high production herds, and lameness (Broom and Corke, 2002:134) and also deterioration in fertility due to high milk yield (Pryce et al., 1997:353). The data obtained during the deterioration of the condition of the animals due to the disease and the recovery after treatment are a clear quantitative indicator of the welfare of the animals (Broom and Corke, 2002:135).

Broilers, on the other hand, are the most intensively produced poultry group, and at the same time, global welfare inspections are possible because they are the most standardized form of animal production in terms of genotypes, feeding, housing, and management. Many retailers and restaurant chains develop or enforce global standards that include animal welfare as well as quality issues to be audited. There are numerous challenges in meeting cultural and religious beliefs (such as halal, and kosher) and the inevitable differences of opinion, not only in what constitutes well-being but also in how to measure them. Monitoring of broiler welfare is in progress as it is a developing area and rationalization is expected as soon as possible. The need for potential inspectors and controllers for broiler units, threats to biosecurity, time required, and related management are among the practical problems in this sector (Weeks, 2004:13).

Despite many improvements in animal welfare in the last 50 years, particularly in poultry production, including selective breeding, controlled environments (lighting, temperature, and humidity), and the supplementation of feeds containing vitamins and minerals, many healthy animals are unnecessarily frequently exposed to antibiotics (Graham et al., 2007:79; Abdallah et al., 2022:6379). This situation creates favorable conditions for the emergence, development, spread, and continuity of antibiotic-resistant bacteria that cause

infections in animals and humans. In general, resistant zoonotic bacteria carried by farm animals cause contamination, creating a public health concern (Addis, 2015:37). Demir et al., (2005:114) reported that it is possible to use herbal natural feed additives as an alternative to growth-promoting antibiotics in broiler production. While these natural feed additives meet the producers' expectations for higher broiler performance, they are also on the agenda as strong alternatives due to the consumers' demand for broiler production to be done under environmentally friendly conditions.

One of the animal production industry's greatest achievements in recent years has been the transformation of chicken from a luxury food into a common and inexpensive source of meat. This success has been achieved through a better understanding of the genetic background and nutrition necessary for birds to grow rapidly and have a good feed conversion ratio (Broom, 2001:61; Broom and Corke, 2002:134). The average market weight of broilers increased by approximately 50%, and the time required to reach market weight, and the amount of feed required to produce one pound of meat was reduced by approximately 35% (Graham et al., 2007:80). With the birds maturing to a weight of two kg in ten weeks in the 1960s, reaching this maturity in five weeks in the 2020s (Broom, 2001:67; Broom and Corke, 2002:134), the disability and lameness caused by rapid growth forced the producers to kill another 1-2% of these animals (Singer, 1995:125).

Very rapid growth can cause problems associated with cardiovascular disorders leading to ascites in birds, leg diseases such as tibial dyschondroplasia, femoral head necrosis, and valgus-varus syndrome resulting in reduced walking ability, chest blisters, and knee burns caused by decreased ability to walk or stand (Broom and Corke, 2002:134), lameness, chronic arthritis in 4% (32 million), bone fractures in 3% (24 million), other injuries in 30%, and cardiorespiratory problems in 1%. In addition, 6% (48 million) of animals lose their lives during breeding and two million animals during transportation every year (Morton, 2004:246).

Cannibalism, which is seen as a result of the stress caused by overcrowded cages, suffocation due to crowding in panic, unsanitary conditions due to beak trimming, and insufficient ventilation are examples of poor welfare conditions that animals are exposed to. In factory farming in the USA, 100 million mammals and 5 billion poultry die annually (DeGrazia, 2002:90). In the beak-cutting process applied to 15 chicks per minute, the temperature and sharpness of the blade used are not always at the same level and sloppy cuts cause serious injuries (Singer, 1995:101). The use of an extremely hot knife causes blisters in the mouth, and the use of a cold or blunt knife causes a tender lump of flesh at

the tip of the beak (Thornberry et al., 1974:634). The Brambell report, published by a panel of experts affiliated with the British government, states that "*between the hairs on the beak and the bone is a thin and very sensitive tissue similar to living tissue where the human fingernail meets the flesh. The heated blade used in the beak cutting process completely cuts the whole consisting of hair, bone, and sensitive tissue, causing severe pain*" (Brambell, 1965:97).

One of Thompson's reasonable assumptions is that breeding to increase rapid growth in broilers reduces aggression and dominance-related traits by simultaneously increasing "*juvenile*" traits. With breeding, broilers tend to show timid behavior while growing rapidly. A different perspective is brought to the fore with the following questions (Thompson, 2015:274): "*Does the hypothesis that the pecking problem can thus be eliminated in a production system in which a herd of one hundred thousand or more broilers is kept without cages justifies this situation?*" or "*If we can solve the pecking problem by genetically modifying the bird, would we be harming this species because we changed the genetic basis for this species-specific behavior?*".

2. Ethics, Welfare, and Ecological Sustainability in Consumption Preferences

Ethical consumers consider various ethical issues in their consumer behavior choices. The increase in consumption levels accompanying our consumer-oriented culture causes some consumers to question their individual consumption preferences, and most of them prefer consumption simplicity (Shaw and Newholm, 2002:167). In recent years, it has been stated that many consumers have acquired an ethical taste, consumers have changed, and they are now more interested in green, ethical, and charitable situations, and attention is drawn to the issue of food ethics (Newholm et al., 2015:291).

Moral concern about food consumption is as old as morality itself (Zwart, 2000:113). Therefore, food ethics issues are not a new issue. Many religions include ethical values in their food laws so that consumer groups can make food choices based on certain ethical principles (Early, 2002:340). Individuals with high ethical sensitivity tend to purchase alternative foods labeled with concepts such as "*vegetarian*", "*ecological*", "*fair trade*", "*free-range*" and "*sustainable farming*" and to associate such practices with food ethics to a large extent. Not only safety and health but also animal welfare and environmental concerns are among the targeted ethical ideals (Korthals, 2001:202; Thompson, 2016:201).

Food choice itself may not be an ethical concept, but many ethical issues are linked to this concept (Korthals, 2015:241). Food consumption is associated

with various environmental effects, and consumers' food preferences therefore involve important environmental decisions (Tobler et al., 2011:674). It is stated that ethical concerns and environmental concerns about animal welfare provide high motivation for vegetarians based on emotional and philosophical reasons (Fox and Ward, 2008:427). It has been reported that young vegans prefer a vegan diet due to ethical concerns about animal welfare and rights rather than health reasons (Larsson and Johansson, 2005:1438; Izmirli and Phillips, 2011:436). It has been revealed that taste and environmental reasons arouse the desire of consumers to consume seasonal fruits and vegetables, health and ethical reasons provide a basis for reducing meat consumption, and individuals (especially women) who prefer natural foods are more willing to adopt ecological food consumption habits (Tobler et al., 2011:678).

Especially when the bad conditions of many layer hens are taken into consideration, veganism emerges as a lifestyle accepted by individuals despite all its difficulties. Considering options such as behaviors, values, perspectives, or lifestyles, it can be concluded that vegetarian impulses are actually complex (Fox and Ward, 2008:428). While vegetarianism today constitutes an important consumer group for some reasons (ethical, environmental, socio-political, and religious) (Izmirli and Phillips, 2011:436), vegetarianism based on animal welfare considerations is a controversial argument (Detmer, 2014:1784). Vegan/vegetarian preference is also directly related to individuals' perspectives on life, living things, and nature (Leitzmann, 2014:499; Tunçay Son and Bulut, 2016:831).

Morton (2004:248) states that "*first of all if we stop eating chicken all at once, the suffering of animals will undoubtedly decrease in a short time*". Not eating chicken also raises the question of whether people should become vegetarian or simply consume more animal welfare-friendly meat products. He claims that we do not need to eat meat to survive, and that eating meat is an action performed because it increases the pleasure of life. In this case, "*Does the human benefit outweigh the harm done to the animals?*" is one of the important questions/problems brought up by Morton (2004:248). Is it possible to compare the subjective experiences of human and non-human animals in this context? Is it more important to base our environmentalism on our concern for future generations, our duties to other sentient beings, or a broader understanding of the intrinsic value found in natural ecosystems? An alternative ethical argument being questioned in this regard is the way different food production systems affect animal suffering, biodiversity, climate change, or ecological sustainability (Thompson, 2015:38).

While the global population is estimated to be about 9-10 billion by 2050, it is predicted that the meat industry will need to increase its production capacity by ~50-73%. There are several different options (selective breeding, eco-agricultural systems, animal cloning, and genetic modification) available in the meat industry that have the potential to meet this demand and increase production. While some of these options require advanced technologies, many are considered "*artificial*" by different consumer groups (Bonny et al., 2017:2216). By emphasizing the benefits of artificial meat for animals and society, it is reported that consumer preferences can be increased by activating the sense of compassion in individuals (Septianto et al., 2023:7).

Due to meeting the increasing demands for protein and increasing competition in other sectors, new technologies have started to be adopted in the meat industry according to consumer demands and the changing market. Animal welfare is becoming increasingly important and consumers, especially in developed countries, are concerned about the treatment of farm animals. In this context, it is predicted that artificial meat (in-vitro meat) production has the capacity to greatly reduce the number of animals required to meet the global demand for meat, thus reducing the number of farm animals and improving animal welfare (Bonny et al., 2017:2219; Allahverdiyev, 2023:2). It is also reported that artificial meat has the potential to prevent animal consumption, to be an alternative for ecological sustainability by reducing the carbon footprint caused by meat production, and to meet all nutritional needs and demands of consumers and citizens (Hocquette et al., 2015:283).

As a result of the rapidly growing population and the migration from rural to urban centers, innovations in nutrition science, and interest in animal products, it is tried to meet the demands by increasing production with classical breeding programs and biotechnology studies. However, intensive breeding in order to meet the increasing demand causes an environment where animals stay away from their natural environments and their wastes cause environmental pollution. In breeding, animal and human health are faced with serious dangers by the application of substances such as feed additives, hormones, antibiotics, and insecticides to the animal, or the indirect passing of substances such as agricultural pesticides into the metabolism of the animal. Pesticides taken with non-ecological feeds in intensive animal production are transferred to new generations as a result of accumulation in the adipose tissue and may cause serious diseases such as cancer (Aksakal et al., 2010:73; Wolfart et al., 2023:11).

Heavy metals such as arsenic and mercury accumulating in the body of animals during intensive production periods threaten human life by causing

serious conditions such as allergies, genotoxicity, and poisoning. Intensive and uncontrolled animal production leads to negative situations for the environment, human, and animal welfare, and health (Aksakal et al., 2010:73). These negative situations bring the return to the organic system and the development of sustainability in aquaculture systems. Organic farming has an important potential for animal welfare, elimination of possible damage to the environment, and the continuity of rural life (Koyuncu et al., 2010:84).

Consumers also want to know ethically the rearing status of animals produced from farm to fork. Negative nutritional issues such as food poisoning, animal diseases, or lack of animal welfare are common situations in our daily lives. Although all these problems are evaluated within the scope of "*consumer concerns*", they also contain important topics such as animal welfare, health, sustainability, and fair trade, which are related to consumer concerns (Korthals, 2004:4).

In the EU, including Sweden, organic farming is seen as a promising alternative for sustainable production, human health, animal welfare, and protecting the environment (Basnet et al., 2023:501). Since organic animal production is basically based on the close relationship between the farm and the soil, it restricts the existence of potential hazards such as synthetic and chemical agents, growth-promoting factors, and the use of GMOs as feed additives. For this reason, people in many parts of the world are more in demand for organic products and may prefer to pay higher prices for these products in order to consume quality and reliable food (Atasever et al., 2010:378). Organic and ecological animal production and the behavior of animals in accordance with their ethology creates an inevitable change on production systems for sustainability and animal welfare. For this reason, the concepts of ethology and ecology within the scope of 3E/4E theory (ethics, ecology, ethology, and economy) are very important (Yiğit and Yaşar, 2008:503; Yaşar, 2020:21).

Although the expression of ecological agriculture is mostly used in the field of plant production, since animal products such as meat, milk, and eggs have a great contribution to the development of mind and body, demand has arisen for animal products as well as for plant products in developed countries, and the organic farming process has begun in animal husbandry (Yeşilbağ, 2004:160). Ecological livestock farming is a mode of production in which animal welfare is considered ethically as well as ecological balance and health criteria are prioritized in product quality rather than product quantity. In industrial (intensive) production, conditions such as the excess number of animals in the shelter due to intensive production, the density of settlement, insufficient movement area, insufficient labor, careless care, and agricultural pesticide

residues increase the level of stress hormones and therefore cause deterioration in the immune system of animals. The use of feed additives such as hormones and antibiotics in animal nutrition leaves residues in animal products and causes significant health problems in people consuming these products. Animal welfare is gaining increasing social importance due to the interest in animal rights in developed countries. For all these reasons, ecological animal husbandry has recently been suggested as an alternative to intensive production (Yeşilbağ, 2004:161).

The reason why poultry products are in great demand among the products that have differentiated recently is that they are suitable for intensive production, their price is cheaper than other products and they are frequently consumed products. With the increase in the level of consciousness of consumers, products that are harmless to human health and environmentally friendly, and suitable for ecological or organic production models are preferred. The demand for animal products produced with such models is also of great importance for the welfare of animals and the future of animal products. Consumers may prefer to pay more for ecological (Armağan and Özdoğan, 2005:20) and sustainable labeled products compared to normal products (Sigurdsson et al., 2022:1107; Osawe et al., 2023:9).

CONCLUSION

Modern biotechnological methods have the potential to adversely affect animal welfare and health, and ecological sustainability, and cause public health concerns compared to conventional production. The increase in consumption concerns of individuals with high ethical sensitivity may result in individuals preferring a diet that does not include animal products in order to reduce their carbon footprint and eliminate possible harm to animals. The fact that animal products, which are in a very important position in nutrition, are removed from the diet of individuals with ethical concerns, has a great potential to cause a major blow to the economy and sustainability of the livestock sector. Nutrition with high nutritious and safe foods is an ideal level of welfare that every society should achieve. The production and sustainability of such foods are possible by considering consumer concerns and ensuring animal welfare.

It has been revealed by researches that consumers want to know the breeding conditions of the animals produced and to consume animals raised in accordance with animal welfare standards. It is very important to support producers and supervise them within the framework of legislation in order to meet the increase in consumer demands for products obtained from healthy, happy, free animals with organic, ecological, and sustainable methods. While

the safety assessment of biotechnology-produced foods raises new challenges, it is necessary to apply the concepts of hazard critical control point and risk analysis to the food supply chain in order to ensure a safe food supply, and to actively enforce the relevant legislation to ensure the realization of education, monitoring, follow-up, and surveillance.

As a result, food ethics, animal welfare, and ecological sustainability are "*universal*" concepts, and these concepts have their own ethical values. In order to solve the problems of animal welfare and sustainability, which are considered within the scope of food ethics, it is one of the most important steps to reveal the attitudes and behaviors suitable for the ethical decision-making process while reaching the solution of the practical problems. It is obvious that there is a need for training to inform consumers about all kinds of food-borne risks.

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Chapter 15

Fracture and Dislocation of Proximal Interphalangeal Joint

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ABSTRACT

Proximal interphalangeal joint (PIPJ) injuries, involving fractures and dislocations, present significant challenges due to their complex nature and potential for disability, especially in individuals requiring fine motor skills. These injuries, often overlooked initially due to swelling, range from purely ligamentous dislocations to those with accompanying fractures.

The extent of these injuries necessitates a broad spectrum of treatments, from conservative to surgical, aimed at restoring joint stability, congruity, and facilitating early motion. Extension block pinning, closed reduction-K wire, volar plate arthroplasty are typically employed for less severe cases, resulting in promising outcomes. Conversely, severe injuries require interventions like hemihamate arthroplasty, dynamic external fixation, and open reduction internal fixation, although these lead to lower postoperative PIPJ range of motion.

Osteoarthritis post-surgery remains a frequent complication, underscoring the need for continued research to refine treatment strategies and manage complications, enhancing patient outcomes.

Keywords: Proximal Interphalangeal Joint, Fractures, Dislocations, Treatment, Postoperative Complications.

INTRODUCTION

Proximal interphalangeal joint (PIPJ) fractures and dislocations are seen as challenging, unforgiving injuries. Purely ligamentous PIP joint dislocations can be observed, or those accompanied by fractures(Hammert et al., 2012a). The development of pain, post-traumatic arthritis, swelling, and stiffness may occur despite being treated carefully and appropriately. Disability in athletes, musicians, and those whose work necessitates fine motor activity can be caused by these conditions. PIP joint injuries are commonly encountered but due to swelling, the possibility of them being overlooked by the initial examiner exists(Bentley, 2014).

Epidemiology

There is limited demographic information on PIP joint fractures and dislocations. In the USA, finger fractures occur at a rate of 67.9 per 100,000 and finger dislocations at a rate of 11.3 per 100,000(Ootes et al., 2012). Another study indicated that finger dislocations occur at a rate of 11.1 per 100,000. Most commonly, 38.6% of these injuries are seen in the 15-19 age range and 78.8% are male. The majority, 35.9%, occur during sporting or recreational activities(Golan et al., 2016). In another study conducted in Taiwan, the rate of finger dislocations was found to be 4.6 per 100,000(Yang et al., 2011).

Anatomy

The PIPJ is a synovial joint, make flexion up to 110 degrees as a hinge type joint. The proximal part of the PIP joint is formed by two concentric symmetrical condyles and is divided into two by the intercondylar sulcus. The size of the radial condyle is longer in 2nd & 3rd fingers, equal to the ulnar condyle in the 4th finger, and shorter than the ulnar condyle in the 5th finger (Hammert et al., 2012b). This condition forms the rotational movement of the fingers, causing the fingertips to converge when the fingers are flexed.

The distal part of the PIP joint, which is biconcave in shape, is formed by the base of the middle phalanx. The volar surface is important for joint stability and consists of two thickened corners and lateral tubercles.

The volar surface of the PIP joint is formed by the volar plate (Kamnerdnakta et al., 2018). The volar plate originates from the P1 with the checkrein ligaments and also from C1 pulley & A2 pulley, attaching its thick lateral fibers to corners of P2. Its thin central fibers attach to volar part.

There are proper collateral and accessory collateral ligaments in the PIP joint (Pang & Yao, 2018). The these ligaments originate from the lateral part of P1 and attach to the volar and lateral part of the P2. The principle task of the proper

collateral ligament is to provide stability in lateral plane. The purpose of the accessory ligament is more limited and it ensures the fit between the volar plate and the surfaces.

The volar plate and collateral ligaments form a box, ensure stability to PIPJ (Figure1). For a dislocation to occur, these structures need to be compromised in at least two planes.

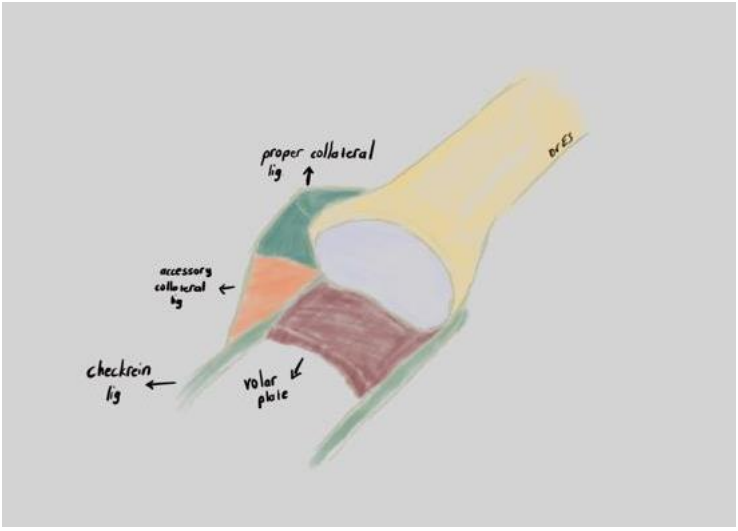


Figure 1. PIP joint anatomy

FRACTURE TYPE AND MECHANISM OF INJURY

Dislocations of the PIP joint can occur either with or without a fracture, and can be dislocated dorsally, volarly, or laterally (Figure 2).

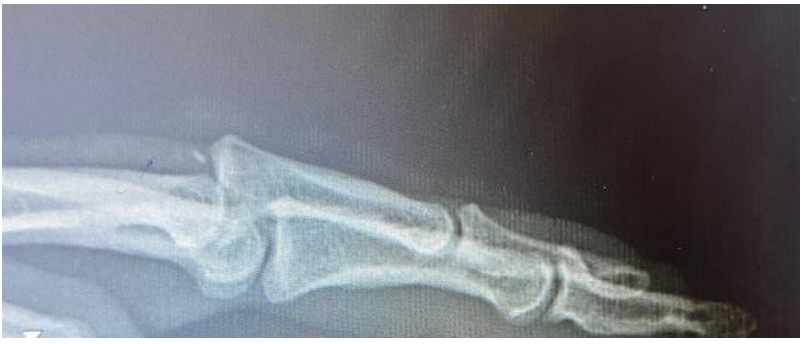


Figure 2. Dorsal fracture dislocation PIP joint

Dorsal dislocations

Dorsal dislocations occur due to hyperextension and axial loading. Dorsal dislocations are the most common type and there are several classification systems for them. However, the Eaton classification is the most frequently used. Injuries to the volar plate are based on the Eaton classification: Type I is a hyperextension injury, Type II is a dorsal dislocation, and Type III is a fracture dislocation.

Type I involves partial or complete detachment of the volar plate. In Type II, the joint surfaces do not make contact and there is detachment of the volar plate along with splitting of bilateral collateral ligaments. In Type III, there is an avulsed bone fragment in the middle phalanx.

Along with the Eaton classification, the Keifhaber-Stern classification is one of the most frequently used today. According to this classification, injuries from volar plate avulsions are divided into three: stable avulsion fractures, tenuous avulsion fractures, and unstable avulsion fractures. In stable avulsion fractures, there is a fracture on < 30% of the joint surface of the P2 . In tenuous avulsion fractures, this rate is between 30-50%. In unstable fractures, this rate is over 50%.

Volar dislocations

Volar dislocations are rarely occuring injuries. These injuries can be overlooked, often resulting in late-stage deformities. These injuries usually involve a central slip lesion. It can easily be missed, leading to a boutonniere deformity in the late period. These injuries are divided into three: simple volar dislocation, volar fracture dislocation, and volar rotary dislocation.

In volar dislocation, there is damage to the bilateral collateral ligaments, volar plate, and usually a central slip injury. In volar fracture dislocation, there is a fracture fragment, and in volar rotary dislocation, the head of the P1 has entered among the central slip & the lateral band.

Lateral dislocations

Lateral dislocations are rare injuries involving damage to the collateral ligament and the volar plate.

DIAGNOSIS

The diagnosis is initiated with detailed history of the patient and an understanding of the trauma mechanism. Whether there was a subluxation or dislocation following the trauma, and whether a reduction was conducted post-injury, is investigated. The occurrence time of the event is ascertained. On examination, the presence of swelling, redness, lacerations, bruising, and open

wounds is looked for. By palpation, the exact location of the pain and the area of greatest sensitivity are identified. The neurovascular status is recorded.

Anteroposterior (AP) and true lateral radiographs of the fingers are obtained. On the lateral radiograph, the superimposition of the condyles is considered important. Oblique radiographs in partial pronation and supination may also be acquired. On the AP radiograph, the existence of an impaction fracture is checked. On the lateral radiograph, the colinearity of the proximal and middle phalanxes is expected. Malalignment is interpreted as a sign of subluxation. Additionally, the existence of a dorsal "V" sign on lateral radiograph is looked for (Figure 3).

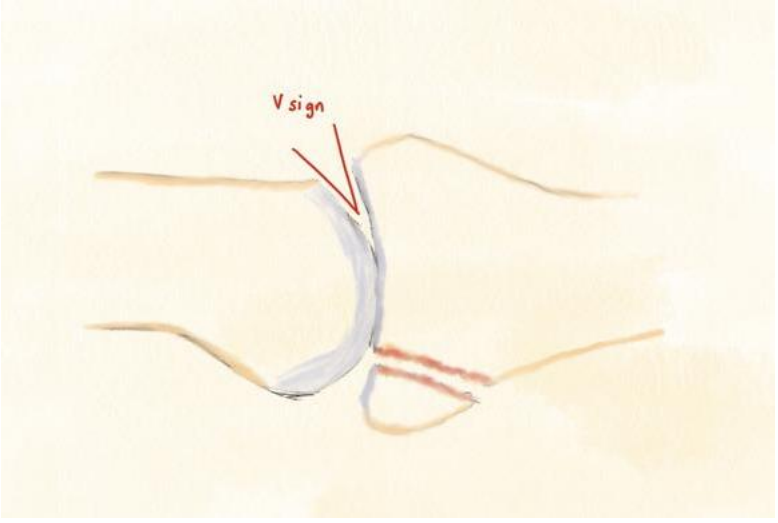


Figure 3. “V” sign

Record the joint range of motion and laxity, and check for instability. If seen as necessary, the examination can be repeated under fluoroscopy.

TREATMENT

In PIP joint injuries, the initiation of early motion is deemed critical for functional outcomes. The treatment decision is mostly influenced by the extent of the trauma, the status of the bone and musculoskelatel tissues. Treatment aims to achieve joint surface congruity, facilitate concentric gliding joint motion, and render the joint stable enough to allow early joint motion. If these conditions aren't fulfilled, chronic pain, joint stiffness, and injury related arthritis can be results of prolonged joint immobilization.

Among treatment options are extension block splinting, closed reduction-K-wire, open reduction internal fixation, volar plate arthroplasty, hemi hamate resurfacing arthroplasty, and dynamic external fixation (Figure 4 &5). Hemi hamate arthroplasty and dynamic external fixator are generally employed for severe injuries(Ellis et al., 2007; Ng & Oliver, 2009).

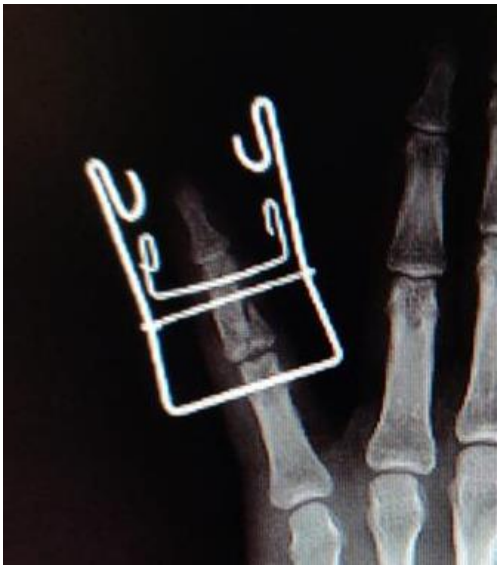
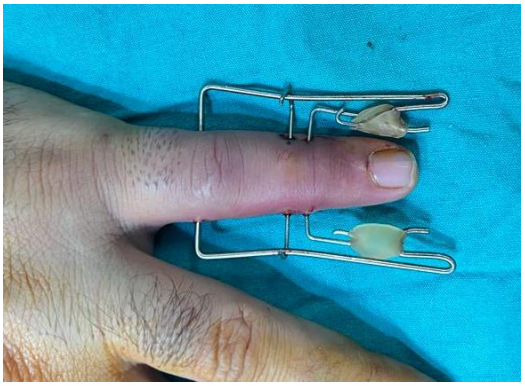


Figure 4. Dynamic external fixator (Suziki frame)



Figüre 5. Suziki frame

Dorsal Dislocations

In dorsal hyperextension injuries, an extension block splinting is followed in mild flexion (around 30 degrees) for 1-2 weeks. In dorsal dislocation cases, a

closed reduction and immobilization in slight flexion are performed for 1-2 weeks.

With dorsal dislocations with fractures, the lenght of the volar fracture fragment, its fragmentation, and angle at which reduction is compromised, are considered important. Extension block splinting (around 20 degrees) is applied and movement is immediately initiated in cases of stable and tenuous dislocation fractures.

For dorsal unstable dislocation fractures, procedures such as extension block pinning, open reduction internal fixation, external fixation, volar plate arthroplasty, or hemi hamate arthroplasty can be performed.

In the presence of a simple and sufficiently sized fracture fragment, open reduction with mini screws, K wires, or tension band wiring can be undertaken(Azari, 2007; Lee & Teoh, 2006).

For external fixation, static or dynamic options can be chosen. It can be used as an addition to internal fixation, allowing immediate joint movement and facilitating fracture reduction via ligamentotaxis (Figure 6) (Damert et al., 2013; Keramidas et al., 2007; Körting et al., 2009). It is contraindicated in proximal phalanx head fractures.

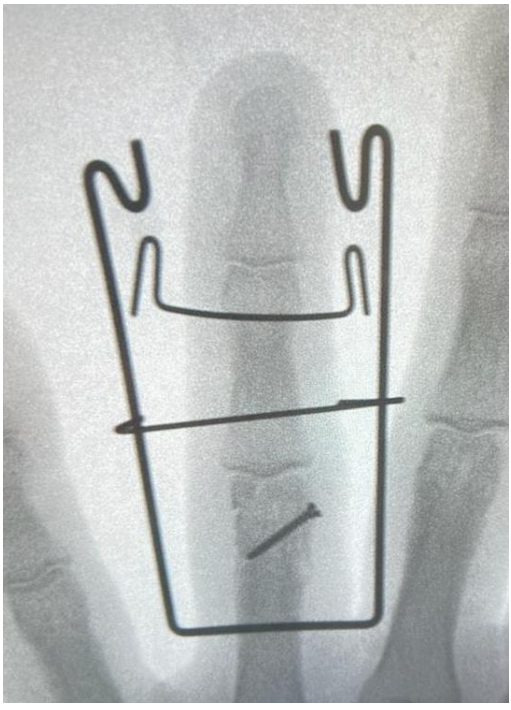


Figure 6. Suziki frame addition to internal fixation

In volar plate arthroplasty, the damaged joint surface is resurfaced with fibrocartilaginous tissue, achieving joint stability. This is typically used in highly fragmented and impacted fractures, with varying clinical results(Dionysian & Eaton, 2000; Rettig et al., 2001).

Hemihamate arthroplasty is used in unstable fragmented or impacted dislocation fractures, fractures involving more than 50% of the joint surface, and as a salvage operation (Afendras et al., 2010; Capo et al., 2008). A part prepared from the ipsilateral dorsal hamate is implanted in the defect area of the middle phalanx.

Volar Dislocations

For simple dislocations, a short-term immobilization is undertaken post-reduction in patients without extension lag, while a central slip repair is performed in those with extension lag. If a closed reduction cannot be achieved, an open reduction can be carried out.

In volar dislocation fractures, If the fragment is smaller than 20% of the articulating surface and is displaced by less than 2 mm, it is managed with a splint similar to Boutonnière injuries. If the fragment displacement is more than 2 mm or more than 20%, an open reduction internal fixation is applied.

Lateral Dislocations

These dislocations often self-reduce and are managed with a buddy splint.

PROGNOSIS AND COMPLICATION

For tenuous fracture patterns, treatments such as extension block pinning, closed reduction-K wire, volar plate arthroplasty have been observed to provide effective clinical and functional outcomes (Kamnerdnakta et al., 2018) (Figure 7). On the contrary, for comminuted and serious PIP joint injuries, interventions like HHA or dynamic external fixation are often required, which typically yield modest postoperative results. Some literature indicates that closed reduction-K wire, volar plate arthroplasty lead to the good postoperative range of motion at the PIP joint and have the lowest reoperation rates (Gianakos et al., 2020).

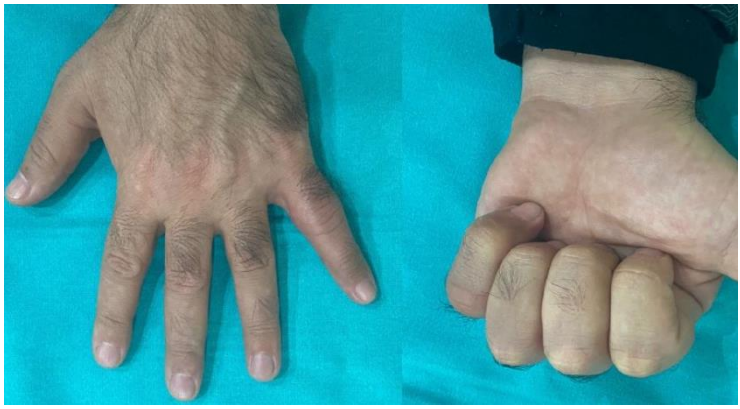


Figure 7. Post op 3 months of PIP joint fracture-dislocation treated by Suziki frame

When addressing severe injuries, particularly those involving extensive articular damage or pilon types, HHA, dynamic external fixation, and open reduction ınternal fixation are commonly used. However, these methods are associated with a lower postoperative PIP joint ROM.

Postoperative osteoarthritis (OA) is a frequent outcome across all surgical treatments, although severe OA cases are relatively few. Notably, extension block pinning was linked with a high rate of postoperative OA and recurring pain, which raises concerns. In addition, significant rates of recurrent subluxation have been reported in studies that examined extension block pinning and ORIF.

CONCLUSION

Proximal interphalangeal joint (PIPJ) injuries are complex and challenging. Treatments range from conservative to surgical, aiming to restore joint stability, congruity, and enable early motion. For tenuous fracture patterns, closed reduction-K wire, extension block pinning, and volar plate arthroplasty yield positive outcomes. Severe injuries often require HHA, external fixation (Suziki frame) or open reduction internal fixation , albeit with reduced postoperative PIP joint ROM. Postoperative osteoarthritis is a common complication across all treatments, particularly notable with extension block pinning. Further research is needed to refine treatment algorithms and develop strategies to prevent and manage complications, ultimately improving patient outcomes.

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Chapter 16

Immune Response to H. Pylori Infection

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ABSTRACT

Introduction

The gastric microenvironment, as illustrated in Figure 1, possesses a robust defense mechanism against a range of pathogenic bacteria, which can be attributed to the low oxygen pressures and low pH-gastric environment, as well as the presence of digestive enzymes (Burkitt & Duckworth, 2017). Furthermore, the epithelial surface of the human gastric stomach is coated with a layer of mucus that is secreted to protect the mucosa from potential pathogens. Despite the hostile conditions of the human stomach that typically eliminate invading microorganisms, *Helicobacter pylori* (*H. pylori*), a gram-negative bacterium, is capable of adapting to these harsh conditions by increasing the pH. Additionally, *Helicobacter pylori* possesses a flagellar structure that confers motility and facilitates colonization of the gastric mucosa.

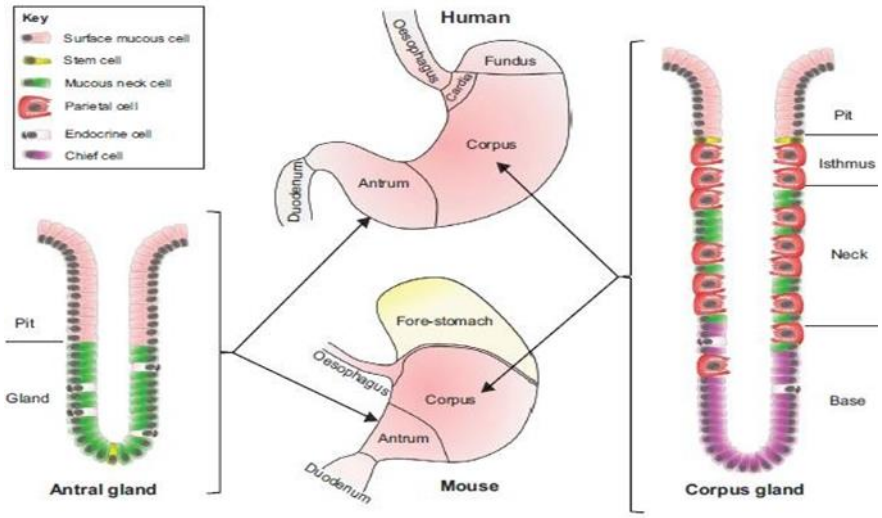

Figure 1 A depiction of the anatomical structure of the stomach in both humans and mice (Burkitt & Duckworth, 2017)

Helicobacter pylori, also known as *H. pylori*, is a type of bacteria that is small in size (0.5-3μm) and has a spiral and curved shape. It is classified as gram-negative and is capable of thriving in high temperature environments with low levels of oxygen. Additionally, it is a highly pathogenic bacteria that is commonly found in the human stomach. The majority of Helicobacter species exhibit a basic morphology characterized by a sheathed flagella structure that enables bacterial motility. The flagella of *H. pylori* enable the bacteria to move within the mucous layer of the gastric epithelium (O'Rourke & Bode, 2001).

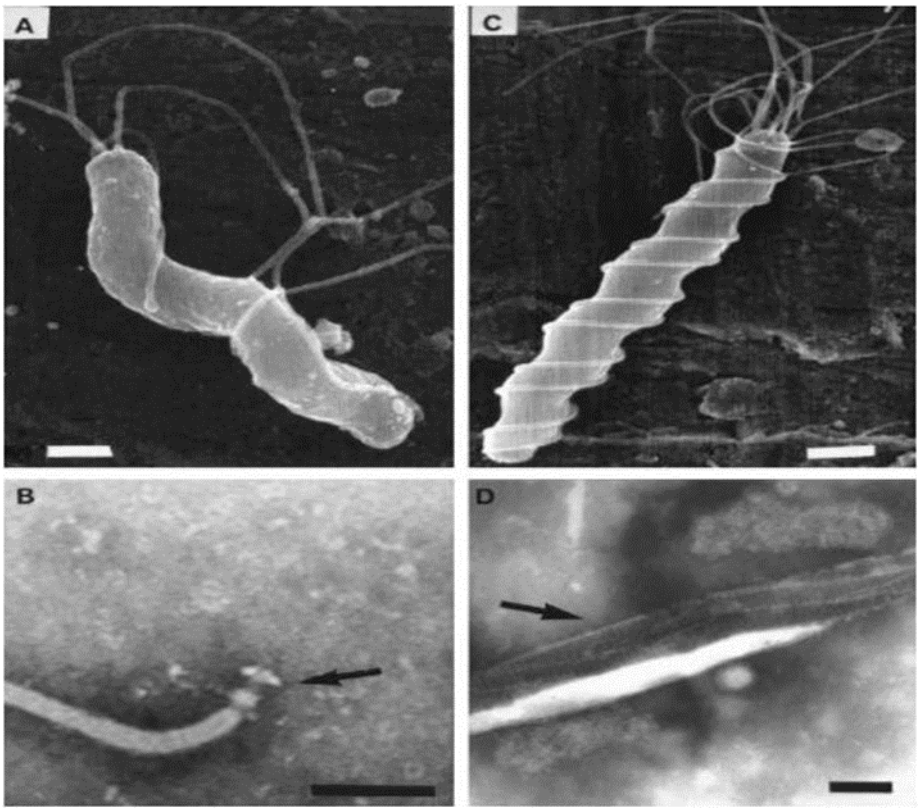

Figure 2 The structure of *H. pylori*.
(A) S-shaped *H. pylori* with five to seven polar flagella that are sheathed. Field emission SEM, bar = 0.5 μm. (B) Specification of the flagella hook. Negative stain, bar = 0.05 micrometers. (C) Helically shaped H. felis with paired periplasmic fibers and bipolar flagella sheaths. Field emission SEM, bar marker = 0.5 μm. (D) A periplasmic fiber detail exhibiting a striated appearance. Negative stain, scale bar = 0.05 m, modified from (O'Rourke & Bode, 2001).

In 1979, John Robin Warren, an Australian pathologist, drew attention to the potential correlation between bacteria and gastric cancer. The researcher detected bacterial presence on the exterior of a solitary gastric biopsy specimen obtained from his subject. Warren (1983) established the presence of Campylobacter-like bacteria in biopsy specimens of patients with active gastritis in the gastric antral mucosa. In 1981, Warren engaged in a collaboration with Berry James Marshall, who was undergoing clinical training at Royal Perth Hospital, on the topic of gastroenterology. Furthermore, Marshall discovered the presence of curved bacteria that were linked to gastric inflammatory lesions in biopsy specimens obtained from his patient. During the

year 1982, the prevailing belief among scholars was that bacteria were unable to survive in the human stomach owing to the stomach's production of copious amounts of acid. Notwithstanding these considerations, he subjected an organism that was isolated from his patient to himself. The individual in question fell ill and subsequent gastric biopsy results indicated the presence of identical organisms found in the gastric biopsy specimens of both his and Warren's patients, thereby establishing a causal link to the development of gastritis. The 2005 Nobel Prize in Medicine was awarded to Dr. Barry J. Marshall and Dr. J. Robin Warren. Marshall and Warren made significant contributions to the understanding of the etiology of gastritis and gastric ulcers, resulting in a revision of the prevailing knowledge on the subject matter. Several papers published between 1989 and 1992 established that *H. pylori* is a significant contributor to inflammatory and neoplastic conditions of the stomach, including gastritis, gastric ulcer, gastric cancer, and lymphoma (Lichtman, 2017).

Gastric cancer development is classified into four stages: chronic gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia. The Turkish population exhibits varying degrees of gastric precancerous lesions due to a high prevalence of H.pylori infection. Some patients are at risk of developing gastric cancer. The pathogenesis of gastric cancer is not fully understood in terms of genetics and immunology, making it impossible to predict its development in patients. The relationship between the virulence factors of *H. pylori* and the host response remains unclear. *H. pylori* can persist for extended periods within the gastric mucosa. This suggests inadequate host immunity in pathogen elimination. This could be attributed to alterations in cytokine expression related to the immune response. *H. pylori* infection elicits a Th1-biased adaptive immune response, while Treg cells suppress inflammation in the host, promoting the bacteria's prolonged survival in the gastric environment. The interaction between programmed death-1 (PD-1) checkpoint proteins found on active T and B cells and programmed death-ligand-1 (PD-L1) results in the suppression of the T cell response. This section examines the correlation between IFN-γ levels, a cytokine produced by Th1 cells, and FOXP3, a transcription factor produced by Treg cells, as well as the relationship between H.pylori infection and precarcinogens, based on relevant literature.

Background

Diseases associated with *Helicobacter pylori*

In the context of developing nations like Turkey, a significant proportion of the populace, approximately 80%, is afflicted with *Helicobacter pylori* (*H.*

pylori) owing to inadequate socio-economic and health-related circumstances.The observed decline in the prevalence of *H. pylori* in developed nations is regrettably not mirrored in Turkey. The outcomes of *H. pylori* infection exhibit variability among the afflicted individuals. Although chronic gastritis is observed in all infected individuals, only a minority (20-30%) of those affected develop associated pathologies such as peptic ulcer, gastric cancer, and Mucosa Associated Lymphoid Tissue (MALT) Lymphoma. The etiology of this variation remains incompletely understood; however, it is postulated that the pathogenesis of infection is influenced by the genetic, immunological, and environmental factors of the host, as well as the bacterial pathogenic properties. Understanding the immunological profile of a host infected with *H. pylori* is crucial in comprehending the etiology of pathologies that may arise in the host's mucosal lining. In this particular context, it is crucial to establish the roles of immune regulatory cells in averting the development of pathological conditions, as well as mitigating the detrimental consequences of hyperactive immune responses directed towards *H. pylori* in the epithelial cells of the gastric mucosa.

Peptic ulcer is a gastrointestinal mucosal defect commonly caused by *H. pylori* infection. *H. pylori* colonization results in elevated gastrin levels in the gastric mucosa. Elevated levels of gastrin stimulate acid secretion, resulting in damage to the intestinal lining. Gastric ulcer is linked to corpus-predominant *H. pylori* gastritis, hyperacidity, and low intragastric nitrosamin levels. On the other hand, duodenal ulcer is associated with antrum-predominant *H. pylori* gastritis, low acid production, and high intragastric N-nitrosamine concentration (Reed & Haines, 1981).

Gastritis, primarily caused by *H. pylori* infection, is a prevalent inflammation of the stomach (Burkitt & Duckworth, 2017). *H. pylori* infection elicits acute and chronic immune responses. T-helper cells subsets, Th1 and Th2, direct response subtypes. During the acute phase of *H. pylori* infection, the bacterium penetrates the mucus layer of the stomach's surface epithelial cells. The epithelium responds to bacterial infection by depleting mucin, exfoliating cells, and undergoing compensatory growth changes. The acute response is associated with the release of bacterial lipopolysaccharides that penetrate the epithelial surface of the gastric mucosa. *H. pylori* stimulates the production of TNF-α and IL-1 by the epithelium in response to bacterial lipopolysaccharide secretion (Dixon, 2001).

Mucosa-associated lymphoid tissue (MALT) lymphoma is another cancer type associated with chronic *H. pylori* inflammation. If host immune response against to bacterium fails to eliminate infection in following 3 or 4 weeks of

penetration, accumulation of lymphoid folliciles, to prevent damaging effects of pathogen, becomes a constant characteristic of chronic H.pylori gastritis. Accumulation of lymphoid cells in gastrit mucosa at the next stages of infection results H.pylori associated lymphoma in the stomach.

Stomach Cancer is the third most common cancer in the world. The high mortality rate in this type of cancer is the end result of late diagnosis and inefficient prognosis. The occurrence of stomach cancer depends on many factors such as genetic susceptibility, environmental factors, diet and age. Among them, chronic inflammation resulting from *H. pylori* infection is the main reason for triggering carcinogenesis. *H. pylori*, defined as a main cause of gastric cancer in epidemiological and experimental studies, has been described as an important risk factor besides nutrition and hygiene. For this reason, *H. pylori* was declared a Class I carcinogen by the World Health Organization (WHO) in 1994.

Gastric cancer is divided histologically in two types; diffuse type (large fibrous stroma in infiltrated, slightly differentiated and non-cohesive cancer cells) and intestinal type (such as cohesive, basal cells groups) (Pecca, 1965). Epidemiological correlation between *H. pylori* and these two pathologies was observed. Pre- cancerogenic lesions (chronic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia) before the occuration of the intestinal type gastric cancer are epidemiologically linked to *H. pylori* infection (Correa, 1995; Portal-Celhay & Perez, 2006).

Precancerous Lesions of Gastric Cancer

Atrophic gastritis. Atrophy is defined as loss of glandular tissue in the stomach due to mucosal damages including H.pylori infection. Atrophy causes different levels of inflammation in gastric mucosa. When such loss of tissue occurs, it may follows erosion or ulceration of gastric mucosa. However, atrophy of the gastric glands is often associated with intestinal metaplasia, which causes changes in the antrum of the stomach more commonly. Atrophy in H.pylori infection may occur as a result of directly bacterial effects or alternatively host inflammatory or immune responses (Dixon., 2001). Patients with atrophic gastritis are statistically at increased risk group about gastric cancer. Even the most of gastric cancers develop on a basis of atrophic gastritis, intestinal metaplasia is the statistically most prevelant precancerous condition that changes the epithelium and causes malignancy.

Gastric ıntestinal metaplasia. Metaplasia is defined as replacing of fully differentiated cell type with another differantiated cell type. Intestinal metaplasia is described as differantiation of gastric mucosa epithelium cells to

small intestinal morphology resembling cells. Intestinal metaplasia has two subtypes based on histological classification; complete and incomplete type. Also, there are patients showing both types of intestinal metaplasia. Complete metaplasia is diagnosed with the presence of small intestinal resembling epithelium cells and also eosinophils, goblet cells and Paneth cells in stomach. On the other hand, in incomplete type metaplasia, there are differentiated cells that resemble colonic epithelium morphology and most of digestive enzymes required for small intestinal system are absent. Another classification of intestinal metaplasia is based on mucin expressions. Complete IM expresses only sialomucins and incomplete IM expresses both sulfomucins and sialomucins (Liu & Wong, 2016).

Gastric dysplasia. Dysplasia in the gastrointestinal system has been described as the formation of a neoplastic epithelium independently from tissue invasion according to the World Health Organization (WHO) (Morson & Sobin, 1980). In addition, WHO defines gastric adenomas as polypoid lesions consisting of tubular and / or villous structures surrounded by neoplastic epithelium. Most gastric dysplasia was discovered incidentally during endoscopic examinations. Gastric dysplasia can occur in any region of the stomach, but most commonly in the antrum, which is also the main site of intestinal type gastric cancer (Lauwers & Riddell, 1999). Gastric dysplasia is defined as a precancerous lesion and predominant event in gastric carcinogenesis, particularly in intestinal type cancers. The relationship between the displaced epithelium and the gastric adenocarcinoma and the definition of the dysplasia as a precancerous lesion have been identified through retrospective analysis of cancer gastrectomy specimens.

Intestinal Type Gastric Cancer

Intestinal-type adenocarcinoma is defined as the transition from normal mucosa to chronic superficial gastritis followed by atrophic gastritis and intestinal metaplasia resulting with dysplasia and adenocarcinoma. In Turkish population, there is high frequency of patients with gastric precancerous lesions at different levels due to the high incidence of *H. pylori* infection. A small proportion of these patients have a risk to developed gastric cancer in the future. However, the identification of whether these patients develop gastric cancer or not, can not be done because the pathogenesis of the stomach is not fully known in the prospect of genetic and immunological aspects. At the same time, there are many unknowns about the connection between the characteristics of *H. pylori* and the host immune response.

Pathogenesis of *H. pylori* infection. At the beginning of the infection, once *H. pylori* enters the host, it uses its own urease activity to neutralize the acidic conditions of host stomach. Flagella-mediated motility allows *H. pylori* to migrate into the host gastric epithelial cells. Subsequently, specific interactions between host cell receptors and bacterial adhesins lead to successful colonization and permanent infection. *H. pylori* secretes many effector proteins, toxins including cytotoxin associated gene (CagA) and vacuolating cytotoxin (VacA), which cause host tissue damage. The gastric epithelial layer, which forms the main interface between the host and *H. pylori*, secretes chemokines to initiate innate immunity and activate neutrophils. It also leads to the formation of clinical diseases such as gastritis and ulcers (Kao & Sheu, 2016).

***H. pylori* colonization.** *H. pylori* colonizition occurs on the mucous layer of the gastric epithelium. There are three critical factors for H.pylori colonization of host stomach; which are urease activity for survival under acidic conditions of the host stomach, movement through the epithelial cells via flagella-mediated motility and adhesion to the gastric epithelium. Adhesion to the gastric epithelium prevents the bacteria being eliminated from the stomach by mucus turnover and gastric peristalsis. Adhesion ability of *H. pylori* is required for protection of *H. pylori* from the host gastric mucosa mediated mechanisms such as mucus, acidic pH and exfoliation. *H. pylori* adhesins, considered as bacterial virulence factors, are effective in numerous stages in the early and chronic stages of infection, affect disease development and cause different pathologies in infected patients.

Virulence Factors of *H. pylori*

UreaseA gene (ureA) and UreaseB gene (ureB). Urease is a required enzyme for decreasing stomach acidity by generating ammonia and carbondioxide from urea. This enzyme is essential for *H. pylori* colonization on the gastric mucosa. *H. pylori* has an acidification mechanism that promotes regulation of periplasmic pH conditions in the harsh acidic environment of the stomach by regulating its urease activity. *H. pylori* urease encoding genes are located as a single 6.13-kb gene cluster on the chromosome of the bacterium. The urease gene cluster is composed of seven genes, including catalytic subunits (ureA / B), acid-gated urea channel (ureI), and accessory assembly proteins (ureE-H) (Mobley & Island, 1995). The metal cofactor Nickel should be added into the apoenzymes for heterodimer urease activity with the effect of four accesory proteins, among which UreE acts as an important urease metallochaperone (Yang & Li, 2014).

Vacuolating cytotoxin gene A (vacA). The vacuolating cytotoxin A (vacA) is an important virulence factor of *H. pylori* that found in all strains and encodes 87 kD protein. In the first studies on vacA, two main polymorphic regions were identified, the signal sequence (s1 and s2) and two central regions (m1 and m2) (Umit & Tezel, 2009). However, recent studies have identified an intermediate (i1 and i2) region between the s and m region (Rahimiana & Sanei, 2014). The mosaic combination of allelic types of the s and m regions determines the production of cytotoxin and is associated with the pathogenesis of bacteria. Cytotoxin that encoded by VacA gene of *H. pylori*, act as cellular toxin which has multiple effects in various host cell types (Rhead & Letley, 2007). Toxin enters the host cell membrane and forms anion-conducting channels. VacA toxin; interferes autophagic pathways of gastric cells that causes gastrophilic epithelial erosions in mice, inducating cell apoptosis and a cell necrosis. It also acts as an immunomodulator by inhibiting the proliferation and activation of T and B lymphocytes (Yamaoka & Kodama, 2001).

Cytotoxin- associated gene A (cagA). *H. pylori* strains are divided into two major subpopulations, according to their ability to produce an immunodominant protein (120-145 kDa) called cytotoxin-associated gene (CagA) antigen. The cagA gene is found in the cag PAI (cag Pathogenicity Island) region, a 40 kb DNA segment in the *H. pylori* genome and encodes approximately 30 genes which are multicistronic and involved in the biogenesis of type IV secretion system (Hatakeyama & Higashi, 2005). Cag PAI has two regions; which are cagI and cagII. The genes on the cagI and cag II regions do not just responsible in developing gastric diseases (Baghaei & Shokrzadeh, 2009). Cag PAI region contains some pathogenic factors; CagE, -G, -H, -I, -L, -M, -T, and -Y. Cag E,-G, -H, -I, -L, and –M; which are necessary for the production of NF- Kb.NF-Kb production causes apoptosis of gastric epithelial cells. CagT and CagY factors are able to synthesize needle-shaped structures that allow CagA toxin to penetrate gastric epithelial cells (Censini & Lange, 1996).

Cag type IV secretion system (Cag-T4SS). The CagPAI DNA segment encodes approximately 30 genes including cagA which encodes the components of a molecular syringe like structure that called the type IV secretion system (T4SS) (Hatakeyama & Higashi, 2005). Additionally, cagL gene on cagPAI region encodes a cagL protein that expressing on the surface of *H. pylori* as a part of the T4SS (Schuelein & Everingham, 2011). Upon adherence of cagA expressing *H. pylori* srain to the gastric epithelial cell, the cagA protein is injected directly into the cell by T4SS (Odenbreit & Püls, 2000). After the injection, the non- phosphorylated cagA interacts with host cell proteins and then, phosphorylated in its EPIYA motif. This causes irregularity of epithelial

structure and induction of pro- inflammatory and mitogenic responses of host stomach.

Outer inflammatory protein A (oipA). The outer membrane proteins of *H. pylori* play an important role in bacterial pathogenesis. One of the important outer membrane protein is outer inflammatory protein (OipA) which is a 35 kDa proinflammatory protein. Bacterial adherence to gastric cells and increased level of IL-8 production by host cells are associated with oipA gene expression. It also has functions in the upregulation of oipA matrix metalloproteinase 1 (MMP-1) (Yoshio & Kudo, 2004).

Blood group antigen-binding adhesion protein A (BabA). Blood group antigen-binding adhesion protein A (BabA) is one of the major outer membrane proteins (OMPs) and described in three allelic types; babA1, babA2 and babB. The coding sequences of babA1 and babA2 are quite similarexcept that babA1 has a 10-bp deletion of the translational initiation codon. The molecular mass of the babA protein is about 78 kDa and is encoded by babA2. When the host stomach is infected with *H. pylori*, the bacteria mediates BabA to bind to the antigen of the fucosylated Lewis B blood group (Leb). BabA mainly associated with the terminal fucose residues found on the blood group ABO antigens that are expressed on the surface of the gastric epithelial cell (Ilver & Arnqvist, 1998). The structure of the babA receptor is similar to type O blood antigen, and there is a correlation between type O blood and stomach-related diseases (Aspholm-Hurtig & Dailide, 2004) .

Duodenal ulcer promoting gene A (dupA). Duodenal ulcer promoting gene A (dupA) is a novel *H. pylori* virulence marker located in the "plasticity region" of the *H. pylori* genome and associated with increased risk for duodenal ulcer. There is a correlation between the increased level of IL-8 expression and DupA in the gastric mucosa of *H. pylori*-infected individuals (Jung & Sugimoto, 2012). Also presence of dupA is associatd with increased levels of proinflammatory cytokines expression such as IL-12p70 and IL-23, IL-12p40 which are released by CD14 + mononuclear cells. (Hussein & Argent, 2010)

Neutrophil - activating protein A (NapA). In vitro assays showed that *H. pylori* directly activates neutrophils. Although the mechanism of direct neutrophil stimulation by *H. pylori* is unkown, a protein that partly responsible is identified and termed as neutrophil activating protein (NapA). Neutrophil-activating protein A (NapA) is a water-soluble 150kDa dodecameric protein that has been identified as a major *H. pylori* infection related virulence factor. NapA stimulates production of high oxygen radicals from neutrophils, causes damage in local tissues and promotes neutrophil adhesion to endothelial cells during *H. pylori* infection. (Evans Jr. & Evans, 1995). This NapA-induced

adhesion is associated with the high affinity of the b2 integrin on the neutrophil surface membrane. In addition to, increase in production of reactive oxygen species, NapA also increases the expression and release of IL-8, macrophage inflammatory protein (MIP) -1a and MIP-1b. (Polenghi & Bossi, 2007) The stimulation of reactive oxygen radical production from neutrophils is a key component of the innate immune system. Also, neutrophils are effective antimicrobial agents against *H. pylori* as well as maintaining the mucosal damage and gastritis.

Putative neuraminyllactose-binding hemagglutinin homologue A (HpaA). *H. pylori* hemagglutinin (hpa) is a binding protein of *H. pylori* that is coded by the hpa gene and enables the *H. pylori* to adhere host gastric epithelial cells. hpa is also described as the main flagellar sheath protein and is considered as a colonization factor for *H. pylori*. It only gives rise to low immune responses after infection. Hpa act as haemagglutinin found on the surface of the bacteria and aggregates with assembly of fibriller structure

Diagnosis of *Helicobacter pylori*

The diagnosis tests for *H. pylori* infection is used to determine whether patients have an *H. pylori* infection or not. There are two main group of these tests; invasive and non-invasive. Invasive tests contains histological, culture and rapid urease tests from endoscopy biopsy specimens of patients as well as molecular methods (Wang & Kuo, 2015). Non-invasive tests have three main methods; serology, urea breath test and stool antigen test. In these non-invasive techniques, biopsy specimens are not required. Also, there are other molecular methods include both invasive and noninvasive tests. Collacation of these methods are important for accurate diagnosis of infection (Lopes & Vale, 2014).

Invasive Methods

To diagnose *H. pylori* related diseases such as gastrit, peptic ulcer, gastric cancer and MALT Lymphoma, most widely used invasive method is endoscopy. Endoscopic process contains screening of gastrointestinal tract by miniature video equipment that is inserted through the patients and enables to real time observing of gastrointestinal tract. During observation, biopsy specimens are taken from gastric to investigate further diagnosis analysis (Wang & Kuo, 2015).

Histology is based on staining methods and described as the golden standart for direct detection of *H. pylori*. Main stains that used for diagnonis of H.pylori are hematoxylin and eosin staining, Giemsa, Warthine-Starry, Hp silver stain,

toluidine blue, acridine orange, McMullen and Genta, Dieterle and immunohistochemical stain (Wang & Kuo, 2015).

Culture is another invasive method and especially used as a standard for highest specifity for H.pylori detection. But also, this method has some disadvantages such as les sensitivity long time period and high cost. On account of this, culture method is not commonly used for detection of H.pylori infection (Wang & Kuo, 2015).

Last invasive method is Rapid Urease Test (RUT) which is mostly used in clinical diagnosis of *H. pylori*. In contrast to culture method, this test is cheap and rapid, also has high sensitivity. Most common type of RUT test is CLOtest. RUT method is mainly depends on the presence of the urease enzyme. After 24 hours, if there is any

H. pylori colonization in biopsy specimen, test turns positive and color changes observed due to increase of pH in the environment (Wang & Kuo, 2015).

Non-Invasive Methods

Non-invasive methods are applied without the need for endoscopic biopsy specimens and examines the urine, whole blood, saliva, serum and expired air samples for diagnosis of *H. pylori*. Urea breath test is one of the important non invasive H.pylori detection method. This technique is based on the measurement of exhaled CO2 released due to *H. pylori* urease activity and extremely accurate (95% sensitivity and specificity) to detect the presence of active *H. pylori* infection (Wang & Kuo, 2015).

Stool antigen test (SAT) is other non-invasive, antigen testing method that could be used with immunoassay (EIA) and immunochromatography assay (ICA). In addition to antigen testing, antibody response of patient could be used for diagnosis of H.pylori infection. Specific antibodies that present at high levels in H.pylori infected patients could be examine from serum and urine by using ELISA method.

Molecular Methods

Molecular methods include both invasive and non-invasive methods. Collacation of these methods are important for accurate diagnosis of infection. Most common moleculer method is polymerase chain reaction (PCR). By detection of H.pylori DNA by using PCR, each virulence factors of H.pylori could be characterisized (Lage & Godfroid, 1995). Most accurated results are obtained by using RT-PCR and multiplex PCR methods. Also, in situ

hybridization (FISH) technique is another molecular method which could be used for H.pylori detection in patients.

Treatment of H.pylori Infection

Eradication of bacterium is important for prognosis of H.pylori related diseases. Many drug therapies have been described for H.pylori eradication; standard first-line threapy, alternatively second and third line threapies. Drugs that include the combinations of antimicrobial agents and anti-secretory agents are key factors for treatment (Yang & Lu, 2014). Most important antimicrobial agents are; clarithromycin, levofloxacin, metronidazole and amoxicillin. Anti-secretory agents H2-receptor antagonists and proton pump inhibitors (PPIs) are anti-secretory agents and used for enhance the antibiotic activity (Yang & Lu, 2014). Standart first line therapy include combination of amoxicillin, clarithromycin as an antibiotic and a proton-pump inhibitor (PPI) as an anti-secretory agent. If the standart first line threapy is failed due to H.pylori resistance to antibiotics, alternative second and third line therapies could be applied. In second line therapy, amoxicillin dose could be increased and lexofloxacin preferentially is used rather than clarithromycin. Also it could be the combination of proton-pump inhibitor (PPI), bismuth, tetracycline and metronidazole. If second line therapy is failed also, third line therapy applied. Third line therapy based on the combination of amoxicillin, sitafloxacin and PPI (Papastergiou & Georgopoulos, 2014).

Resistance to these antimicrobial agents is the main reason of eradication failure. Resistance to metronizadole is highly prevelant and the responsible mechanism for resistance is identified as H.pylori mutations. Studies provided that the main reason for metronizadole resistance is null muatations in rdxA gene (H0954 in the H.pylori genome database) and three point mutations at A2143G, A2142G, and A2142C are related with the resistance to metronidazole (Mirzaei & Poursina, 2014). Also, point mutations at 2142C, A2142G, and A2143G in 23S rRNA gene point muations are responsible for clarithromycin resistance (Kim & Choi, 2015) Therefore, alternative solutions such as probiotics can be used for the treatment of H.pylori. Probiotics lead to increase of mucin secretion in stomach and modifications in the immunologic response of the host. L. acidophilus 4356, L. casei 193, L. Lactis BH5, B. subtilis, W. confusa PL9001 are some probiotic microorganisms used for the tretment of *H. pylori* infection (Rial, 2000).

Immune response to H.pylori Infection

Despite the 20% of *H. pylori*-infected individuals in the community shows the pathology of developing progress through the gastric cancer, the rest of the infected individuals do not show the same progression. It indicates the presence of regulatory mechanisms that suppress inflammatory immunologic responses in the stomach or peripheral lymph nodes. (Belkaid, 2007). Immune responses against bacterial pathogens are divided into innate and adaptive responses. The innate response to bacterial infection usually begins as a nonspecific process. It reacts rapidly with various bacterial molecules and gives an infectious danger signal to kill the pathogenic bacterium. Recognition of bacterial antigens by the innate immune system is mediated by TLRs (Toll-like receptors) expressing on APCs (antigen-presenting cells), such as monocytes and DCs (dendritic cells). Cytokines are part of an extracellular signaling network that controls functions of the innate and adaptive immune system and have the ability to regulate the type of immune response generated and its extent. Interaction of monocytes and other APCs with bacteria leads to the secretion of proinflammatory cytokines such as TNF-α (tumor necrosis factor-a), IL (interleukin) -1β and IL-8. *H. pylori* infection is associated with increased levels of these cytokines, which act as a local chemoattractants. On the other hand, the adaptive immune response is delayed and highly specific to bacterial antigens. It activates T cells, B cells and memory cells and it is shaped by the innate immune response (Portal-Celhay & Perez, 2006) (Figure 3).

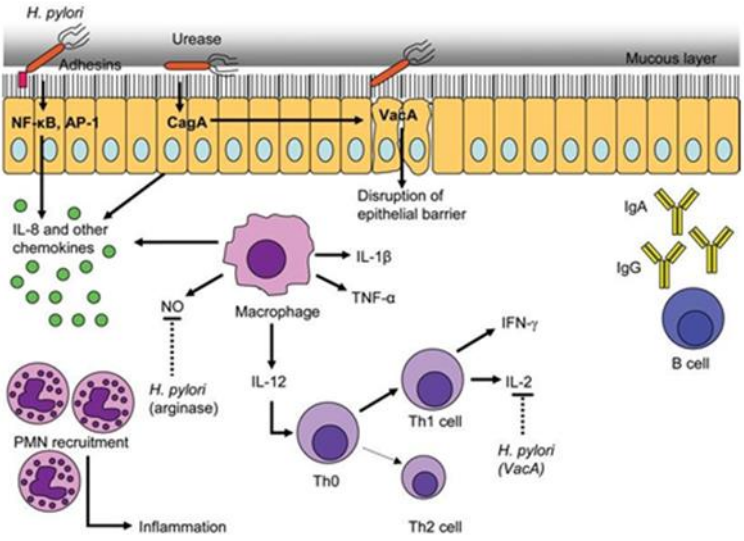

Figure 3 *H. pylori* pathogenesis and the host immune response (Portal-Celhay & Perez, 2006)

T cell Developments and Types of T cells. T cells are a subset of lymphocytes that play a central role in cell-mediated immunity. A naive lymphocyte is not able to mount an immune response until it has been activated to become an effector cell. Activation of a naive T cell occurs in specialized microenvironments within secondary lymphoid tissue (e.g. peripheral lymph nodes, Peyer's patches, tonsils, and spleen). Within these microenvironments, dendritic cells capture antigen and present it to the naive lymphocyte, resulting in its activation. Naive T cells are distinguished upon cytokine profies and divided into two subtypes; CD4 + effector T cells which secrete various cytokines that affect other immune cells and inflammatory mechanisms or CD8 + cytotoxic T cells which directly kills cells carrying foreign antigen.

CD4+ effector T cells. Naive T cells is distinguished into different types of CD4+ T cells depending on activation signals, cytokine profiles, type of antigen presenting cells, and regulation of co-stimulatory molecules in the microenvironment. CD4+ T cells are classified into two main classes; T helper cell 1 (Th1) and T helper cell 2 (Th2) due to their cytokines and transcription factors. Also, T helper 17 (Th17), T helper 9 (Th9) and T helper 22 (Th22) are described as a lineage CD4+ T cells that have a crucical role in the immune system (Alberts, Johnson, & Lewis, 2002). Th1 cells secrete signature cytokines; interferon-γ (IFN-γ), Interleukin 2 (IL-2) with other pro-inflammatory cytokines; like tumour necrosis factor-α (TNF-α). Th1 cells have critical functions in cell mediated immunity and inflammation and autoimmunity, macrophage activation, leading to phagocytosis and destruction of intracellular pathogens. Also, T-bet is member of T-box family of transcription factor and Th1-specific transcription factor and an important regulator of Th1 differentiation (Hsieh & Steven, 1993).

T helper 2 cells (Th2) are second main subtype of CD4+ effector T cell that secretes IL-4, IL-5, IL-9, IL-13, IL-10 and IL-17E/IL-25. Also Th2 cells are associated with humoral immunity. GATA binding protein 3 (GATA-3) is a member of the GATA family of transcription factors and key transcription factor that is required for Th2 differentiation (Gros & Ben-Sasson, 1990).

T helper 17 cell is a recently identified subset of T helper cells that secretes Interleukin 17 (IL-17) cytokine family, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25) and IL-17F. Th17 cells have a function in host defense against especially fungal and bacterial infection (Weaver & Harrington, 2006). Retinoic acid-related orphan receptor gamma (RORγ) is a "master-regulator" transcription factor that controls differentiation of Th17 cells. ROR-α and ROR-γt are required for maintaining Th17 cell differentiation and development (Jin & Martynowski, 2010). RORγt is expressed in cells

differentiated Th17 cells in the presence of TGF- β and IL-6 (Ivanov & McKenzie, 2006).

T helper 9 (Th9) cells are discribed by expression of high level of interleukin 9 (IL- 9) and important function of Th9 cells is enhancing the pro-inflammatory Th17 cell- driven immune response (Annunziato & Romagnani, 2009).

Regulatory T cell (Treg). Regulatory T cell (Treg) is one of the major suppressor T cell type in the anti- inflammatory immune response (Belkaid, 2007). Forkhead Box P3 (FOXP3) is a specific genetic marker for CD4 + CD25 +T regulatory cells and major transcription factor that expressed in Treg cells. Also, FOXP3 is known as the master regulator of Treg function and acts as master switch gene in the development of regulatory T cells (Kim & Leonard, 2007).

The most important function of Treg cells is maintaining self-tolerance, immune homeostasis and prevent excessive collateral tissue damage that can occur during the immune response. It has been shown that Tregs' infiltrate into the stomach tissue during H.pylori infection, providing a balance between the host and Helicobacter, allowing the bacterium to maintains long-term durability in the stomach and preventing destructive inflammation to the tissue (Kao, Zhang, Miller, & Mills, 2010).

Costimulatory Pathways in T cell Regulation

B7-CD28 pathway. Costimulatory molecules play a critical role in the initiation and termination of immune responses and are important in the control of T cell activation. The best characterized T cell costimulatory pathway consists of the B7-1 (CD80) and B7-2 (CD86) ligands, which bind same two receptors CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) (CD152). B7-1 and B7-2 are expressed on antigen presenting cells (APCs). While B7-1 expression is upregulated on APCs as a response to maturational stimuli, B7-2 expression is promoted at low levels and is induced by rapid kinetics (Inaba & Witmer-Pack, 1994). Both CD28 and CTLA-4 bind to the B7 molecules with the conserved MYPPPY motif. CTLA-4 has a higher ligand binding affinity than CD28.

CD28 is mainly expressed on T cells and induces T cell activation. While CD28 provide signals compatible with TCR which enhance and maintain T cell activation, CTLA-4 antagonizes TCR signals to induce tolerance to secondary immune responses. The balance between positive and negative signals provided by CD28 and CTLA-4 is responsible for T-cell proliferation, differentiation, survival and regulation of T cell dependent B-cell responses. The B7/CD28 family has recently been expanded and two new pathways have been identified;

inducible costimulator (ICOS) and its ligand, B7h (also known as GL50, B7RP1, LICOS and B7-H2) and programmed death-1 (PD-1) and its ligands PD-L1 and PD-L2 (Figure 7) (Keir & Sharpe, 2005). Appropriate manipulation of these co-signaling pathways may promote immune responses against bacterial infection and cancer development.

B7-H2/ICOS pathway. ICOS was identified as an inducible T-cell costimulator, homologous to CD28 and induced in both CD4 + and CD8 + T cells by activation with TCR. CD28 costimulation increases ICOS upregulation. ICOS contains an FDPPPF motif in place of the MYPPPY motif in its immunoglobulin (Ig) V-like domain and binds a unique B7 family member, B7h which is expressed on B cells and macrophages.

In contrast to CD28, ICOS is expressed on activated, but not resting T cells. Its ligand, B7h is expressed on B cells, monocytes, DCs. B7-H2/ICOS pathway preferentially regulates effector function of T cells (Parry & Rumbley, 2003).

B7-H1/ B7-DC/ PD-1 pathway. B7-H1/ B7-DC/ PD-1 pathway has both positive and negative effects in T cell responses as independent from the B7-CD28 pathway. B7-H1 and B7-DC are two B7 homologous molecules, which were initally identified as T cell costimulatory molecules and bind to programmed cell death-1 (PD-1), an Ig superfamily member. PD-1 has funtional and structural homology to CD28 and CTLA-4 and contains that both immunoreceptor tyrosine-based inhibitor motif (ITIM) and an immunoreceptor tyrosine-based key motif (ITSM). PD-1 is an inhibitory receptor and down-regulates T-cell responses. PD-1 is expressed on activated T cells, B cells and myeloid cells (Keir & Sharpe, 2005).

Programmed Death Ligand-1 (PD-L1, B7-H1). Programmed Death Ligand-1 (PD-L1) , also known as B7-H1, is a member of the B7-CD28 family. It is ligand of programmed death-1 (PD-1) and widely expressed on T cells, B cells, macrophages, monocytes, DCs, and non-lymphoid cells including endothelial cells, syncytiotrophoblasts in the placenta, muscle, and pancreatic islets. Immunohistochemical analysis showed that PD-L1 expression is increased by tumour cells in the tumour microenvironment. The interaction of PD-1 and PD-L1 blocked T-cell receptor signalling and therefore down-regulates T-cell response (Wang & Chen, 2004).

Clinical studies have shown that the expression of PD-L1 is mostly associated with a worse prognose in patients with cancer compared with those without PD-L1 expression and suggested that blockade of PD-1 and PD-L1 interaction, by using anti-PD-1 or anti-PD-L1 antibodies, can upregulate T cell activity against tumour cells and make improvement in prognosis of patients who have cancer disease.

Result and Discussion

Gastric carcinogenesis is a multi-step process that is affected by environmental, genetic, epigenetic factors, and *Helicobacter pylori* (*H. pylori*) infection. In the literature, it was shown that in gastritis and ulcer patients, there is a relation between *H. pylori* specific virulence factors and T cell mediated adaptive immunity against the infection (Yamaoka, 2010). The progression of gastric cancer is through chronic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia stages (Gurzu, Sugimura, Orlowska, Szentirmay, & Jung, 2015). Active chronic gastritis is the inflammatory state of the stomach mucosa and is characterized by the presence of mononuclear cells, lymphocytes and plasma cells. In intestinal metaplasia, the normal stomach glandular epithelium is degraded and mucin-producing cells that resembling intestinal goblet cells are replaced with normal epithelium. The risk of gastric cancer development from incomplete intestinal metaplasia pathology is more prevalent than complete type (Yoon & Kim, 2015). Within these 24 patients; 23 of them were *H. pylori*-positive and 1 patient was *H. pylori*-negative according to patient pathology reports. To survive in acidic environment of stomach, *H. pylori* must express one of the ureA and ureB virulence factors (Cheng-YenKaoa, Sheu, & Wu, 2016). This may be explained by the weakly positive *H. pylori* infection in those patients. In pathology reports, the classification of *H. pylori* infection level is divided into 3 subgroups; mild, moderate and strong. These, inactive chronic gastritis patients reported as mildly infected by *H. pylori*. Therefore amplification of ureA and ureB genes are not strong enough to detect in agarose gel electrophoresis visualization system. After detection of H.pylori infection status of patients, we detaily examined the prevalence of H.pylori specific virulence factors. Among the all active chronic gastritis patients, the most common virulence factors were napA (81%), vacA s1/s2 (56%) and hpaA (56%). This high prevelance of napA in active chronic gastritis was also shown in the literature (Mobley., 2001).The least prevalent virulence factors were oipA (19%), dupA (12%) and babA (6%) in active chronic gastritis patients. Furthermore, all intestinal metaplasia patients had ureA and hpaA virulence factors. VacA m1/m2, oipA, dupA and babA virulence factors were not detected in any intestinal metaplasia patient. VacA m1 / m2 was not detected in any patient for active chronic gastritis and intestinal metaplasia patients. There was no significant difference in virulence factors between the active chronic gastritis and intestinal metaplasia patients. It is known from the literature that, the presence of the cagA virulence factor repress inflammatory response and increase the risk of gastric cancer development (Suriani, Colozza,

Cardesi, & Zeneroli, 2008). Also, vacA increase the vacuolization of gastric epithelial cells (Rahimiana & Sanei, 2014).

H. pylori can survive for a long time in the gastric mucosa. Infections by *H. pylori* can cause the stimulation of host immune response (Robinson, Argent, & Atherthon, 2007). Initially, H.pylori infection, causes an infiltration of innate immune cells such as macrophages, monocytes and neutrophils to the site of infection, mediated through the induction of cytokines (Peek, Fiske, & Wilson, 2010). However, this innate immunity response is not specific to pathogenic antigens of H.pylori . Because of this non-specific feature of innate immunity, the clearance of H.pylori is generally failed. Then, more specific adaptive immune cells starts to accumulate into the site of infection (Sundquist & Quiding-Jarbrink, 2010). In literature, it was shown that T helper 1(Th1) and T helper 17 (Th17) are functional in clearance of H.pylori infection and Tregs are responsible for regulation of T cells (Larussa, Leone, Suraci, Imeneo, & Luzza, 2015) . So that, in this study we detaily examined the expression levels of the interferon-γ (IFN-γ), which is secreted from Th1 cells, and FOXP3, which is a Treg-specific transcription factor, in *H. pylori* -infected and -uninfected patients in gastric pathologies; active chronic gastritis, inactive chronic gastritis, intestinal metaplasia, and gastric cancer. Firstly, in this study, we investigated the expression levels of IFN-ɣ in gastric pathogenesis. We detected the lowest IFN-γ expression level in active chronic gastritis patients. This decrease was found as significant when compared to control group. The results of this study had shown that IFN-ɣ expression declined at a significant level in precancerous lesions when compared to control group. In previous study of our lab, it was shown that Th17 cells are predominantly expressed in H.pylori-infected active chronic gastritis patients (Oktem-Okullu & Tiftikci, 2015). This decrease of IFN-ɣ expression levels from uneffected, uninfected control to precancerous lesions may be explained by this dominantly expression profile of Th17 cells than Th1 cells in precancerous lesions. When we analysed the expression levels of IFN-ɣ depend on H.pylori-infection status, we observed that H.pylori infection did not affect the IFN-ɣ expression significantly. This may also due to the predominance of Th17 cells and their cytokines in *H. pylori* infection. As a future aspect, we may detaily examine the characterization of Th1 and Th17 cell profiles and their cytokine expression levels in precancerous lesions and gastric cancer.

It is known that in the literature, FOXP3 expression level increases in gastric cancer compared to control group (Chmiela & Karwowska, 2017). Surprisingly, in this current study, FOXP3 expression was detected in significantly lower level in gastric cancer patients than precancerous lesions and normal control

group. In literature, the effect of neoadjuvant chemotherapy in gastric cancer on tumor microenvironment has been already studied (Li, Chen, & Xie, 2016). Over the past few years neoadjuvant chemotherapy (NACT) has been used as main therapeutic strategy for gastric cancer. This treatment method directly affects the immune cell profile in tumor microenvironment (Li, Chen, & Xie, 2016). It was detected that,in response to NACT, Treg cells in tumor microenvironment decreased. So that, Treg specific transcription factor FOXP3 expression also decreased.

Conclusion

Gastrit cancer is the third most common cancer in the world. The occurrence of stomach cancer depends on many factors such as genetic susceptibility, environmental factors, diet and age. Among them, *Helicobacter pylori* (*H. pylori*) infection due to chronic inflammation is the main reason for developing gastric carcinogenesis. The stages of development of gastric cancer follow chronic gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia, respectively. In Turkish population, there are patients with gastric precancerous lesions at different levels due to the high incidence of *H. pylori* infection. Cytotoxin associated gene A (cagA), vacuolating cytotoxin A (VacA), outer inflammatory protein A (OipA), blood group antigen-binding adhesion (BabA), putative neuraminyllactose-binding hemagglutinin homolog A (HpaA), neutrophil activating protein A (NapA), duodenal ulcer promoting gene A (dupA) and urease subunit A (UreA), urease subunit B (UreB) are the main virulence factors of H.pylori which are related with H.pylori infection.

Virulence factors have important role colonization and adherence of bacteria on the gastric epithelium. However, there are many unknowns in the connection between the characteristic factors of *H. pylori* and the response of the host. It has been provided that CD4+CD25+ regulatory T (Tregs) cells infiltrate into the stomach tissue during H.pylori infection to providing a balance between the host and bacteria, also PD-L1 inhibits the T-cell receptor signalling and down-regulates T-cell response.

Recent studies suggest that *H. pylori* infection causes an increase in PD-L1 expression levels in human gastric epithelial cells and infected mice (Wu, Chen et al. 2011). However, in patients with precancerous lesions the expression status of PD- L1 and its relationship with *H. pylori*infection status is unknown. So that, for the first time the examination of PD-L1 expression levels in precancerous lesions and *H. pylori* infection were studied in this study. In our results, PD-L1 expression was detected in higher level in gastric cancer and precancerous lesions patients compared to control group. In literature it was

known that, tumor cells and some microorganisms could use PD-1/ PD-L1 regulatory mechanism as a way to suppress immune response in tumor microenvironment (Gong, Chehrazi-Raffle, Reddi, & Salgia, 2018). Especially, tumor cells mimics PD-L1 to inactivate T cells by binding to active T cells (Lu, Redd, Lee, Savage, & Liu, 2016). T cells are inactivated when their surface receptor PD-1 binds to its ligand PD-L1 (Shi, Chen, Yang, & Li, 2013). Therefore, the increase of PD-L1 expression in gastric cancer and precancerous lesions compared to control is an expected result. Furthermore, even it was not significant, there was an increase of PD-L1 expression in H.pylori-infected patients. This could be explained with H.pylori may use this mechanism to escape immune response. As a future aspect, by increasing the H.pylori-infected patient numbers, PD-L1 expression analysis will be extended.

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Chapter 17

Evaluation of the Relationship Between Temporomandibular Joint Disorders and Primary Headaches: A Multidisciplinary Approach to Orofacial Pain

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ABSTRACT

This chapter reviews the relationship between temporomandibular joint (TMJ) disorders (TMD) and primary headaches. TMD is a condition characterized by the TMJ's anatomical, morphological, and functional disorders. In this review, the etiologic relationships between TMD-related orofacial pain and primary headaches, as well as the current diagnostic and therapeutic modalities of these disorders, have been discussed. The link between headaches and TMD-related pain is complex and multifaceted. Among the factors that play a role in this relationship, central sensitization, muscle and joint fatigue, loss of function and fear of mobility, and cervical mobility seen in individuals with TMD and headaches are discussed, and pathophysiology is emphasized to increase the success of treatment by establishing a cause-effect relationship. Studies, diagnosis, and classification of TMD, types of headaches, mechanisms and risk factors that are blamed for the relationship between TMD-related pain and headaches are discussed in detail in this chapter. In addition, treatment modalities for both conditions are examined. Generally, these include patient education and lifestyle changes, pharmacologic treatment regimens, and non-pharmacologic treatment alternatives such as physiotherapy, postural therapy, elimination of parafunctions, elimination of respiratory problems such as sleep apnea, nasal concha hypertrophy, etc., and invasive methods such as botulinum toxin and local anesthetic injections and surgical procedures preferred in persistent cases.

Consequently, this chapter aims to develop new and up-to-date approaches to understanding the relationship between TMD-induced facial-jaw pain and headaches in close proximity and to increase treatment efficacy with a synergistic effect by treating both conditions. This chapter provides comprehensive information on etiology, diagnostic methods, and current treatment approaches for healthcare professionals interested in TMD and headaches. We hope this chapter will be useful for clinicians and researchers in these fields.

Keywords: Temporomandibular joint disorders, headaches, etiology, diagnostic methods, treatment approaches

INTRODUCTION

Temporomandibular joint disorders (TMD) are disorders in which the TMJ or masticatory structures are affected. The International Society for the Study of Pain (ISAP) has defined orofacial pain and detailed the relationship between them. According to ISAP, facial pain is defined as pain inferior to the orbitomeatal line, anterior to the auricle and superior to the neck. Headache is pain that occurs superior to the orbitomeatal line (International Classification of Orofacial Pain 1st Edition, 2020). The aim of this book chapter is to examine the relationship between TMD and headaches, one of the factors causing facial pain, and to contribute to the development of a multidisciplinary perspective for the treatment of orofacial pain.

In order to understand the possible relationship between headaches and TMD, it is first necessary to review the research on this subject. These studies have also shown that factors such as TMJ fatigue, kinesiophobia and cervical mobility have an effect on headache in individuals with TMD (Arıkan et al., 2018:40-42).

Thus, this chapter aims to provide information about the etiology of the relationship between TMD and headache, the methods used in the diagnosis and current treatment approaches. The diagnosis and classification of TMD, types of headaches, mechanisms and risk factors blamed for the relationship between TMD and headache, methods used in the treatment of TMD, and headache will be discussed. The primary purpose of this chapter can also be summarized as providing a holistic perspective for health professionals dealing with TMD and headaches.

TEMPOROMANDİBULAR JOINT DISORDERS AND PRIMARY HEADACHES

Temporomandibular Joint Disorders

Definition, Pathophysiology, Classification and Epidemiology: Temporomandibular joint disorders affect the masticatory muscles or the joint separately or together. Such disorders are prevalent in the population. They can cause significant limitations in the quality of daily life of the patient as they affect essential functions such as eating and speaking. The most common symptoms are pain starting from the ear and temporal region, which may radiate to the face, neck and other parts of the head, tenderness in the masticatory muscles, clicking sound from the joint during mandibular function and restriction in mandibular movements (Okeson, 2019).

The resting position of the TMJ is defined as the position in which the mouth is slightly ajar, the lips are together, the teeth do not touch each other, and the first half of the tongue is positioned on the hard palate (Aksoy, 2000, p. 1391).

The oral aperture within normal limits is between 35-50 mm. TMJ, masticatory muscles and surrounding soft tissues are called the “stomatognathic system”. The structures that make up this system are the TMJ, teeth, muscles and soft tissues (temporal, masseter, medial and lateral pterygoid muscles that close the TMJ and suprahyoid, infrahyoid and lateral pterygoid muscles that open the TMJ) and osseous structures (cranium, mandible, cervical and upper dorsal vertebrae, sternum) (Gezer Albayrak & Levendoğlu, 2016, p. 34).

Being a synovial joint, the TMJ's long-term joint health and function depends on the mechanisms that control stress on the articular surfaces. The synovial membrane, which covers the articular surfaces, synovial fluid, disc and fibrocartilage (articular cartilage) are involved in the lubrication of the TMJ. The lubrication mechanism of the joint is related to the movement of synovial fluid from one area to another during mandibular movements or the limited amount of fluid absorbed by the articular cartilage (Poveda-Roda, 2007:293).

TMJ is classified in many different ways. Bell (1982) introduced the classification system, which was later modified by Okeson (1998) and Wilkes (1989) and is now commonly used (Yalçın, 2010).

According to Wilkes' classification system, internal disorders of the TMJ are made by evaluating early, intermediate, and late clinical and radiographic findings.

- Stage 1: There is neither pain nor restriction of mandibular movements, only a reciprocal click during or after chewing. Radiographically, mild anterior disc displacement is detected.
- Stage 2: Mild to moderate pain with a reciprocal click and periodic locking. Radiologic examination may show a change in disc position.
- Stage 3: There is frequent pain, tenderness in the joint, and occasional and persistent locking. The radiologic evaluation shows changes in disc position with deformation and adhesions.
- Stage 4: There are severe chronic pain and restriction of mandibular movements. There are changes in the shape and position of the disc and condyle shape. Numerous adhesions are observed.
- Stage 5: There is occasional pain with crepitation. Chronic restriction of mandibular movements, anterior disc displacement and changes in its morphology occur. Large anatomical deformations and disc perforations are observed (Yaltırık et al., 2017).

The classification made by the Headache Society and modified by Okeson is as follows (Ekizer et al., 2016):

I. Disorders of the masticatory muscles:

Excessive use of the masticator muscles due to parafunctional movements (lip/cheek biting, teeth clenching, nail-biting, etc.) causes tense banding of the muscle tissue and associated pain trigger points, which can result in muscle spasm, tenderness, fatigue and dysfunction. With the aggravation of this condition, a common problem called myofascial pain syndrome (MPS) may occur (Özcan, 2005; Okeson, 2022).

II. Temporomandibular Joint Disorders:

It is based on a disorder in the condyle-disc complex. It is a disorder of the condyle-disc complex that interferes with the movement of the TMJ and causes a temporary feeling of the condyle catching the disc, clicking, popping and locking, depending on the position of the disc (usually in anterior or medial directions). There are three types of disorders of the condyle-disc complex:

Disc displacement: at rest, the condyle is more in contact with the posterior part of the disc and makes a sliding movement on the disc during function. This sliding movement occurs abnormally due to the displacement of the disc**.**

Disc dislocation with reduction: If the condyle starts an abnormal sliding movement after a short rotation but catches the disc, this is called reduction, during which a clicking sound is heard, and the mandible opens. If the click is heard only during the opening, it is called a single click; if it is heard during both opening and closing, it is called a reciprocal click. The reciprocal click is pathognomic for early-stage disc deposition (Özcan B, 2005). In disc dislocation with reduction, the disc is in the wrong position when the mouth is closed and in the correct position when the mouth is open.

Disc dislocation without reduction: When the elasticity of the superior retro-discal ligament decreases, it becomes difficult for the condyle to capture the disc during function, and the disc is positioned anterior and medial to the condyle in the mouth open and closed positions because it cannot be reduced. There is no clicking sound and a restriction in mouth opening. During mouth opening, the mandible shifts in the affected direction (deviation/deflection) depending on the limitation of function on the affected side. If the condition becomes chronic, the collagen fibers of the ligaments lose their tension, leading to increased mandibular movements. At this stage, crepitation is felt. This sound is caused by disc perforation (Manfredini, 2009, p. 213).

TMJ dysfunction is characterised by conditions in which the morphology of the articular surfaces is disrupted. In cases such as trauma, pathological causes, forcing the mouth to open too much, etc., sharpness and protrusions may occur on the osseous surfaces of the joint, and perforations may occur in the disc. As a

result, adhesions may occur, and changes occur in the normal function and movements of the joint (Melad, 2009).

Subluxation (Hypermobility): A clinical condition where the condyle moves anterior to the articular eminence (Melad, 2009). Patients with subluxation state that their jaws dislocate when they open their mouths too wide. In some patients, a clicking sound may be heard, but this sound is different from that heard in disc displacement. In fact, this sound may be best described as a popping sound. Usually, this clinical condition is painless (Okeson, 2019).

Spontaneous Dislocations: It can be described as open locking and is observed when the mouth is opened wide. At the maximum opening, the condyle makes a translational movement up to its anterior border. Meanwhile, the disc is able to rotate over the condyle up to its last posterior border. From this moment on, when the condyle moves beyond this limit, the disc is also forced forward. Due to the collapse of the anterior disc space, the disc is compressed in this space. Spontaneous dislocation may also occur due to contraction of the superior lateral pterygoid muscle at the last limit of translation.

Clinically, depression is observed in the preauricular region. There is severe pain, and patients usually cannot close their mouths (Yaltırık, 2017, p. 45).

Inflammatory diseases of the TMJ lead to degenerative changes in the TMJ and supporting anatomical structures due to parafunctional movements or some systemic or autoimmune diseases (Table 1). The inflammatory cascade caused by this type of degeneration can result in TMJ pain, joint tightness, limitation of function and joint noise.

I. Chronic Mandibular Hypomobility: It is a long-term but painless restriction of the mandible. Pain is observed as a result of excessive forces applied when the mandible is forced to open.

Ankylosis: It is formed due to adhesions developing on the joint's articular surfaces, preventing the joint's movements (Okeson JF, 2019). Ankylosis that develops due to fibrous adhesions or fibrotic changes in the capsular ligament is called fibrous ankylosis. As a result of the proliferation of bone cells, the fusion of osseous structures and complete immobility of the joint is called osseous ankylosis (Yaltırık, 2017, p. 46). Apart from these, myo-static or myo-fibrotic muscle contractions and impedance (resistance) of the coronoid process can also lead to chronic limitation of mandibular movements (Gezer Albayrak & Levendoğlu, 2016, p. 36).

II. Developmental Disorders and Others: TMD in this group are congenital or developmental conditions such as hypoplasia, hyperplasia, neoplasia, etc., of bone, cartilage or muscle tissues and cause asymmetry and loss of function rather than pain (Gezer Albayrak & Levendoğlu, 2016, p. 34).

TMD is the common name for a series of disorders that can be seen at any age, most commonly in young people, and whose symptoms include pain in the jaw and surrounding tissues during mandibular movements, limitation in mandibular movements and sounds such as clicking, crepitation, head, neck, ear, and toothache (Buescher, 2007, p. 1477). Symptoms related to TMJ disorders can be seen in 20% of the population at any time. The lifetime prevalence of TMJ disorders is between 3-15% in Western societies (Gezer Albayrak & Levendoğlu, 2016, p. 34).

Table 1: Classification of TMJ Disorders

Masticatory Muscle Disorders	**TMJ Disorders**	**Chronic Mandibular Hypomobility**	**Developmental Disorders et al.**
Protective contraction	**Disorders in the condyle-disc bones complex**	**Ankylosis**	**Congenital/developmental osteopathies**
Local muscle pain	1.Disk dislocation	1.Fibrous	1.Agenesis
Myofascial pain	2. Disc dislocation with reduction	2. Osseous	2. Hypoplasia
Myositis and others	3.Disc dislocation without reduction	**Muscle contractures**	3. Hyperplasia
	Malposition of the articular surfaces	1.Myostatic	4.Neoplasia
	1. Changes in form	2.Myofibrotic	**Congenital/developmental myopathies**
	2.Adhesions	**Coronoid impedance**	1.Hypotrophy
	Inflammatory diseases		2.Hypertrophy
	1.Synovitis/Capsulitis		3.Neoplasia
	2.Retrodiscitis		
	3.Arthritis		

Kaynak: Gezer Albayrak & Levendoğlu, 2016; 34-40

Evaluation of Temporomandibular Joint Disorders: Diagnostic Examinations, Scales and Imaging Methods

Diagnosis is based on clinical and physical examination. Palpation, auscultation, and muscle examination are the most essentialt steps of clinical examination. The TMJ should be palpated anteriorly to the tragus, over the condyle heads and from the external auditory canal. During palpation, the movements of the condyles, whether they are symmetrical or not, whether there is pain or not, and whether it is unilateral or bilateral should be determined by

opening and closing the mouth. The patient is asked to open and close the mouth and make protrusion and lateral movements. Restriction in movements, clicking or other joint sounds are noted. Mandibular movements should be straight during mouth opening and closing. If deviation is observed, it should be noted. With a stethoscope placed on the joint, it is determined whether there is a clicking sound or crepitation during opening and closing, whether unilateral or bilateral. The purpose of the muscle examination is to identify sensitive areas and to recognize referred pain. Referred pains mostly follow the anatomical structure of the muscle and show symptoms at a certain distance from the spastic muscle (Okeson, 2019).

The most accepted diagnostic method used in the diagnosis of TMJ is the Diagnostic Criteria of TMD: DC/TMD Form (Schiffman et al., 2014, p. 6). This form consists of two main parts (Axis I and II) and is developed by the authorities to make an accurate diagnosis with a holistic approach by analyzing forms for detailed examination of the TMJ and external criteria, including stress and psychological factors and parafunction.

Although many imaging methods such as conventional radiography (transcranial plain radiography when the mouth is in the open-closed position), panoramic mandibular radiography, ultrasonography (USG), computed tomography (CT), cone beam computed tomography (CIBT), magnetic resonance imaging (MRI), cephalometric radiography and radio nuclear imaging techniques can be used for diagnosis, the most valuable imaging method for the articular disc and surrounding structures is MRI, which is considered the gold standard today (Tognini et al., 2005, p. 252; Fallon SD et al., 2006, p. 223). On the other hand, CT or CIBT is more successful in detecting degeneration of the bone surface and hard tissues. However, its clinical use is more limited due to its high radiation dose (Jeon, 2020:3599). Arthrography, one of the imaging methods used in the diagnosis of TMD, is a technique in which the image of the disc is obtained by injecting radiopaque contrast material into the joint cavity. Pathologies such as disc displacements, disc perforation, degenerative diseases, etc., can be evaluated in this imaging method. It is not widely used in clinical practice due to its invasiveness, radiation effect, allergic reaction to the contrast material and uncomfortable approach. Arthroscopy, on the other hand, is a surgical technique applied with the help of optical instruments, which is used to examine the joint surfaces with an endoscopic approach in advanced degenerative joint diseases, as well as to wash the joint surfaces for treatment purposes and to separate the adhesions in the synovial membrane (Yaltırık et al., 2017, p. 48).

Headaches: Definition, Physiology, Classification and Epidemiology

The leading pain problem, headaches, is a health problem that seriously affects many individuals' daily lives. It has been reported that a high rate of 25% of the reasons why patients apply to primary health care services is headaches (Kaygısız et al., 2016). Headaches are analyzed into two main groups: primary and secondary headaches, and approximately 90% are primary.

Migraine: Among the most common primary headaches, migraine is characterized by symptoms such as nausea, aura, sensitivity to light and sound, and its pathophysiology and treatment are the subject of ongoing research in the field of neurology. Migraine is defined as a recurrent neurological disorder that causes severe headaches. Migraine-type headaches, mostly pulsatile, are usually unilateral and rarely bilateral. Migraine is a disorder characterized by attacks, and although the duration and severity of these attacks vary, they usually cause moderate pain. Typical migraine symptoms include:

- Headache
- Nausea
- Sensitivity to light and sound
- Aura (transient neurological symptoms seen in some migraine patients before the onset of the headache and symptoms such as visual impairment, numbness, or tingling sensation, etc., which warn the patient that the headache and migraine attack will begin) (Goncalves, 2013).

Tension-type headache: Among primary headaches, tension-type headaches are highly prevalent in the community and are generally associated with psychological stress and muscle tension (IHS, 2013). It has also been reported that the prevalence of headaches in the community is much higher in women than in men (Özbenli, 2014-2015).

If there is a secondary event that causes a headache, this is defined as a secondary headache, and the most common causes include cerebral pathologies, infections, and vascular anomalies. According to the declaration of the International Headache Society (IHS), the physiologic classification of headaches is presented in Table 2 below (IHS, 2013).

Table 2. Classification of Headaches

Primary Headaches	1. Migraine
	1.1. Migraine without aura
	1.2. Migraine with aura
	2. Tension-type headache
	2.1. Infrequent episodic tension-type headache
	2.2. Frequent episodic tension-type headache
	2.3. Chronic tension-type headache
	3. Cluster headache and other trigeminal autonomic cephalalgias
	3.1. Cluster headache
	4. Other primary headaches
Secondary Headaches	5. Headache attributed to head and or neck trauma
	5.1. Chronic post-traumatic headache
	6. Headache attributed to a cranial or cervical vascular disorder
	6.1. Headache attributed to subarachnoid hemorrhage
	6.2. Headache attributed to giant cell arteritis
	7. Headache attributed to non-vascular intracranial disorder
	7.1. Headache attributed to idiopathic intracranial hypertension
	7.2. Headache attributed to an intracranial neoplasm
	8. Headache attributed to a substance or its withdrawal
	8.1. Carbon monoxide-induced headache
	8.2. Alcohol-induced headache
	8.3. Medication-overuse headache
	8.3.1. Ergotamine-overuse headache
	8.3.2. Triptan-overuse headache
	8.3.3. Analgesic-overuse headache
	9. Headache attributed to infection
	9.1. Headache attributed to intracranial infection
	10. Headache attributed to disorder of homeostasis
	11. Headache or facial pain attributed to a disorder of the cranium, neck, eyes, ears, nose, sinuses, teeth, mouth or other facial or cranial structures.
	11.1. Cervicogenic headache
	11.2. Headache attributed to acute glaucoma
	12. Headache attributed to psychiatric disorder
Neuralgias and Other Headaches	13. Cranial neuralgias, central and primary facial pain and other headaches
	13.1. Trigeminal neuralgia
	Other headache, cranial neuralgia, central or primary facial pain

Kaynak: Kaygısız vd., 2016

Evaluation of Primary Headaches: Diagnostic Examinations, Scales, and Imaging Methods

The most crucial method in diagnosing headaches is thought to be history taking. The things that should be questioned in an accurate history taking are as follows (Özbenli, 2014-2015):

- Time of occurrence, duration and frequency of pain
- Nature of the pain (throbbing, stabbing, electric shock, etc.)
- Location of the pain
- The character of the pain (episodic, chronic, etc.)
- The onset of pain and triggering factors (physical factors, specific foods, physiological conditions such as menstruation or hunger, etc.)
- Severity of pain
- Symptoms accompanying pain (photophobia, nausea, etc.)
- Conditions that reduce pain (silence, darkness, rest, etc.)
- Family history
- Response to analgesics

Clinicians also frequently prefer to have patients keep a pain diary to determine this history (Rothrock, 2006).

The classification used to diagnose primary headaches is the current classification of the International Headache Society (IHS, 2013) and is still considered the gold standard (Table 2). However, MIDAS (Migraine Disability Assessment), used in the diagnosis of migraine, is a scale used to assess the impact of migraine and was developed to determine the impact of migraine on activities of daily living. This scale assesses the loss of labor force, social interactions, housework, and leisure time activities caused by migraine and helps to determine the limitations and effects of migraine. The MIDAS score is used to assess the impact of migraine. MIDAS scores help to classify migraine as mild, moderate, severe or extremely severe. These scores (Table 3) can also be used to evaluate the effectiveness of migraine treatment and to determine treatment plans (Stewart et al., 2001).

Table 3. MIDAS Rating

Rating
I

Kaynak: Ford JH vd., 2020; 2021.

The ID- Migraine test, which is recommended for the practical and accurate diagnosis of migraine, which ranks first in terms of referral to health institutions, consists of three questions asked to patients and has taken its place in diagnosis

as a method to question the last three months (Table 4). The patient who responded positively to two of these three questions was reported to be diagnosed with migraine with a probability of over 90% (Martelletti, 2013).

Table 4. ID-Migraine test that can be used for Migraine Diagnosis During Primary Care

1.	**1. Do you experience nausea during your headache?**
2.	**2. Does your headache prevent you from working or doing your daily activities?**
3.	**3. Do you feel sensitive to light during your headache?**

Kaynak: Kaygısız Ş, 2016.

TREATMENT AND MANAGEMENT APPROACHES

Treatment of Temporomandibular Joint Disorders: Pharmacological, Physical, Behavioral and Surgical Methods

Since TMJ disorders are mostly musculoskeletal disorders, the first approach to be used in treatment is to reprogram the routine life activities of the patients. The patient should start function restriction to reduce the pressure on the TMJ and masticatory muscles, open the mouth within painless limits, avoid hard foods, and fast and intense chewing. Parafunctions such as clenching teeth, nail-biting, etc., should be abandoned. The patient should learn deep and nasodiaphragmatic breathing instead of oral and superficial breathing (Gezer Albayrak & Levendoğlu, 2016, p. 37). Apart from habitual conditions, if there is an obstructive airway problem such as sleep apnea or nasal turbinate hypertrophy, it should be treated accordingly. The patient should also be subjected to an approach that includes postural and physical therapy (Sheats, 2017, p. 111).

Pharmacologic Treatment: Analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, muscle relaxants for the central or peripheral nervous system, and antidepressants are the agents used in the pharmacologic treatment of TMD. Although there is no clear consensus on the doses and combinations of these medications, pharmacologic treatments are generally preferred for conditions involving the masticatory muscles, myofascial pain syndrome and inflammatory diseases of the TMJ. Simple analgesics are preferred in mild to moderate pain cases, and narcotic analgesics may be used in cases of very severe pain (Fernandez, 2002, p. 1161). Most patients with chronic pain have depression and sleep disorders. Therefore, tricyclic antidepressants have been reported to be the main treatment in such cases (List, 2010). In conclusion, analgesics and local corticosteroids should be preferred in acute TMJ pain, NSAIDs and muscle relaxants in acute and chronic pain, and tricyclic antidepressants in chronic pain.

Physical therapy methods: Transcutaneous electrical stimulation (TENS), superficial and deep hot applications, cold application, massage, biofeedback (applied with EMG), iontophoresis, phonophoresis, galvanic stimulation, ultrasound, laser, microwave, manual therapy techniques, trigger point injection, acupuncture and exercise are the most commonly used physical therapy agents. Common mechanisms of action of physical therapy methods are to reduce pain, relieve muscle spasms, reduce inflammation, accelerate muscle coordination and tissue regeneration, and increase function (Çapan, 2010, p. 56; Okeson, 2022).

Oculus therapy: The orthopedic appliances used in occlusal therapy, splints, are removable appliances made of hard acrylic. They cover the occlusal and incisal surfaces of the teeth in the arch and contact the teeth in the opposite arch in the determined occlusion. These appliances have different types, such as occlusal stabilization splints, anterior positioning splints, anterior bite plates, posterior bite plates, and tripod (pivoting) splints (Ramoğlu, 2010:914-16.).

Trigger point injection therapy: Trigger point injection can be performed in case of myofascial pain. Severe pain is felt when the trigger point is reached during the injection. This method is often preferred to reduce pain by relieving muscle spasms in trigger points using local anesthetic agents or dry needling (Gezer Albayrak & Levendoğlu, 2016, p. 38).

Intra-articular injection therapy: Injections are preferred in cases of internal degeneration in the acute exacerbation period or in cases of internal degeneration that do not respond to conservative treatment, especially in unreduced disc displacement. Local anesthetics or corticosteroids can be administered intra-articularly to treat inflammation in the capsule. However, repeated corticosteroid injections are not recommended because of possible damage to the articular cartilage. However, intra-articular sodium hyaluronate injections are among the most common treatment options (Güven, 2004, p. 47).

Botulinum toxin injection therapy: It has been reported that neurolysis of botulinum toxin-A administered intramuscularly (usually masseter and temporal muscles) has become a necessary treatment regimen by temporarily paralyzing the muscles. In addition, local anesthetics or injection of botulinum toxin into myofascial trigger points are recommended in the treatment of chronic bruxism (Zhang, 2017, p. 736).

Surgical treatment: The surgical approach is applied to patients with TMD who do not respond to conservative treatment, and the rate of these cases in all TMD cases has been reported to be approximately 5%. The surgical approach is mainly indicated in cases such as infection, fracture, neoplasia, inflammation of the TMJ, acute non-reduced disc replacement, or ankylosis (Gezer Albayrak & Levendoğlu, 2016:38-39).

Treatment of Primary Headaches: Pharmacological, Physical, Behavioral and Surgical Methods

Lifestyle counselling and patient education: For treatment of primary headaches, especially migraine and tension-type pain the first step in the treatment of primary headaches, especially migraine and tension-type pain, is to create daily routines that will avoid the factors that trigger these pains and include mitigating factors by making arrangements to improve living conditions and quality. Regular, adequate and high-quality sleep and nutrition, adopting lifestyle changes away from foods that trigger migraine attacks and psychological stress reduces attacks in most patients (Kaygısız et al., 2016).

Pharmacologic treatment: Patients should be made aware of how to control their pain attacks; general information about the importance of taking acute attack medications as early as possible in the attack, the importance of using metchloropamide-derived antiemetics before analgesics, and the need to avoid overuse should be adopted (Özbenli et al., 2014-2015). Although the efficacy of triptans (serotonin agonists) in migraine patients with peripheral and central sensitization is controversial, these drugs have a place in treating primary headaches (Silberstein, 2004). Generally, it has been reported that NSAIDs, muscle relaxants and antidepressants can also be used in treatment (Okeson, 2019).

Prophylaxis: Prevention of migraine includes prophylactic practices aimed at reducing the frequency, duration and severity of migraine attacks. For this purpose, a drug regimen called migraine prophylaxis is used. For preventive treatment, vasodilator drugs (beta blockers) and monoclonal antibody therapy (vaccine), such as galcanezumab, are generally preferred (Ford JH, 2020-2021). The basic principle is to start with small doses and gradually increase the recommended medication to control the side effect process. Likewise, the drug should be discontinued a gradual reduction when terminating treatment to prevent the return of attacks (Kaygısız et al., 2016).

Anticonvulsants are also frequently used in prophylactic treatment in patients diagnosed with migraine with aura or drug overuse headaches with frequent and severe attacks. The correct prophylaxis regimen should be given considering the patient's personal characteristics and should be determined according to the patient's clinical course for at least six months. Techniques such as acupuncture, massage and meditation can also be tried as paramedical treatment methods for tension headaches.

Verapamil is the first choice drug in the treatment of cluster headache attacks. Oxygen therapy, sumatriptan injection, or intranasal lidocaine in resistant cases

have been reported. Other options include lithium, valproate, topiramate or melatonin, and prednisolone can be used in short-term preventive treatment (İnan, 2011; Romero-Reyes & Uyanik, 2014; Kaygısız et al., 2016).

Invasive treatments: Intramuscular injections of botulinum toxin (Botox) are frequently used in migraine treatment and have been proven in clinical studies to help patients reduce the frequency, duration and severity of migraine attacks. Nerve blockage aims to stop pain stimulation by blocking specific nerves with local anesthetic drugs, and the target nerves in migraine treatment are the occipital nerve and its branches located in the back of the head and sometimes the ocular nerve for headaches triggered by certain eye diseases. Neuromodulation therapy is another minimally invasive treatment option, using modulation devices such as transcranial magnetic stimulation (TNS) devices to alter nerve impulses and thereby reduce pain sensation. Finally, migraine surgery can be preferred in rare cases, significantly to reduce chronic migraine pain by removing the compression on the nerve. The place of surgical migraine treatment is in the last step; surgery is used when all conservative methods are not successful.

Which of these treatment methods should be used in combination with each other should be determined by comparing success and effectiveness with empirical methods, considering the personal characteristics and conditions of the patient. (Romero-Reyes & Uyanik, 2014; Kaygısız et al., 2016; Jenkins, 2020; Suri & Ailani, 2021).

THE RELATIONSHIP BETWEEN TEMPOROMANDIBULAR JOINT DISORDERS AND PRIMARY HEADACHES

Headache and TMJ disorders have been important research topics in recent decades (Graff-Radford & Abbott, 2016, p. 335). The focus of research on this topic has been on the similarities in the pathophysiology of these two painful conditions and identifying triggers for each other.

The TMD is characterized by pain and dysfunction in the mandibular joint and associated muscles. Headaches, on the other hand, are an area of clinical neurology that continues to be studied and researched. Although TMJ may originate as the cause of TMJ pain, it may also occur as a reflection of another disease or pain. It has been emphasized that masticatory muscle tenderness may develop in chronic types, and pain modulation may also change. This group of patients may present to the clinic with various clinical presentations (tinnitus, dizziness, neck pain, etc.). The relationship between orofacial pain and primary headaches and the pathophysiology of the clinical findings are still under investigation, and some hypotheses have been proposed. For example, tension-type headache is associated with many types of back and neck pain and psycho-

stress. Research has shown that episodic tension-type headache is more commonly associated with TMD than migraine headaches (Schiffman et al., 2014). There are several reasons for the link between TMD and headaches:

- Muscle tension and pain trigger points: Individuals with TMD usually experience pain in the maxillofacial and neck muscles. The stress associated with these pains often follows a pattern that radiates towards the temple, forehead and back of the head (Schiffman et al., 1995, p. 122).
- Loss of function in the TMJ: It was reported that soft and hard tissue tensions resulting from dysfunction in the mandibular joint could trigger tension-type headaches or migraine headaches (Ekizer et al., 2016, p. 15).
- Bruxism: The habit of involuntary clenching and grinding of teeth may cause muscle fatigue and increased tension in the muscle groups adjacent to the maxillofacial and TMJ, triggering headaches (Okeson, 2019).
- Central sensitization (central sensitization): TMD can cause chronic painful episodes, especially in the later stages. Such chronic pain conditions make the individual increasingly sensitive to pain signals generated by the central nervous system (CNS) (Graff-Radford & Abbott, 2016, p. 339).

Multidisciplinary Approach in the Treatment of Temporomandibular Joint Disorders and Headaches:

Adequate treatment of headaches and TMD requires a multidisciplinary approach. The disciplines that should be included in the treatment team of such an approach are dentists, neurologists, physiotherapists and other health professionals when necessary. The treatment approach should be determined according to the individual complaints and needs of the patients (Fernandes, Franco et al., 2013:19). Treatment principles are as follows (Graff-Radford & Abbott, 2016 p. 340):

- Education: Patients should first have detailed information about TMD and their headaches. This will increase patient cooperation and awareness to achieve an effective treatment outcome.
- Self-care: This includes exercises, massage, and stress management methods that patients should do at home, as well as habits and actions to avoid.

- Behavioral therapy and referrals: These are methods specifically aimed at reducing patients' stress levels and improving their quality of life.
- Pharmacological treatment: Mainly includes analgesics and anti-inflammatory drugs for pain, central and peripheral muscle relaxants, tricyclic antidepressants and vasodilators for migraine prophylaxis. Pharmacologic therapy should be tailored to the individual patient and should be multidisciplinary.
- Physical therapy: These methods, which are used in treating problems related to the kinetic system of the body and aimed at goals such as increasing muscle strength and flexibility and correcting posture, consist of massages and exercises performed and performed by professionals.
- Dental treatment: It usually includes orthopedic mandibular appliances, dental occlusion correction, intramuscular and intra-articular injections and, in advanced cases, surgical treatment.

DISCUSSION AND CONCLUSION

The primary headaches, especially migraine and tension-type headaches, may present as facial pain. The activation of the ophthalmic and maxillary branches of the trigeminal nerve causes the spread of this pain. As known, the trigeminal nerve's ophthalmic branch is found to be responsible for migraine's pathophysiology, but sometimes the maxillary and mandibular branches are rarely involved in this process. This rare regional distribution may present with migraine-like symptoms such as photophobia-phonophobia, nausea-vomiting or autonomic symptoms. According to some authors, it has also been called lower facial migraine or neurovascular orofacial pain. In an orofacial study, the frequency of headaches spread to the face was shown to be 10% (Mitrirattanakul & Merrill, 2006).

Central sensitization has been defined as sensitization involving nociceptive neurons in the central nervous system (a high-threshold sensory nerve response of the peripheral somatosensory nervous system that converts and encodes transmitter stimuli) (ICOP, 2020). In this relatively high sensitization, even a mild stimulus can elicit a pain response, increasing the likelihood of an individual experiencing headaches. Some research suggests that the trigeminal nerve, which plays a vital role in pain perception in the maxillofacial and head regions, may be an essential bridge in this relationship (Graff-Radford & Abbott, 2016, p. 339).

The neuroanatomical and functional connections between the masticatory and cervical regions are the physiologic relationship that best explains the association of maxillofacial and head and neck symptoms. It is also emphasized that there may be a synergistic relationship between the presence of pain in the masticatory

system and the occurrence of cervical spine dysfunctions and related pain (Arıkan, 2018, p. 40).

Although primary headaches are among the most common pathophysiological conditions encountered by neurology, TMD-related headaches are neurologically considered secondary headaches. This type of pain can be unilateral or bilateral but is usually seen in the masseter and temporal regions of the face (HIS, 2013). Researchers emphasize correctly identifying possible motor behavior changes in people suffering from TMD. In particular, the occurrence of pain during TMJ function causes difficulties in mandibular movements and knowing the masticatory fatigue is very important in determining the correct treatment approach (Schiffman et al., 2014, p. 6; Arıkan et al., 2018, p. 40).

Garrigós-Pedrón et al. (2018) drew attention to the relationship between TMD and chronic migraine in their clinical study; they emphasized that these two conditions show a similar etiological scheme through neuron sensitivity in the trigeminocervical complex. However, they also reported that TMD increases the frequency of headaches and contributes to the chronicization of migraine; therefore, it is necessary to address both conditions together in treatment approaches. For this purpose, they mentioned that cervical physical therapy should be added to orofacial pain treatment.

The trigeminovascular theory is the most accepted view of the mechanisms

of primary headaches today. According to the trigeminovascular theory, which is the most accepted theory in the pathophysiology of migraine, it is noteworthy that the pain is clinically expected to be mainly in the facial region, but it is rare. One of the most critical issues here is to emphasize that the fact that the concepts of facial pain and headache are considered afferent entities is not a clear distinction because, as we mentioned before, all of these pains are symptoms that occur as a result of intertwined mechanisms (Goadsby PJ, 2012). Studies have been conducted on the dual innervation of the facial nerve as an example of such a relationship.

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Chapter 18

Is "Infantile" Hypertrophic Pyloric Stenosis An Accurate Definition?

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ABSTRACT

Infantile hypertrophic pyloric stenosis (IHPS) is a disease characterized by projectile nonbilious vomiting, typically in the first 3-6 weeks of life. In this study, it was aimed to discuss whether the disease is a congenital or an infantile pathology by examining the diagnosis time of patients who were operated for IHPS.

The data of patients who were operated with the diagnosis of IHPS between 01/01/2016 - 01/12/2020 were examined. The data regarding the diagnosis time of the patients in this patient group were analyzed retrospectively.

Twenty-two patients were operated with the diagnosis of IHPS. The median value of diagnosis time 28,5 days. 4 (18%) of patients were diagnosed with clinical symptoms within the first week of life. One patient in the patient group was diagnosed with gastric dilatation and hypertrophic pyloric stenosis in ultrasonography performed in the third trimester during antenatal period.

IHPS can be diagnosed earlier in life and even in the antenatal period. This situation suggests that the disease may actually be a congenital pathology as in its old definition.

Keywords: Congenital disorders, newborn, pyloric stenosis, ultrasound.

1.Introduction

Infantile hypertrophic pyloric stenosis (IHPS) is a disease caused by pyloric hypertrophy that can progress to almost complete obstruction of the gastric outlet, leading to severe vomiting. IHPS is the most common cause of gastric outlet obstruction in newborns (Puri P et al.,1999:266). Although the frequency of IHPS varies from region to region, its frequency is approximately 2 to 3.5 per 1000 live births (To T et al.,2005:159). The etiology of IHPS is still unclear. It is considered to be multifactorial. It is a disease characterized by projectile nonbilious vomiting, first several times and then increasing in frequency, typically in the first 3-6 weeks of life. In this study, it was aimed to discuss whether the disease is a congenital or an infantile pathology by examining the diagnosis time of patients who were operated for IHPS in Health Sciences University Van Education and Research Hospital.

2.Materials and methods

2.1.Ethics Committee Approval

The study was conducted after obtaining approval from the Health Sciences University Van Education and Research Hospital Clinical Research Ethics Committee (approval#10.12.2020-2020/24).

2.2.Study Design

The data of patients who were operated with the diagnosis of IHPS between 01/01/2016 - 01/12/2020 in the department of pediatric surgery of XXX XXX University XXX Education and Research Hospital were examined. The data regarding the diagnosis time of the patients in this patient group were analyzed retrospectively.

2.3.Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA). Relationships between continuous variables were evaluated using Spearman's correlation test and Student's t-test. A value of $p<0.05$ was considered statistically significant.

3.Results

Twenty-two patients with the diagnosis of IHPS were operated between 01/01/2016 - 01/12/2020 in our clinic. The diagnosis of the patients were made by ultrasonography, laboratory findings and physical examination. The diagnosis times and ultrasound findings of the patients are shown in Table-1. The median value at the diagnosis time was found to be 28,5 days. One patient in the patient group was diagnosed with gastric dilatation and hypertrophic pyloric stenosis in ultrasonography performed in the third trimester during antenatal period. The postnatal plain abdominal x-ray of this patient is shown in Fig. 1. The diagnosis of IHPS was also confirmed with the postnatal ultrasonography.

In ultrasonographic findings, the average pyloric muscle thickness was 5,2mm, pyloric muscle length was 20,8mm and pyloric diameter was 14,1mm. "Olive" was palpated in 8 of 22 patients.

The patient group was divided into two groups as before and after the diagnosis time 21 days. There was no statistically significant difference between these two groups in ultrasonographic data.

Four of 22 patients were born before 37 weeks of gestation. 18 patients were breastfed postnatally by the mother. 3 patients were formula fed with a bottle and 1 patient was diagnosed and operated on without oral feeding.

4.Discussion

Infantile hypertrophic pyloric stenosis is an interesting disease that usually presents in the first 3 to 6 weeks of life, is characterized by projectile and non-bilious vomiting, and presents with severe malnutrition, dehydration and acid-base imbalance if not recognized and treated in time. The full definition of the disease was made by Hirschsprung in 1888 (Hirschsprung H, 1988:61). Its etiology is still unclear. Neonatal hypergastrinemia and gastric hyperacidity are thought to play a role (Rogers Im, 1997:6). In addition, prematurity (<37 weeks of gestation) is thought to be effective (Svenningsson A et al, 2014:1226). Maternal smoking during pregnancy increases the risk of IHPS 1.5 to 2 times (Svenningsson A et al, 2014:1226). It is also suggested that feeding with a bottle rather than breastfeeding increases the risk of IHPS (McAteer JP et al, 2013:1143). In addition, several genetic loci that predispose to IHPS have also been identified. It has also been suggested that the use of both erythromycin and azithromycin increases the risk of IHPS, especially in infants younger than two weeks (Eberly MD et al, 2015:483; Centers for Disease Control and Prevention, 1999:1117)). In patient group, it was found that 4 out of 22 patients were born before 37 weeks of gestation. None of the mothers of the patients had a history of smoking and / or drug use during pregnancy and it was observed that there was no gestational pathology. None of the patients had a history of postnatal use of erythromycin or azithromycin. It was found that 18 of 22 patients were breastfed postnatally by their mothers, and 3 patients were fed formula with a bottle. One patient was diagnosed and operated on without postnatal oral feeding.

Although the word "congenital" is mentioned in the definition of the disease, it is still a matter of debate whether the disease is congenital or not. The general opinion is that hypertrophic pyloric stenosis is not congenital and is an infantile disease. The most important reason why the disease is thought to be infantile is that no pyloric stenosis was found in autopsies of stillborn babies. The disease occurs within the first week of life, but clinical symptoms become evident after

2-3 weeks (Geer LL et al, 1985:205). Therefore, hypertrophic pyloric stenosis is accepted as an acquired disease rather than congenital (Rollins MD et al, 1989:138). However, although rare, there are case reports diagnosed antenatally (Katz S et al, 1988:1021). In our department, 9 (40%) of 22 patients who had clinical findings due to IHPS and were operated after the diagnosis was confirmed by ultrasonography, were diagnosed within the first 3 weeks postnatal. 4 (18%) of these 22 patients were diagnosed with clinical symptoms within the first week of life. In addition, an appearance compatible with gastric dilatation and hypertrophic pyloric stenosis was detected in the ultrasonography performed in the antenatal third trimester of a patient born at 34 gestational weeks with a weight of 2860 g. The diagnosis of this patient was verified by postnatal plain abdominal x-ray and abdominal ultrasonography. All patients were treated with Ramstedt pyloromyotomy and no recurrence was observed (Fig. 2). Contrary to conventional knowledge, 18% of the patients in our study were diagnosed in the first week of life. In addition, one patient was diagnosed in the antenatal period. Contrary to conventional knowledge, 18% of the patients in our study were diagnosed in the first week of life. In addition, one patient was diagnosed in the antenatal period. This information is remarkable. Diagnosis of the patients during this period brought the agenda again that the disease might actually be a congenital pathology. In addition, the widespread use of ultrasonography, the experience of the ultrasonographist, and the increase in the awareness of physicians about IHPS may have caused the early diagnosis of the disease.

The sensitivity and specificity of ultrasonography for IHPS performed in experienced hands is above 95 percent, but this rate depends on the expert performing ultrasonography (Niedzielski J et al, 2011:508). The most commonly used measurements in ultrasonography are pyloric muscle thickness, pyloric muscle length and pyloric diameter. Criteria defining the upper limits of normal range from pyloric muscle thickness 3 to 4 mm, pyloric muscle length 15 to 19 mm, and pyloric diameter 10 to 14 mm. (Hallam D et al, 1995:261). Measurements within or above these ranges support the diagnosis of IHPS. In ultrasonographic findings, the average pyloric muscle thickness was 5,2mm, pyloric muscle length was 20,8mm and pyloric diameter was 14,1mm. These findings are consistent with the literature. In 8 of 22 patients “olive” was palpated. The reason for this low rate is probably due to the widespread use of ultrasonography. The use of ultrasonography in diagnosis has become gold standard. Therefore, physicians rely more on ultrasonographic findings than on physical examination. The median diagnosis time of the patient group was 28.5 days. The patient group was divided into two groups as before and after the diagnosis time 21 days. There was no significant difference in ultrasonographic

measurements between these two groups. In addition, there was no correlation between the diagnosis time of the patients and ultrasonographic findings. In line with this information, it is not possible to determine how long the pathology occurred in patients according to ultrasonographic measurements. Moreover, although the disease is considered a progressive process, it is not possible to predict when the pathology first appeared. The fact that the patients do not have ultrasonographic measurements made periodically by the same ultranographist is one of the reasons why the progressive process cannot be proven. However, since these patients are operated after their preoperative preparations as soon as they are diagnosed, waiting for surgery or trying to demonstrate the progressive process with repeated ultrasonographic measurements will create ethical problems. For these reasons, it is not possible to say clearly whether the disease develops in a congenital process or occurs postnatally. However, the presence of antenatal cases described in the literature, the fact that there is a patient with an antenatal diagnosis in our study group and that 18% of the patients in the study group were diagnosed in the first week of life made the idea that this disease develops congenitally. Of course, it is not possible to say "hypertrophic pyloric stenosis is a congenital pathology" based on these data alone. There is a need for larger and multicenter case series, experimental studies and genetic studies. However, we think that this article can be a guide in this direction.

5.Conclusion

Infantile hypertrophic pyloric stenosis is a pathology whose etiology is still unclear, usually manifests in the first 3-6 weeks of life with its classical clinical presentation and is diagnosed ultrasonographically. IHPS can be diagnosed earlier in life and even in the antenatal period. This situation suggests that the disease may actually be a congenital pathology as in its old definition. However, this situation still remains a mystery. As Nietzsche said, "*Every profound spirit needs a mask: even more, around every profound spirit a mask is continually growing*" (Parkes G, 1987:65). Nevertheless, of course, multicenter case series, experimental and genetic studies are needed to clarify such a situation.

6. Figures

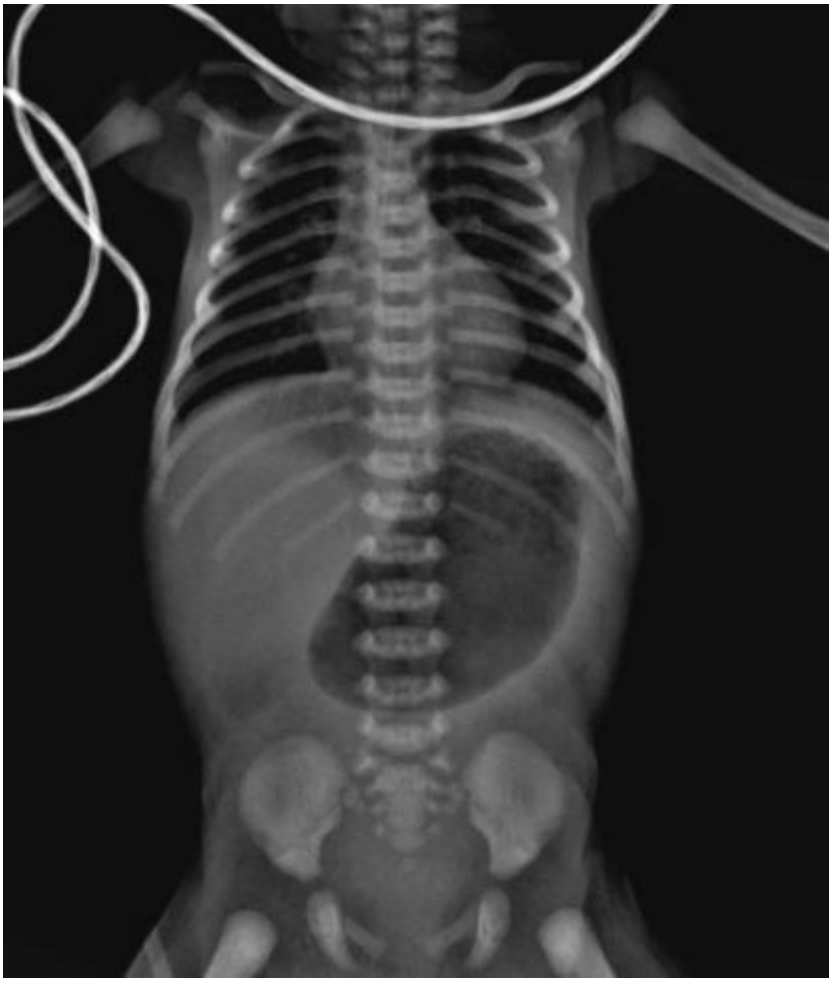

Figure 1: Plain abdominal x-ray of patient diagnosed antenatal infantile hypertrophic pyloric stenosis.

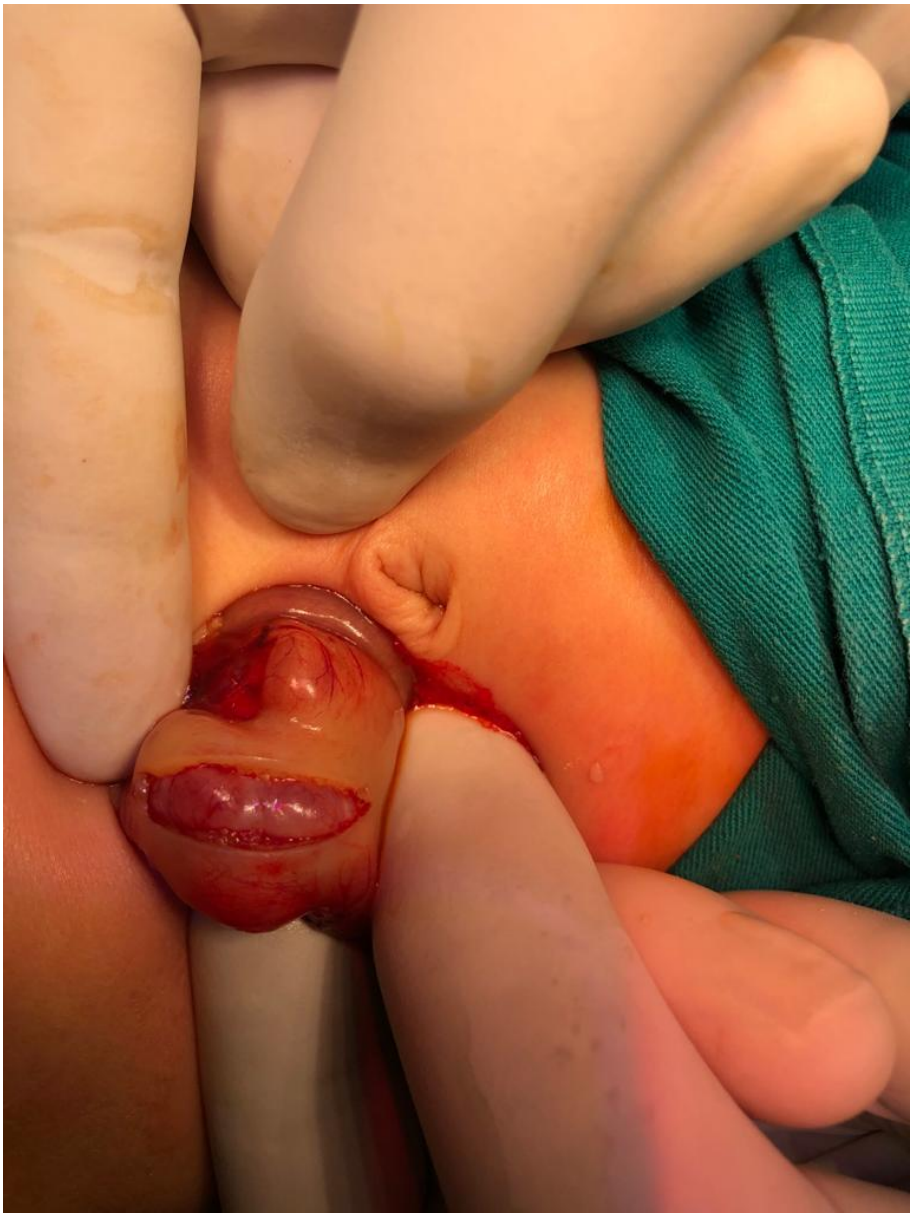

Figure 2: Ramstedt pyloromyotomy.

7.Table

Table 1: Ultrasonographic measurements and diagnosis time of patients operated for infantile hypertrophic pyloric stenosis (PMT: Pyloric muscle thickness, PML: Pyloric muscle length, PD: Pyloric diameter).

Patient		Diagnosis time (day)	Ultrasonographic findings		
			PMT (mm)	PML (mm)	PD (mm)
1		51	6	20	15
2		50	6,7	20	14
3		45	6,5	23	16
4		44	4,6	18	13
5		42	5,5	21	16
6		42	6	22	18
7		40	4,8	19	15
8		40	4,8	19	15
9		36	4	24	11
10		33	5	18	14
11		29	4,5	19	13
12		28	5	20	13
13		26	4,5	19	13
14		19	7	23	17
15		17	6	23	13
16		17	6,7	20	17
17		17	4,5	21	11
18		13	4	16	12
19		3	5	25	12
20		3	4	22	15
21		2	4	25	11
22		0	7,3	22	18

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Chapter 19

Effects of Physical Activity on Neurogenesis and Cognitive Function

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ABSTRACT

Physical activity is known for its positive effects on overall health and well-being. In recent years, research has focused on the impact of physical activity on neurogenesis and cognitive function. Physical activity has been found to enhance neurogenesis in the hippocampus, a region crucial for learning and memory. Mechanisms such as increased blood flow, neurotrophic factors, and reduced inflammation are thought to contribute to exercise-induced neurogenesis. These factors create a favorable environment for the generation and survival of new neurons, leading to structural and functional changes in the brain. Beyond the hippocampus, physical activity also promotes neurogenesis in other brain regions, including the prefrontal cortex. This suggests that exercise may have broader cognitive benefits beyond memory and learning. Numerous studies have reported positive associations between physical activity and cognitive function. Regular exercise improves attention, executive function, processing speed, and memory. These cognitive enhancements are believed to be mediated by the structural and functional changes resulting from physical activity-induced neurogenesis. Physical activity also shows promise in mitigating age-related cognitive decline and reducing the risk of neurodegenerative diseases, such as Alzheimer's disease. The neuroprotective effects of exercise may involve promoting neuronal plasticity, enhancing brain connectivity, and preserving cognitive function. Regular exercise promotes the generation of new neurons in the hippocampus and other brain regions, leading to improvements in various cognitive domains. Incorporating physical activity into lifestyle interventions can help promote brain health and optimize cognitive function across different stages of life. This chapter provides an overview of the relationship between physical activity, neurogenesis, and cognitive function.

Keywords: Cognitive function, Neurogenesis, Physical activity.

INTRODUCTION

The intricate workings of the human brain have long fascinated researchers, seeking to unravel its mysteries and unlock the potential for improved cognitive function and brain health. In recent years, the relationship between exercise and neurogenesis has emerged as a captivating area of study, shedding light on the profound impact physical activity can have on the generation of new neurons in the brain. The discoveries stemming from research have sparked a lively debate regarding the long-standing dogma that nerve cells do not regenerate throughout one's lifetime. In fact, recent publications have emerged, unveiling the existence of neurogenesis, the formation of new neurons, in the brain, challenging and even displacing this previously held view (Aimone et al., 2014). Various studies on this topic have demonstrated that, in addition to non-exercise factors such as engaging in mental activities, playing a musical instrument, and listening to music, aerobic exercises, in particular, can induce significant neurogenesis (Sachdeva et al., 2015).

The beneficial impact of exercise on our overall health has been extensively supported by numerous studies (Demirel & Ozyener, 2016; Demirel & Ozyener, 2022). Exercise has been shown to yield significant effects across multiple domains, ranging from cardiovascular health to diabetes prevention, from obesity management to bolstering the immune system (Yilmaz, 2022c). In fact, exercise can impede the advancement of chronic diseases, while also slowing down their progression and reducing their severity. It holds substantial potential in enhancing physical endurance, extending lifespan, and improving quality of life. Moreover, exercise is widely employed as both a preventive and therapeutic measure for various physical ailments (Yilmaz, 2021, 2022a; Yilmaz & Dege, 2021).

One of the most intriguing aspects of exercise revolves around its impact on nerve regeneration, cortical plasticity, and cognitive enhancement (Buonomano & Merzenich, 1998; Okyar et al., 2022). Particularly fascinating is the discovery that newly formed neurons primarily localize in the Hippocampus region and the Dentate Gyrus region (Jonas & Lisman, 2014). This finding holds great promise, as these regions are closely associated with memory function, and it is noteworthy that forgetfulness and dementia often originate from these specific areas.

The Relationship Between Neurogenesis and Exercise

Neuronal development is generally believed to proceed rapidly until the ages of 8 to 9, and then continue at a slower pace until the age of 18, after which no new neurons are thought to be generated. This paradigm was widely accepted for rodents, primates, and humans during the latter half of the 20th century

(Lledo et al., 2006). However, the advent of cutting-edge techniques and methodologies has recently challenged this perspective. Previously, it was commonly held that the number of neurons in the brain remained constant after birth, with only the potential for a decrease in numbers. This unexpected discovery defied the established dogma at the time. In recent years, the field of neuroscience has witnessed one of the most important developments, especially in neural regeneration and plasticity, through the discovery of mature neurons in the brain. Exercises have been shown to activate neurogenesis in the dentate gyri, proving this for the first time (Olson et al., 2006).

New neurons are created through a biological process known as neurogenesis (Fares et al., 2019). Neural stem cells undergo proliferation, migration, and maturation to form the neurons that comprise the central nervous system during embryogenesis. After proliferation, differentiation, and migration, recent research has shown that exercise plays a crucial role in the integration of newborn neurons in the dentate gyrus into neural circuits of the hippocampus and other brain regions that are important for memory consolidation and learning (Camuso et al., 2022).

Only three distinct neurogenic regions in the human brain, namely the striatum, the subventricular region of the lateral ventricles, and the hippocampal formation, have been demonstrated to undergo neurogenesis during exercise (Lucassen et al., 2010).

What happens during and after exercise that leads to exercise-induced neurogenesis? The answer to this question is not yet fully understood. Recent studies have placed emphasis on a chemical called brain-derived neurotrophic factor (BDNF), although the precise mechanisms are not yet fully understood (Sun et al., 2023). BDNF is a member of the neurotrophin family and plays a crucial role in various processes associated with neurogenesis, including proliferation, differentiation, maturation, and survival. In addition to BDNF, signaling pathways such as insulin-like growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2), and vascular-endothelial growth factor (VEGF) have also been found to effectively promote neuroplasticity and hippocampal neurogenesis (Ma et al., 2022).

Exercise has been found to induce increased expression of FNDC5 (fibronectin type III domain-containing protein 5) in muscle tissue (Wrann et al., 2013). FNDC5 is a membrane protein that undergoes cleavage and secretion, resulting in the production of the 112 amino acid peptide hormone known as irisin. Irisin has the ability to cross the blood-brain barrier (BBB) and has been shown to activate adult neurogenesis and enhance cognitive functions through the upregulation of BDNF (Wrann, 2015). Interestingly, voluntary

running in mice has been observed to upregulate the expression of FNDC5 in the hippocampus, but not in other brain regions (Wrann et al., 2013). However, the specific contribution of hippocampal FNDC5/irisin to exercise-induced neurogenesis remains unclear. Irisin has been proposed as a potential therapeutic agent for age-related conditions, including Alzheimer's disease (de Freitas et al., 2020). Additionally, studies have shown that exercise increases the expression of cathepsin B in peripheral muscles. Cathepsin B can diffuse through the BBB and enhance the expression of BDNF in the hippocampus, thereby improving adult neurogenesis, learning, and memory formation (Liu et al., 2019). Furthermore, the production of lactate, a molecule generated by exercising muscles, can induce BDNF expression and activation through the TrkB receptor in the hippocampus. Lactate is capable of crossing the BBB (Müller et al., 2020). Interestingly, BDNF is not only expressed in the brain but can also cross the BBB and be expressed in muscle progenitor cells. Therefore, BDNF may act as a target in the brain for the peripheral factors induced by exercise (Wrann et al., 2013).

Adiponectin, VEGF, nerve growth factor (NGF), IGF-1, and VEGF are among the additional neurotrophins that are upregulated as a result of physical exercise and play crucial roles in neural development (Mahalakshmi et al., 2020). Additionally, leptin, angiotensin II, glucocorticoids, adrenaline, reactive oxygen species, AMP-kinase, peroxisome proliferator-activated receptor (PPAR), PPAR coactivator 1 (PGC-1), ciliary neurotrophic factor (CNTF), and pro-inflammatory cytokines are among the other neurotrophins, growth factors, and cellular signaling molecules that are upregulated by physical activity (Lee et al., 2021). However, there is still uncertainty regarding the specific mechanisms through which these neurotrophins and growth factors mediate exercise-induced neurogenesis, as well as the causal relationship between them.

Physical activity has been shown to elevate the levels of various peripheral factors in the bloodstream, including platelet factor 4, which mediates platelet activation (Peñín-Grandes et al., 2022), liver-derived glycosylphosphatidylinositol-specific phospholipase D1, involved in modulating coagulation and complement pathways (Horowitz et al., 2020), and the antioxidant selenium transport protein selenoprotein P (SEPP1) in the plasma, which has been demonstrated to increase neurogenesis and enhance cognitive functions in aged mice (Leiter et al., 2022). However, the specific sources of these peripheral factors, particularly SEPP1, have yet to be identified. A member of the neurotrophin family of proteins, BDNF is predominantly present in the cerebellum, hippocampus, cerebral cortex, hypothalamus, and other regions of the central nervous system. The

neurotrophic protein BDNF plays a vital role in the development, differentiation, and survival of neurons, all of which contribute to learning. For BDNF to exert its effects, it must effectively penetrate the brain. Therefore, understanding the factors that facilitate the entry of BDNF into the brain is crucial (Pan et al., 1998).

The circulating BDNF consists of two components: the serum component and the plasma component. Both platelet-associated BDNF and freely circulating BDNF in the blood are included in the serum component, which represents the total measurable amount of circulating BDNF. In contrast, the plasma component can freely circulate in the bloodstream and cross the blood-brain barrier in both directions. While BDNF can be found in musculoskeletal tissue, peripheral blood mononuclear cells, and vascular endothelial cells, the brain and vascular endothelial cells are the primary sources of plasma BDNF. Platelets, on the other hand, contain a much higher concentration of BDNF compared to plasma, with approximately 100-200 times more BDNF. The release of BDNF from both plasma and platelets under conditions of physiological stress induced by exercise is an interesting similarity between these two sources (Wrann et al., 2013). In a study conducted to examine the effects of exercise intensity and BDNF on memory in adolescents, it was revealed that after 12 weeks of exercise, there were increases in brain tissue oxygenation and nutrition, as well as increases in BDNF levels (Jeon & Ha, 2017). Furthermore, improvements in memory were observed as a result of the exercise regimen. Similar positive findings have been observed in both children and elderly individuals (Felin Fochesatto et al., 2023; Fleitas et al., 2022).

Recent meta-analyses have demonstrated a dose-response relationship between exercise intensity and peripheral concentrations of BDNF in response to exercise (Jemni et al., 2023; Wang et al., 2022). In their comprehensive meta-analysis, Wang et al. examined the impact of physical exercise on brain- BDNF levels in healthy subjects through randomized controlled trials. The meta-analysis included 21 articles, encompassing 809 participants. According to the findings, both acute exercise (5 trials, standardized mean difference (SMD): 1.20, 95% confidence intervals (CI): 0.36 to 2.04, $p = 0.005$) and long-term exercise (17 trials, SMD: 0.68, 95% CI: 0.27 to 1.08, $p = 0.001$) significantly increased BDNF levels. According to subgroup analysis, long-term exercise, particularly with larger sample sizes, female participants, individuals over 60, and aerobic activities, demonstrated a more significant improvement in BDNF levels compared to the overall results. Both short-term and long-term exercise significantly increased circulating BDNF levels in healthy participants. This review suggests that acute exercise and sustained aerobic exercise are effective

physical activities that enhance the neurotrophic effect, especially in female participants or individuals over the age of 60 (Wang et al., 2022).

Chapman et al., in their study investigating the effects of physical activity and cognitive training on cerebral blood flow and memory, divided the participants into two groups. The first group underwent physical activity, while the second group received cognitive training, both for three hours a week over a period of 12 weeks. Magnetic resonance imaging (MRI) scans and physiological measurements were conducted at the beginning, during, and after the training period. The study reported that brain functions improved and cerebral blood flow increased in the brain regions of the cognitive training group. On the other hand, the physical exercise group exhibited more significant memory development. These findings indicate that a combination of both types of training may yield better results for enhancing brain health.In a study on the effectiveness of long-term exercise training in improving cognitive function, it was shown that 6 months of treadmill exercise, physical activity, and aerobic exercises resulted in improved cognitive functions in individuals with cognitive decline (Chapman et al., 2013).

In a recent study by Kleinloog et al., the researchers investigated the effects of physical exercise on cerebral blood flow (CBF), which serves as a physiological marker of cerebrovascular function. Additionally, the study examined the relationship between changes in CBF and cognitive performance. The review encompassed 45 intervention studies with diverse designs. Among these, 16 studies (median duration: 14 weeks) employed MRI to evaluate CBF markers, while transcranial Doppler ultrasound was used in 20 studies (median duration: 14 weeks), and near-infrared spectroscopy was utilized in eight studies (median duration: 8 weeks). The MRI studies consistently demonstrated increased CBF in the anterior cingulate cortex and hippocampus, although no significant changes were observed in whole-brain CBF. The effects of exercise training on resting CBF, as assessed by transcranial Doppler ultrasound and near-infrared spectroscopy, displayed variability. However, certain studies reported elevated middle cerebral artery blood flow velocity subsequent to exercise or hypercapnic stimuli. Remarkably, alterations in regional CBF were observed in conjunction with improvements in physical fitness, and a correlation was detected between exercise-induced effects on CBF and cognitive performance. To summarize, exercise training was determined to enhance cerebrovascular function through modifications in regional CBF (Kleinloog et al., 2023).

In the adolescent brain, BDNF and exercise intensity have a synergistic effect on memory development. The beneficial neuroendocrinological effects of

BDNF and its role as a mediator of synaptic plasticity are highlighted. This highlights the effectiveness of high-intensity chronic aerobic exercise in improving working memory in adolescents. Furthermore, this study indicates that exercise can improve memory during a vital developmental stage (adolescence) and potentially reduce age-related cognitive decline later in life (Tharmaratnam et al., 2018).

The most affected brain region in physical activities is the hippocampus (Lattanzi et al., 2022). Moderate aerobic exercises have been found to significantly increase the size and function of the hippocampus. This region is crucial for learning and memory storage. By preventing or reducing atrophy in this region through exercise, individuals can maintain their memory and cognitive abilities and mitigate the negative effects of aging (Tarumi et al., 2022).

The weight of the brain may decrease with age, and specific neuronal populations may deteriorate due to cell death. Age-related declines in enzyme activity can also affect the production of various neurotransmitters, including acetylcholine, norepinephrine, and dopamine. Clinical manifestations of aging are believed to be influenced by changes occurring in specific neural circuits, including those involved in memory, motor function, mood regulation, sleep patterns, appetite, and neuroendocrine activities. (Vaseghi et al., 2022). Exercise is known to reduce the risk of age-related forgetfulness and the onset and progression of dementia, particularly Alzheimer's disease, by either preventing cognitive decline or slowing its rate. Long-term exercise is thought to decrease the risk of developing Alzheimer's disease due to its inflammation-suppressing effects. Additionally, exercise is currently recommended as a treatment option for major depression, partly due to its antidepressant effects (Rafii & Aisen, 2023).

Exercise intensity and peripheral BDNF concentrations are inversely correlated with dose. It has been demonstrated that individuals who engage in high-intensity exercise retain newly learned knowledge more effectively than the control group, thereby enhancing memory. Chronic aerobic exercises have also been found to improve cognitive and motor skills (Statton et al., 2015). MR imaging has revealed an increase in the volume of the hippocampus region, and physically active children have been found to have a larger hippocampal volume compared to sedentary children (Latino & Tafuri, 2023).

Exercise and BDNF may enhance physiological parameters, academic performance, achievements, and maintain a healthy metabolic profile due to their complementary effects. These features suggest that cardiovascular exercise during the crucial period from childhood to adolescence in healthy children may

potentially improve memory and prevent age-related cognitive decline. Examinations using 5-bromo-2-deoxyuridine (BrdU) staining and radiocarbon dating have shown an increase in dentate gyrus neurons as a result of exercise (Gault & Szele, 2021).

Additionally, exercise, especially when performed in moderation, has been demonstrated to increase the size of the hippocampus in humans, which is associated with improved memory (Voss et al., 2019).

Exercise has been demonstrated to enhance memory functions and promote hippocampal neurogenesis in animal experiments, providing support for the notion that exercise plays a crucial role in maintaining the structure and function of the hippocampus (Park et al., 2018; Vilela et al., 2017). In human intervention and randomized control trials utilizing structural MRI, it has been observed that individuals who are more physically active or engage in regular exercise tend to have larger hippocampal volumes, predominantly in the hippocampal head region (Erickson et al., 2011; Wilckens et al., 2021). However, conflicting results have also been reported, with some studies indicating no significant effects or even adverse consequences of exercise on hippocampal size (Venkatraman et al., 2020; Wagner et al., 2015). Similarly, there are conflicting findings regarding the relationship between human cognition and exercise or physical activity (Sokołowski et al., 2021; Young et al., 2015)

High-intensity aerobic exercises have been shown to promote the formation of approximately 700 new neurons per day, indicating that the brain has the ability to regenerate itself at a rate of 1.7% per year (Mateus-Pinheiro et al., 2021). These findings have provided optimism for potential therapeutic interventions in neurological disorders. Currently, exercise is utilized as a treatment approach for major depression and is also employed to prevent or delay the onset of conditions such as Alzheimer's disease, dementia, and age-related cognitive decline (Yilmaz, 2022b).

Stress and Neurogenesis

It is crucial to avoid turning exercise into a stressor. Stress can hinder neurogenesis by elevating glucocorticoid secretion in the hypothalamo-pituitary-adrenal axis and impeding the formation of new neurons.

Stress is a response that occurs to cope with challenging or threatening situations in an individual's environment. A stressor, perceived physically or psychologically, can trigger the stress response. Stress can cause the release of stress hormones such as cortisol and lead to changes in brain structures. Research suggests that stress may be a triggering factor in the emergence of

neurological diseases. In addition, other hormones such as oxytocin may also have an effect on neuroplasticity (Yilmaz, 2023).

The effect of stress on neurogenesis is complex and depends on many factors. Acute stress can cause increased neurogenesis for a short time. An interesting conclusion was reached in a recent study conducted by Sannino et al. The results of the study imply that acute stress causes broad modifications in gene expression in the hippocampus. The researchers looked at the regulation of gene expression in the hippocampus at several time intervals (3, 12, and 24 hours) after the stress exposure using a mouse model of short-term restraint stress. Mice exposed to acute restraint stress showed unique transcriptional responses in the hippocampus when compared to non-stressed controls, according to microarray research. Ttr, Rab6, Gh, Prl, Ndufb9, and Ndufa6 were among the transcripts whose up-regulation was specifically associated with neurogenesis and neuronal protection. A considerable enrichment of biological processes related to neurogenesis, neuron morphogenesis, and cognitive functioning was also revealed by system-level analysis. These findings lend support to the hypothesis that acute stress positively impacts the hippocampus, promoting the formation and preservation of neurons (Sannino et al., 2016). However, the situation is different in situations of prolonged or chronic stress.

Chronic stress occurs as a result of prolonged exposure to or repeated stressors. Chronic stress can cause decreased neurogenesis in the hippocampus. This can result in the hippocampus shrinking and lead to impairments in functions such as learning, memory, and emotional regulation.Stress is a physiological and psychological response that arises in order to adapt to challenging or threatening circumstances in an individual's environment. Stressors, whether perceived physically or psychologically, can initiate the stress response. This activation of stress can lead to the release of stress hormones, such as cortisol, and induce structural alterations within the brain (Jones et al., 2022).

Chronic stress emerges as a consequence of sustained exposure to stressors or recurrent stress-inducing circumstances. Prolonged or chronic stress has been linked to a decline in neurogenesis within the hippocampus. Consequently, this diminishment in neurogenesis can lead to hippocampal atrophy and impairments in cognitive functions, including learning, memory, and emotional regulation. Moreover, even voluntary exercises, if performed excessively or at an overwhelming intensity, can inhibit neurogenesis through the same mechanisms. Therefore, it is imperative that exercise programs are tailored to the individual and do not exert excessive strain leading to exhaustion (Pant et al., 2022).

Studies highlight the significance of encouraging healthy individuals to engage in exercise and its potential as an adjunct treatment in various pathological conditions (Izquierdo et al., 2021). The results of these studies offer valuable insights into the potential benefits of aerobic exercise as a supportive therapy for individuals with obesity or type 2 diabetes mellitus. It has been demonstrated that the increase in BDNF resulting from exercise can help alleviate symptoms such as overeating (polyphagia), lower blood sugar levels, and improve insulin sensitivity and glucose oxidation (Zhou et al., 2019). As a result, the combined effects of exercise and BDNF may play a role in improving various physiological parameters, academic performance, achievements, and maintaining a healthy metabolic profile.

Recreational Activities and Neurogenesis

There is evidence suggesting that activities such as listening to music, playing a musical instrument, engaging in social and intellectual environments, and learning new activities, in addition to exercise, have comparable cognitive and neural effects. Dance activities that combine music and physical movement have been found to be particularly effective in promoting the generation of new neurons (Matziorinis & Koelsch, 2022). Other factors, such as diet or sexual activity, should also be taken into consideration (Ghaddar et al., 2020). Regular exercise offers numerous mental and physical benefits. Currently, nutritional therapies are used in the treatment of neurological diseases (Cagiran & Yilmaz, 2022).

Preclinical investigations conducted in rodent models have elucidated the adverse impact of high-fat and/or high-sugar diets on adult hippocampal neurogenesis (Mota et al., 2023). Conversely, diets enriched with bioactive compounds, such as polyunsaturated fatty acids and polyphenols, as well as interventions like intermittent fasting or caloric restriction, have been shown to promote hippocampal neurogenesis. Notably, emerging evidence indicates that maternal nutrition during the perinatal period can influence offspring hippocampal neurogenesis. Consequently, interventions targeting nutrition in early developmental stages and throughout life hold promise as a therapeutic approach to ameliorate neurodegenerative disorders by augmenting neurogenesis. The precise mechanisms through which nutrients and dietary factors modulate adult hippocampal neurogenesis are still under investigation. Intriguingly, recent findings suggest the involvement of peripheral mediators in this process. In this context, the microbiota-gut-brain axis, which facilitates bidirectional communication between the gut and the brain, emerges as a

potential link between nutritional factors and hippocampal neurogenesis (Melgar-Locatelli et al., 2023).

Regular fluid and electrolyte intake is associated with neurogenesis. Fluid intake is vital for maintaining brain functions and promoting the formation of healthy nerve cells. Insufficient fluid intake can negatively impact brain functions and the neurogenesis process. It is important to have adequate and regular fluid and electrolyte intake to support brain health and enhance neurogenesis. However, neurogenesis is a complex process influenced by various factors. Therefore, individuals may benefit from incorporating natural spring waters into their fluid intake routine for a healthier lifestyle (Cagiran et al., 2023).

CONCLUSION

It is critical to keep in mind that exercise should be personalized, accessible to everyone, and that a medical examination is required prior to initiating any exercise program. Looking ahead, further studies are expected to provide additional insights and solidify the evidence supporting the therapeutic potential of aerobic exercise in neurological and psychiatric disorders. These studies will help refine exercise protocols, identify optimal exercise parameters, and shed light on the underlying mechanisms by which exercise exerts its beneficial effects on the brain. In conclusion, personalized and accessible exercise programs, guided by medical examination and professional advice, hold great potential as adjunctive therapies for individuals with neurological and psychiatric disorders. The ongoing research in this field is paving the way for exercise to be recognized as a valuable and cost-effective intervention to improve brain health and overall well-being.

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Chapter 20

Respiration in Exercise: Parameters and Dynamics

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ABSTRACT

Respiration plays a crucial role in exercise, providing the necessary oxygen for energy production and removing carbon dioxide as a waste product. Understanding the parameters and dynamics of respiration during exercise is essential for optimizing athletic performance and ensuring the safety and efficiency of physical activity. The dynamics of respiration during exercise are influenced by various factors such as exercise intensity, duration, and training status. Higher intensity exercise requires a more rapid and forceful breathing pattern to meet the heightened oxygen demand. Prolonged exercise can lead to respiratory muscle fatigue, affecting ventilation and potentially impacting performance. Regular training can improve respiratory muscle strength and endurance, enhancing the overall efficiency of respiration during exercise. Moreover, respiratory adaptations occur with exercise training, resulting in increased lung capacity, improved oxygen uptake, and enhanced respiratory muscle function. These adaptations contribute to better oxygen delivery to working muscles and the ability to sustain exercise for longer durations. Respiration also interacts with other physiological systems during exercise. The respiratory system collaborates with the cardiovascular system to ensure efficient oxygen delivery and carbon dioxide removal. The respiratory system also plays a role in maintaining acid-base balance by regulating carbon dioxide levels and blood pH. Respiratory adaptations occur with exercise training, resulting in increased lung capacity, improved oxygen uptake, and enhanced respiratory muscle function. These adaptations contribute to better oxygen delivery to working muscles and the ability to sustain exercise for longer durations. Understanding the parameters and dynamics of respiration during exercise provides insights into optimizing training strategies and ensuring adequate oxygen supply and carbon dioxide removal. This chapter provides a concise overview of the key aspects related to respiration in exercise.

Keywords : Exercise, Physiological adaptations, Respiration

INTRODUCTION

"Physical activity" refers to everyday activities that include the use of muscles and joints, increase heart and respiratory rates, and cause exhaustion at varying intensities. "Exercise", on the other hand, is defined as planned, structured, voluntary, repetitive body movements that aim to develop and maintain one or more elements of physical fitness such as strength, endurance, speed, and balance (Dasso, 2019; Yilmaz, 2021;Yilmaz, 2022a; Yilmaz, 2022b). Both exercise and physical activity are affected by a variety of internal and external factors. Internally, hormones play an important role in shaping our propensity and ability to engage in physical activity (Yilmaz et al., 2022).

Aerobic exercise is rhythmic and continuous exercises that involve large muscle groups and increase the body's oxygen consumption. In this type of exercise, which is of relatively low intensity but continues for a long time, such as long-distance running, there is an increase in the number of mitochondria involved and the capillaries around the muscle fibers. The goal of aerobic exercise is to increase endurance with minimal fatigue. In this way, aerobic exercise programs bring about changes in skeletal muscle, respiratory, and circulatory systems (Fritzen et al., 2020;Yilmaz, 2022c). Aerobic training aims to improve aerobic capacity. The ability of the pulmonary and cardiovascular systems to provide the most oxygen to the muscles is known as aerobic capacity (Joyner & Dominelli, 2021).

Contrary to aerobic exercise, short-term and high-intensity exercise such as weightlifting is defined as resistance exercise. Fast fibers are affected by resistance training; these fibers increase their diameter (hypertrophy) and enhance muscle strength due to the activation of satellite cells and the increase in the synthesis of actin and myosin filaments, which form more myofibrils. Although the muscles hypertrophied by resistance training are very strong, their endurance capacity is low (Reggiani & Schiaffino, 2020; Yilmaz & Dege, 2021).

Respiration plays a crucial role in exercise, serving as a fundamental aspect of optimal performance and overall well-being. During physical activity, the body's demand for oxygen increases, and proper breathing techniques enable the efficient delivery of oxygen to the muscles while facilitating the removal of waste products. Additionally, mindful and controlled breathing enhances mental focus, promotes relaxation, and helps regulate effort and intensity during various types of exercise. By understanding the importance of breathing and employing appropriate techniques, individuals can enhance their exercise experience, maximize performance, and achieve their fitness goals more effectively (Fritzen et al., 2020). In this section, we will explore the respiratory

mechanics involved in exercise and delve into the dynamics of respiration during physical activity.

Respiration in Exercise

The capacity to exercise is correlated with both the pulmonary system's capacity to eliminate carbon dioxide (CO_2) from the blood through the lungs and the cardiovascular system's capacity to provide oxygen (O_2) to the muscles. The respiratory and circulatory systems work in tandem to deliver oxygen to the tissues and remove carbon dioxide (Karbing & Rees, 2020). There are four processes at work here (Figure 1):

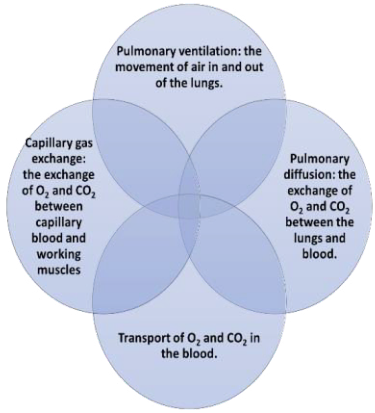

Figure 1: The Processes of Respiratory Physiology During Exercise

The first two processes are called external respiration, and the fourth process is called internal respiration. Exercise can expose anomalies that may not be seen at rest by taxing the systems in charge of both internal and external breathing. Cardiovascular output (CO), which can rise up to six times above resting levels, facilitates the increase in O_2 uptake by exercising muscles. (Lador et al., 2006).

Besides, myokines such as irisin, which are released mainly from skeletal muscles during and after exercise, have beneficial effects on the respiratory system by relaxing airway smooth muscles (Demirel & Ozyener, 2022).

Maximal Exercise Capacity: VO_2max or Peak VO_2

The traditional measure of oxygen delivery and utilization in the body is maximal oxygen uptake (VO_2max). VO_2max is an objective measure of exercise capacity during exercise and determines the upper limits of the cardiopulmonary system. Expressed by the Fick principle, it is equal to the

product of the difference in CO and (a-v)O_2 (arteriovenous oxygen difference) at peak exercise (Mezzani et al., 2009).

$VO_2max = (CO \times SV) \times [C(a\text{-}v)\ O_2]$

CO is determined by the product of heart rate (HR) and stroke volume (SV). CO normally increases linearly in relation to oxygen uptake (VO_2). The linear increase in CO as a function of VO_2 reflects increases in HR and SV. Increases in CO are initially achieved by increases in SV and HR, and later by increases in HR, particularly during moderate to vigorous exercise (Crisafulli et al., 2011). The increase in SV reflects an increase in end-diastolic volume or a decrease in end-systolic volume, or both, depending on ventricular function, body position, and exercise intensity (Stöhr et al., 2011).

HR increases linearly with workload and VO_2. Increases in HR occur primarily during diastole. The amount of O_2 taken up by the tissues during exercise reflects the difference between the oxygen content in the arteries (usually 18 to 20 ml O_2/100 ml at rest) and the oxygen content in the veins (usually 13 to 15 ml O_2/100 ml at rest), typically at rest (a-v)O_2 is around 4 to 5 ml O_2/100 ml, approximately 23% uptake (Rivera et al., 1989). This difference increases hyperbolically as the working tissues take up more oxygen during exercise. The amount of venous oxygen decreases to very low levels, and (a-v)O_2 can increase up to 16 to 18 ml O_2/100 ml in vigorous exercise (oxygen extraction from the blood at VO_2max exceeds 85%) (Smith & Fernhall, 2022).

The increased CO is largely redistributed to the contracting muscle units through local vasodilation in contractile units by the action of local metabolites on arteriolar smooth muscle. Although the details of this process are still not completely understood, it is thought to include increases in intramuscular potassium ions (K^+), hydrogen ions (H^+), PO_2, osmolarity, temperature, catecholamines, nitric oxide, and degradation stress (Sagiv, 2012). During exercise, 80% of the increase in CO is directed to the muscles (Harms, 2000).

The most accurate measure of aerobic capacity is VO_2max, which is also the benchmark for cardiorespiratory fitness. It stands for the highest level of oxidative metabolism that can be achieved in big muscle groups (Lee & Zhang, 2021). The actual VO_2max (physiological VO_2max) is defined by a plateau in VO_2 during the last two workloads. It requires reaching and maintaining maximal effort over a period of time. Determining VO_2max is subjective and can be difficult. The term "peak VO_2" is commonly used to describe exercise capacity when individuals with pulmonary disease are tested. The term VO_2max

is often used to define exercise capacity in healthy individuals who are more likely to achieve a maximal physiological response (Santisteban et al., 2022).

VO_2 can increase from a resting value of approximately 3.5 ml/kg/min to a maximal value of 30-50 ml/kg/min, which is 15 times higher. Low peak VO_2 values may reflect problems related to oxygen transport (cardiac output, O_2 carrying capacity of blood), pulmonary limitations (mechanics, respiratory control, and gas exchange), O_2 uptake in tissues (tissue perfusion, tissue diffusion), and limitations associated with exertion, neuromuscular, or musculoskeletal factors (Smith & Fernhall, 2022).

CO_2 Production (VCO_2)

VCO_2 is mostly produced during exercise from two different sources. The first source is oxidative metabolism, which generates metabolic CO_2. Approximately 75% of the O_2 consumed by the body is converted to CO_2. This CO_2, carried by venous blood, returns to the right side of the heart, enters the lungs, and is eventually exhaled as VCO_2. The second source of CO_2, referred to as non-metabolic CO_2, arises from the buffering of lactate during higher levels of exercise. It is important to note that elevated levels of CO_2 in the bloodstream can lead to respiratory acidosis (Guazzi & Adami, 2019).

Both VCO_2 and ventilation (V_E) increase in proportion to VO_2 and workload during exercise levels ranging from approximately 50% to 70% of VO_2max. However, at exercise levels above this range, V_E increases disproportionately compared to VO_2. This disproportional increase is attributed to the production of more lactate than can be effectively removed from the bloodstream as exercise intensity rises (Husaini & Emery, 2023).

Ventilation

V_E plays a crucial role in oxygen uptake during exercise. V_E increases linearly with O_2 uptake and CO_2 production up to approximately 50 to 60 percent of VO_2max. Beyond this point, often referred to as the anaerobic threshold (A_T), V_E becomes more closely associated with increased CO_2 production rather than oxygen uptake. The increase in V_E during exercise is accompanied by an elevation in both respiratory frequency and depth (Hagberg et al., 1978).

In healthy individuals, the increase in ventilation at lower levels of exercise is primarily achieved through augmentations in tidal volume (V_T). As exercise intensity progresses, both V_T and respiratory frequency continue to rise until reaching 70-80% of peak exercise, after which respiratory frequency becomes more dominant. V_T typically plateaus at around 50% to 60% of V_C (Demirel &

Ozyener, 2016). In young adults, V_T increases by approximately 3 to 5 times, while in older adults, the increase is around 2 to 4 times. Respiratory frequency typically increases by 1 to 3 times in most individuals. The increase in respiratory frequency during exercise reflects a reduction in both inspiration and expiration times. However, a more significant reduction in expiratory time is observed at moderate or higher ventilatory demands, resulting in an increase in the ratio of inspiratory time to total time from 0.4 at rest to 0.5 to 0.55 at maximal exercise. As a consequence of this greater reduction in expiratory time, the increase in mean expiratory flow rate exceeds the increase in mean inspiratory flow rate (Guenette et al., 2013).

The ventilation-perfusion ratio, with a resting value of about 0.8, is the ratio of alveolar ventilation to alveolar capillary blood flow. During exercise, alveolar blood flow and ventilation both increase, and this ratio may reach levels as high as 5 (Petersson & Glenny, 2014).

$$V_E = 863 \times VCO_2 / PaCO_2 (1 - V_D/V_T).$$

There are three factors that determine the appropriateness of the ventilation response to exercise, namely VCO_2 (metabolic component), $PaCO_2$ (control set point), and the V_D/V_T ratio (reflecting inadequate pulmonary gas exchange) (Cao et al., 2015).

Ventilatory Reserve/Capacity

The assessment of whether ventilatory limitation is a causative factor or contributor to exercise intolerance involves the evaluation of ventilatory reserve, which represents the relationship between ventilatory requirement and ventilatory capacity. Ventilatory capacity is quantified as maximal voluntary ventilation (MVV). Ventilatory reserve is calculated as the percentage of MVV achieved at peak exercise [(VE_{peak}/MVV) x 100], or alternatively, as the disparity between MVV and the actual ventilation (V_E) achieved at peak exercise. In the majority of healthy individuals, V_E approximates 70% of MVV during maximal exercise (Burghard et al., 2022). It is typically expected that ventilatory reserve exceeds 20% of MVV. Conversely, individuals with pulmonary pathologies exhibit a diminished ventilatory capacity coupled with an elevated ventilatory demand, resulting in a reduction in ventilatory reserve (O'Donnell, 2001). Values below 15% are indicative of ventilatory limitation. Notably, individuals with airway obstruction may demonstrate V_E during maximal exercise that equals MVV, signifying the attainment of the ventilatory limit (Lotshaw & Betts, 2022).

Ventilatory Threshold

In numerous everyday activities, maximal effort is not required. The anaerobic or ventilatory threshold (VT) is a commonly utilized submaximal measure of exercise capacity. VT represents a physiological event characterized by the point during exercise at which V_E begins to exhibit an exponential increase in relation to VO_2. It is considered a reflection of the A_T. At this threshold, the supply of oxygen to the muscles becomes inadequate to meet the oxygen demands imposed by the workload. Consequently, there is an augmented reliance on anaerobic glycolysis for energy production, resulting in the production of lactate as a metabolic byproduct. The elevation in V_E is necessary to eliminate the surplus CO_2 generated during the conversion of lactic acid to lactate. The lactate threshold has proven to be a valuable indicator for the onset of exercise-induced metabolic acidosis (Kenney et al., 2021).

Three potential causes of increased blood lactate during exercise are as follows (MacDougall et al., 2022; Takeda et al., 2022):

I- Limitation of O_2 availability: When O_2 is not utilized as the terminal oxidant in the electron transport chain, lactate is produced to sustain adenosine triphosphate (ATP) production.

II- Enzymatic rate limitation: Factors related to oxidative energy transfer, such as the number of mitochondria, existing Krebs cycle enzymes, mitochondrial respiratory chain enzymes, and myoglobin.

III- Fiber-type composition of the muscle: If the increase in strength is primarily due to Type II fibers rather than Type I fibers, there is a higher likelihood of lactate production.

The question of whether muscle hypoxia is the primary stimulus for increased lactate production remains a topic of debate. The terms "anaerobic threshold," "ventilatory threshold," and "lactate threshold" are often used interchangeably. However, it is important to consider them as distinct but related events (Galán-Rioja et al., 2020).

In healthy individuals, the VT typically occurs at approximately 45 to 65% of peak or VO_2max. In individuals with endurance training, it may occur at higher percentages of exercise capacity. The choice between treadmill or bicycle ergometry can influence the VT response. Therefore, consistency in the testing and training methods is essential when using VT to recommend exercise intensity (Pymer et al., 2020).

The determination of VT during exercise has been the subject of ongoing investigation, and various methods have been proposed. However, a consensus on the optimal approach for determining VT has yet to be reached. Among the recommended methods for VT determination are the V-slope method, the point

of systematic increase in the ventilatory equivalent for oxygen (V_E/VO_2) without a concomitant increase in the ventilatory equivalent for carbon dioxide (V_E/VCO_2), and the end-tidal pressure of oxygen ($PETO_2$) without a reduction in the pressure of carbon dioxide ($PETCO_2$).

The V-slope method involves plotting the VCO_2 (carbon dioxide production) against the VO_2 (oxygen consumption) during exercise and determining the VT as the point where the slope of the VCO_2 versus VO_2 relationship begins to increase systematically. This method relies on the premise that the increase in ventilation is directly related to the metabolic rate and the production of carbon dioxide (Galán-Rioja et al., 2020).

Another approach involves identifying the point of systematic increase in V_E/VO_2 without a corresponding increase in V_E/VCO_2. The VT is determined as the point where there is a consistent rise in V_E/VO_2, indicating an increase in ventilation relative to oxygen consumption, without a concurrent rise in V_E/VCO_2.

Additionally, the $PETO_2$ can be monitored during exercise. $PETO_2$ refers to the partial pressure of oxygen in the exhaled breath. By observing the point at which a systematic increase in tidal O_2 pressure is observed without a reduction in the $PETCO_2$, the VT can be determined. This method relies on the assumption that the rise in $PETO_2$ reflects the ventilation-perfusion relationship and the adequacy of oxygen delivery to the working muscles (Pymer et al., 2020).

While these methods offer potential approaches to determine VT, researchers and practitioners continue to explore their strengths, limitations, and applicability across different populations and exercise modalities. The selection of the most appropriate method may depend on the specific research or clinical context, the available equipment, and the expertise of the investigator. (Rovai et al., 2021).

Peak Respiratory Exchange Ratio

The respiratory exchange ratio (RER) represents the relationship between VCO_2 and VO_2 during exercise. It is calculated by dividing the VCO_2 by the VO_2 (Padhy et al., 2023). At rest, the RER is usually between 0.70 and 0.85. As exercise intensity increases, lactic acid buffering contributes to VCO_2 production. This physiological response to exercise makes the peak RER the most accurate and reliable measure of effort. Generally, a peak RER value of 1.1 and above is considered an excellent indicator of effort during a cardiopulmonary exercise test (Goedecke et al., 2000). However, it is important to note that a high peak RER value alone is not an indication to terminate the

test. In general, termination of the exercise test with a peak RER value below 1, without any electrocardiographic or hemodynamic abnormalities, reflects submaximal cardiovascular effort that can be observed in individuals with pulmonary limitations to exercise (Kaminsky et al., 2022). RER values above 1 should suggest lactic acidosis and hyperventilation (Datta et al., 2015).

V_E-VO_2 Relationship

The relationship between V_E and VO_2 reflects ventilatory efficiency during exercise. V_E is influenced by VO_2, but VO_2 is independent of V_E. The V_E-VO_2 relationship is complex, often nonlinear, and challenging to standardize. The ratio of V_E to VO_2 is referred to as the ventilatory equivalent for O_2. This ratio is associated with the ratio of dead space to tidal volume (V_D/V_T), and higher V_E/VO_2 values can be observed when V_D/V_T increases. The normal pattern of change in V_E/VO_2 during exercise includes an initial decrease, reaching its lowest point near the A_T, followed by an increase as the individual approaches maximal exercise capacity (Amann et al., 2004; Arena et al., 2012).

It's worth noting that the V_E-VO_2 relationship can vary among individuals based on factors such as fitness level, training adaptations, and pulmonary function. Additionally, various physiological and environmental factors can influence this relationship, making it important to consider these factors when interpreting V_E and VO_2 data during exercise testing or training.

V_E-VCO_2 Relationship

The V_E/VCO_2 relationship is a widely used index of ventilatory efficiency. During exercise, V_E is regulated by the VCO_2. The V_E/VCO_2 relationship is often quantified as a slope value calculated through linear regression (y=mx+b, where b represents the slope) (Myers et al., 2009). V_E is influenced by factors such as perfusion compliance and cardiac function. When considering age and gender, a V_E/VCO_2 relationship below 30 is generally considered normal (Sarullo et al., 2010). However, patients with conditions like chronic obstructive pulmonary disease or pulmonary hypertension may exhibit values exceeding this normal threshold, with advanced disease severity resulting in values above 60 (Blanco et al., 2010). An abnormal V_E/VCO_2 response is associated with ventilation-perfusion mismatch, abnormal chemoreceptor and ergoreceptor sensitivity leading to an exaggerated ventilatory response to exercise, reduced CO, increased pulmonary pressures, decreased alveolar-capillary membrane conductivity, and decreased HR variability. Elevated V_E/VCO_2 values indicate low partial pressure of carbon dioxide ($PaCO_2$), increased dead space to tidal volume ratio (V_D/V_T), or both (Mehani & Abdeen, 2017).

$PETCO_2$ and $PETO_2$

$PETCO_2$, also known as end-tidal CO_2, reflects ventilation-perfusion compliance and the level of arterial CO_2. Normal resting values for $PETCO_2$ range between 36-42 mmHg. During tidal breathing, $PETCO_2$ increases by 3-8 mmHg from rest to V_T and then decreases as the individual approaches maximal exercise (Loughnan et al., 2021). $PETO_2$, on the other hand, progressively increases until reaching the lactate threshold, after which it shows a systematic increase (Sun et al., 2022).

In the presence of increased dead space or rapid superficial breathing patterns caused by conditions such as acute hyperventilation, emphysema, or other lung diseases, $PETCO_2$ may decrease independent of cardiac function. Numerous studies have demonstrated a significant relationship between resting $PETCO_2$ and CO. Among patients with pulmonary hypertension, resting $PETCO_2$ values, as well as values during V_T and peak exercise, are significantly associated with pulmonary pressures. Thus, $PETCO_2$ can serve as a noninvasive reflection of disease severity (Akizuki et al., 2020).

Ventilatory Dead Space-Tidal Volume (V_D/V_T) Relationship

The V_D/V_T ratio serves as a valuable parameter for evaluating the uniformity of the ventilation-perfusion ratio, both at rest and during exercise. Optimal pulmonary gas exchange relies on the uniformity of the alveolar ventilation-perfusion ratios across alveolar/capillary units. The physiological dead space, which represents the portion of V_T that does not participate in gas exchange, is typically around one-third of the V_T (Feldman et al., 2023).

During exercise, the expansion of the pulmonary vascular bed leads to a reduction in the V_D/V_T ratio, particularly in the early stages of exercise. In individuals without pulmonary disease, the resting V_D/V_T ratio is generally around 0.34, but it can decrease to 0.1 or even lower during peak exercise. Conversely, individuals with pulmonary conditions often exhibit normal or elevated V_D/V_T values at rest. When the V_D/V_T ratio fails to decrease adequately during exercise, it necessitates a compensatory increase in respiratory rate to account for the augmented ventilation in the dead space. Assessing changes in the V_D/V_T ratio provides insights into the efficiency of gas exchange and can help identify abnormalities in pulmonary function. Monitoring the V_D/V_T ratio during exercise offers valuable information about the physiological responses of the respiratory system and the capacity to adapt to increased ventilatory demands. Understanding alterations in the V_D/V_T ratio contributes to the evaluation and management of respiratory conditions, guiding interventions

aimed at optimizing gas exchange and enhancing exercise performance (Feldman et al., 2023).

The V_D/V_T ratio can be calculated using the following formula:

$V_D/V_T = [(PaCO_2 - PECO_2) / PaCO_2] - (VD_{app} / V_T)$

$PaCO_2$: It represents the partial pressure of CO2 in arterial blood. It is typically measured using blood gas analysis.

$PECO_2$: It represents the partial pressure of carbon dioxide in mixed expired gas. PECO2 is obtained by collecting and analyzing a sample of exhaled air during the breathing cycle.

VD_{app}: It stands for the apparent dead space, which is an estimation of the physiological dead space. Physiological dead space refers to the portion of each breath that does not participate in gas exchange with the blood. VD_{app} is typically determined using a formula based on the Bohr equation.

V_T: It represents the tidal volume, which is the volume of air inspired or expired during each breath.

Ideally, the direct measurement of arterial $PaCO_2$ through arterial blood gas analysis is required for accurate V_D/V_T ratio calculation. However, for practical reasons, especially during exercise, noninvasive calculation of $PaCO_2$ using PET CO_2 is commonly employed. Nevertheless, it should be noted that the reliability of calculated $PaCO_2$ may be diminished in individuals with pulmonary disease (Balady et al., 2010; Guazzi et al., 1995).

VO_2/Workload Relationship

Under physiological conditions, there exists linearity in the ratio of the increase in VO_2 to the increase in workload (WR: $\Delta VO_2/\Delta WR$). The slope of this relationship reflects the ability of the exercising muscle to supply oxygen and generate ATP aerobically. In the case of a linear ramp protocol test conducted on healthy sedentary individuals, the reported slope is approximately 10 $mLO_2 \cdot min^{-1} \cdot W^{-1}$, with no significant influence of age, gender, or height. A decrease in the $\Delta VO_2/\Delta WR$ relationship often indicates a deficiency in oxygen transport, which can be observed in patients with lung disease or mitochondrial myopathy, where alterations in the cellular pathways necessary for oxygen utilization occur (Prieur et al., 2005).

O_2 Pulse

O_2 pulse is an indirect measure of cardiopulmonary O_2 transport. It is calculated by dividing O_2 intake (ml/min) by HR and represents the amount of

oxygen delivered with each heartbeat. O_2 pulse can be determined by multiplying SV and (a-v).

During exercise, O_2 pulse exhibits a distinctive pattern. Initially, there is a rapid increase in O_2 pulse as the body's demand for oxygen rises. This is due to an increase in both SV and (a-v)O_2, reflecting the heart's ability to pump more blood and the muscles' enhanced capacity to extract oxygen from the blood. As exercise continues, O_2 pulse reaches a plateau or approaches an asymptotic value. This indicates that the oxygen delivery with each heartbeat has reached its maximum efficiency. Lower values of O_2 pulse may suggest early exercise limitation or reduced cardiopulmonary function. Several factors can contribute to lower O_2 pulse values, including deconditioning, cardiovascular disease, ventilatory limitations, or other symptoms that hinder O_2 transport or utilization. Monitoring O2 pulse can help identify individuals who may experience exercise intolerance or provide insights into the effectiveness of interventions aimed at improving cardiovascular fitness.

By assessing O_2 pulse during exercise testing or monitoring, healthcare professionals can gain valuable information about an individual's cardiopulmonary health, cardiovascular efficiency, and exercise capacity. This metric is particularly useful in evaluating exercise responses, designing targeted training programs, tracking progress, and identifying potential underlying physiological limitations or abnormalities (Balady et al., 2010). Lower O_2 pulse values may indicate early exercise limitation due to factors such as deconditioning, cardiovascular disease, ventilatory limitation, or other symptoms (DAJP et al., 2019; Montes de Oca et al., 1996).

VO_2 Kinetics During Exercise and Recovery

VO_2 kinetics, referring to the response of oxygen consumption (VO_2) during exercise and recovery, exhibits distinctive phases. In the initial 60 to 120 seconds of exercise at a constant workload, the VO_2 response is typically considered undetectable. This phase, known as phase I or the cardiodynamic phase, is characterized by a rapid increase in VO_2, primarily attributed to augmented pulmonary blood flow. Subsequently, phase II ensues, characterized by a slower rise in VO_2, reflecting the delivery of oxygen to the working muscles. If exercise intensity remains below the ventilatory threshold (V_T), these initial phases are followed by a steady state phase (phase III), where VO_2 plateaus. In cases where VO_2 fails to reach equilibrium and exhibits pauses before reaching the steady state, an oxygen deficit occurs. During this period, energy demands are met through high-energy phosphates, such as phosphocreatine, and anaerobic energy sources like anaerobic glycolysis. The

accumulation of lactic acid is directly associated with the duration of the non-steady-state response. Prolonged or unresolved VO_2 response may contribute to exercise dyspnea and exercise intolerance. The recovery phase following exercise also displays characteristic VO_2 kinetics. Initially, there is a rapid decrease in VO_2 during the early recovery period, referred to as the fast component. This decline is primarily attributed to the cessation of muscle contractions and a reduction in energy expenditure. Subsequently, a slower component, termed the slow component, may be observed, and it can contribute to prolonged elevation in VO_2 during the recovery phase (Balci et al., 2020; do Nascimento Salvador et al., 2016).

Recovery VO_2 kinetics refer to the process of restoring energy stores in active muscles after exercise. It reflects the rate at which blood and tissue oxygen stores, as well as phosphocreatine levels, recover following exercise. In healthy individuals, VO_2 decreases rapidly during the recovery period and is not significantly influenced by workload (Caen et al., 2021).

Alveolar-Arterial PO_2 Pressure Difference [$P(A\text{-}a)O_2$]

An important parameter for assessing pulmonary gas exchange is the alveolar-arterial PO_2 pressure difference, denoted as $P(A\text{-}a)O_2$. The normal resting value of $P(A\text{-}a)O_2$ is typically less than 10 mmHg. During exercise, in healthy individuals, it can increase to above 20 mmHg. Values exceeding 35 mmHg may indicate abnormalities in gas exchange, while values surpassing 50 mmHg may suggest pulmonary dysfunction ("ATS/ACCP Statement on cardiopulmonary exercise testing," 2003).

SpO_2

A reduction of more than 5% in arterial saturation, as assessed by pulse oximetry during a cardiopulmonary exercise test, suggests exercise-induced hypoxemia. In certain laboratories, a desaturation level below 85% or a decrease to 85% is considered the termination criterion for the exercise test. During prolonged high-intensity exercise in healthy adults, a decrease of 5% to 10% from the baseline arterial saturation may occur due to diffusion limitation caused by rapid pulmonary vascular transit time associated with elevated cardiac output. It is important to note that this finding does not always indicate the presence of pathology (Ascha et al., 2018; Rowland & Medicine, 2017).

CONCLUSION

Pulmonary exercise physiology is important for understanding how the respiratory system functions during exercise. It provides valuable insights into gas exchange efficiency, oxygen utilization, and ventilatory responses. Key factors include VO_2max, A_T, V_E, VCO_2, and measures like RER and O_2 pulse. Assessing parameters such as $P(A\text{-}a)O_2$, V_D/V_T, and SpO_2 helps identify gas exchange abnormalities and hypoxemia. Understanding pulmonary exercise physiology aids in exercise prescription, monitoring training adaptations, diagnosing respiratory conditions, and optimizing performance.

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Chapter 21

Psychiatric Nutrition
What You Eat, Your Mind

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ABSTRACT

1. INTRODUCTION

The World Health Organization affirms that a quarter of individuals encounter mental or neurological challenges during certain periods throughout their existence. (WHO, 2012) According to recent estimates, mental disorders globally constitute a significant barrier and burden to society, and pharmacologically focused psychotherapeutic intervention, along with additional treatment methods, currently stands as the primary mode of treatment employed in psychiatry. In spite of the traditional approach to therapy, there are anticipations of a surge in mental ailments in the forthcoming years, leading to a global outbreak. (Logan & Jacka, 2014) Increasing substantial proof suggests that the correlation between a person's dietary patterns and nutritional intake plays a vital role in the prevention, progression, and control of mental illnesses. (Sarris, Logan, Akbaraly, Amminger, et al., 2015)

Over time, globalization has led to a shift in food consumption patterns from traditional, high-fiber foods that are abundant in nutrients yet low in calorie density to modern foods that are less nutritious, higher in energy density, and lower in fiber content. This change may have negative effects on nutritional quality and ultimately on mental health. (Kingdom, 2006) In addition to individual characteristics and actions, as well as a person's financial and social standing, and environmental factors, various nutritional factors such as omega-3 fatty acids, vitamin B, vitamin C, vitamin D, and vitamin E, folic acid, selenium, iron, magnesium, zinc, S-adenosyl methylanin, and N-acetyl cysteine can impact neurochemical reactions and mental health. (Kingdom, 2006; Logan & Jacka, 2014) Food-mediated pathways leading to mental disorders can be attributed to chronic inflammation, differentiated metabolism, impaired microbiota, and oxidative stress. (Sarris, Logan, Akbaraly, Amminger, et al., 2015) Studies suggest that nutrients can be used as nutraceuticals through monotherapy, combined therapy, or dietary advice alone. (Sarris, Logan, Akbaraly, Paul, et al., 2015)

Proper nourishment has a pivotal significance in the well-being of the mind as well as various non-transmissible conditions. In addition, the trajectory of mental well-being is intricately intertwined with both direct and indirect associations with dietary factors. Also, nutrition and noncommunicable conditions like diabetes and other long-standing ailments exhibit a relationship with an individual's mental wellbeing comorbidly. Hence, by tackling dietary deficiencies, both the control of non-communicable diseases and mental health disorders can be improved. Even a small alteration in a population's diet can have a major impact on the distribution of noncommunicable diseases in the

population, as well as common mental disorders. It has the potential to invert the present pattern of the illness. (Sarris, Logan, Akbaraly, Paul, et al., 2015)

Research findings indicate that initiation of the combination of micronutrient treatment and conventional therapy leads to a decrease in psychiatric symptoms by 50%. (Logan & Jacka, 2014) Therefore, incorporating nutritional evaluations and treatments must be a fundamental element of mental healthcare provisions. Individuals with mental disorders should be screened with questions and scales questioning the diet. Implementing dietary modifications is an economical approach to enhancing mental health results at a community level. While acknowledging the significance of nourishment in relation to mental wellbeing outcomes, numerous psychiatrists and healthcare practitioners advocate for an inclusive and cooperative strategy to tackle the escalating impact of mental health disorders.

Despite the widely recognized correlation between nutrition and mental health, there exists an insufficiency of a comprehensive roadmap or framework. The primary step should involve contemplation and comprehension of the connection between nutrition and mental well-being before implementing dietary modifications and lifestyle interventions. Once the correlation between nutrition and mental health has been comprehended, a multidisciplinary approach can be employed to formulate interventions and strategies. Thus, great progress can be made in the recovery and prognosis of mental health disorders. In fact, proper nutrition can help prevent mental health disorders, and the adverse side effects of pharmacological treatments can be reduced through appropriate nutrition and diet therapy. This can lead to faster treatment and better outcomes by mitigating the negative effects of medications on living conditions. Certain nutrients can be used alongside nutraceutical or pharmacological treatment or even in place of pharmacological treatment for certain diseases. This review aims to explore the correlation linking nourishment and psychological well-being from a comprehensive perspective of nutritional psychiatry and to assess potential treatments.

2. GENERAL INFORMATION

2.1. The Interconnection of Nourishment and Psychological Well-being

In the realm of mental well-being, the attention of many individuals is often directed towards elements such as stress management, physical activity, and therapeutic interventions, overlooking the significance of nutrition. However, an often-overlooked aspect of mental health is nutrition. Our dietary choices can significantly influence our emotional well-being, and understanding this connection is crucial for maintaining good mental health.

While pharmacologically centered methods have led to modest alleviation of the global impact of mental health disorders, there are signals pointing towards the burden of mental illness will persistently surge worldwide in the coming decade. (Baxter, Patton, Scott, Degenhardt, & Whiteford, 2013; Whiteford et al., 2013) Overall, mental disorders, encompassing a range of conditions, among which major depression and anxiety disorders are just examples, impose a significant load of impairment on a global scale. (Logan & Jacka, 2014)

The urgent challenges of swift urbanization and shifts in conventional ways of life (such as dietary patterns, physical activity, and social dynamics) have been associated with a rise in depression and various other mental ailments. (Jacka, Sacks, Berk, & Allender, 2014; Logan & Jacka, 2014) Within the context of the prevailing comorbidity epidemic between physical and mental well-being, depression and other mental disorders are intrinsically intertwined. Diet unquestionably emerges as a crucial determinant in this intricate relationship. (F. N. Jacka et al., 2014)

The current situation, where both developed and developing countries consume low-quality foods that are high in energy density and highly processed, has led to both over- and under-nutrition. (Parker E, 2014) Many people do not consume the necessary nutrients required for brain development and health, such as B group vitamins, zinc, and magnesium, through their diet. Although there have been some slight improvements in dietary habits, such as reductions in sugar and fat intake, nutrient and fiber-rich foods are still not being consumed as much as recommended. (Bowman, Friday, Thoerig, Clemens, & Moshfegh, 2014) The convergence of adverse dietary transformations, alongside tobacco consumption, inadequate engagement in physical activity, and the misuse of alcohol and drugs, has sparked a widespread outbreak of illnesses. (WHO, 2013)

The pathways through which nutrition influences mental well-being are evident; the human brain, characterized by its heightened metabolic activity, relies on amino acids, fats, vitamins, and minerals for its structural integrity and optimal performance. Dietary patterns have a pivotal function in regulating the efficacy of the immune system, subsequently mitigating the likelihood of experiencing depression. (Berk, Williams, et al., 2013) Insufficient intake of some nutrients can negatively impact the antioxidant defense system that plays a role in mental disorders. Additionally, neurotrophic factors play a significant role in neuronal plasticity and repair mechanisms throughout life. (Molendijk et al., 2011)

The brain's makeup, organization and operations rely on the presence of crucial nutrients in the body, such as amino acids, lipids, vitamins and minerals. (Castro et al., 2018) Therefore, consumption of food and the nutritional value of

the food are expected to influence the functioning of the brain. This leads to diet an important variant for aiming for better psychological well-being, emotional state and cognitive performance. (Dinan et al., 2019) Moreover, the dietary composition has a direct impact on neurotransmitters, neuropeptides, endogenous gut hormones and microbiota. (Van de Wouw, Schellekens, Dinan, & Cryan, 2017) In other words, not only does it influence the arrangement and operation of the brain, but it also contributes to the generation of hormones, neurotransmitters, and the interaction between the gut and the brain. (Adan et al., 2019)

The food we eat has a prominent impact on our mental health and well-being through various mechanisms such as reducing inflammation, promoting antioxidant activity, supporting neurogenesis, regulating the microbiome, and influencing immune responses and epigenetic changes. (Marx, Moseley, Berk, & Jacka, 2017) To understand the connection between nutrition and mental health, it's important to first understand the role of neurotransmitters. Neurotransmitters are chemicals in the brain that are responsible for transmitting signals between neurons. Some of the most important neurotransmitters such as dopamine, serotonin, and norepinephrine are involved in aspects of mental well-being. These are directly affected by the foods we eat. For example, carbohydrates can increase the production of serotonin, which is often referred to as the "feel-good" neurotransmitter. Similarly, foods that are high in protein has the potential to boost the synthesis of dopamine, which is involved in motivation and reward. Conversely, a diet that is high in saturated fat, sugar, and processed foods can have a negative efficacy on mental health. The consumption of such food varieties can induce brain inflammation, a factor consistently associated with the manifestation of depression and various other mental health conditions. (Adan et al., 2019)

Eating a balanced diet and supplementing with vitamins, minerals, and healthy fatty acids have the potential to offer additional benefits due to their various biological roles. (Polavarapu & Hasbani, 2017) For patients with psychiatric disorders, changing their diet can be particularly beneficial in improving depressive symptoms quickly, reducing activation of reward-based eating behavior, and promoting weight reduction or inhibiting weight accumulation. (Fond et al., 2015) However, It is advisable noted that eating disorders such as anorexia require special investigations and do not necessarily comply with these recommendations. (Cuenca-Sánchez, Navas-Carrillo, & Orenes-Piñero, 2015) Diet can assist individuals to modify their dietary intake and educate them on better nutrition practices when they return home; whereas, outpatients may need workshops such as healthy cooking workshops. A recent study with randomized

control showed proof of the efficacy of these training sessions in mitigating symptoms of depression after a period of 3 months. (Parletta et al., 2019) Consumption of fresh organic food has been proven to lower the chances of developing cancer and is thought to improve mental health, but this positive effect continues to be challenged due to access to fresh organic food in hospitals and food storage issues. (Baudry et al., 2018)

Last scientific studies have established links between nutrition and mental health, particularly in the role of healthy diets in reducing depression and suicide risk. (J. S. Lai et al., 2014; Nanri et al., 2013) The content of the healthy diet associated with suicide; It is marked by consuming ample portions of fruits, vegetables including potatoes, mushrooms, soy-based products, sea vegetables and fish. Certain ingredient of the diet (e.g., coffee, and green tea) are also connect to a reduction in depressive symptoms. (Pham et al., 2014)

Maternal and early-life nourishment also play an essential act in mental health outcomes in later life. (Steenweg-de Graaff et al., 2014) Unhealthy eating habits in children and adolescents have also been linked to worse mental health. (O'Neil et al., 2014) Evidence is presented showing that the mother's inadequate home and unhealthy diet and early childhood nutrition are linked to the emotional and behavioral dysregulation of the child. (Steenweg-de Graaff et al., 2014) Thus, nutrition serves as an adaptable focus for intervention that could prevent primary occurrence of prevalent psychological conditions. Specifically linked to improved cognitive results and decreased likelihood of dementia during older age is the Mediterranean eating pattern. (Solfrizzi & Panza, 2014) Moreover, scientific investigations have indicated that the core involvement of the intestinal microbiota is vital in the intricate mechanisms of inflammation, oxidative stress, and neuroplasticity, serving as fundamental pathways for each of these biological processes. (Berk, Williams, et al., 2013; Moylan et al., 2014) Consequently, there has been a suggestion to explore dietary interventions and nutraceutical approaches as prospective therapeutic options for mental ailments, like melancholy and psychosis that fall within the realm of psychiatric disorders. (Sarris et al., 2016) Current studies have also been conducted focusing on dietary interventions for managing clinical depression, which serve as valuable references for further exploration. (Jacka et al., 2017)

In addition, unbalanced and inadequate dietary patterns escalate the likelihood of cardio-metabolic disorders and decreased cognitive performance. Therefore, it can be said that an unfavorable diet of low quality can initiate psychological well-being issues and impaired cognitive function, which are prone to manifest as one grows older. (Prenderville, Kennedy, Dinan, & Cryan, 2015) There is a significant relationship between obesity caused by one's nutritional status,

especially unhealthy diet, and mood regulation, and it can be said that this relationship is also between stress sensitivity. (Dallman, 2010) This situation reveals that there is an undeniable relationship between nutrition, metabolism, and mental health. In addition, it can be said that physical activity may be correlated with indications of depression in connection with metabolic syndrome. (Matta et al., 2019) Considering the findings from both human and animal experiments, a robust correlation between the consumption of a western-style diet rich in fats and sugars and the development of cognitive dysfunction is evident, specifically marked by memory impairments and elevated anxiety-related behaviors. (Attuquayefio, Stevenson, Oaten, & Francis, 2017; Peris-Sampedro et al., 2019) In addition, obesity demonstrates an association with impaired functioning of the hippocampus and declines in episodic memory observed in experiments involving humans and rodents. (Cheke, Simons, & Clayton, 2016)

2.2. Possible Mechanisms in Nutrition and Mental Health

There are ways that play a role in mental health, and these are diet-modifiable ways. These pathways are different from each other, but they can overlap or interact to create a synergistic pathway. (Berk, Williams, et al., 2013; Moylan et al., 2014)

2.2.1. Inflammation Factor

Persistent low-level inflammation, known as chronic low-grade inflammation, manifests as heightened elevated concentrations of inflammatory cytokines and proteins involved in the acute phase response. This inflammatory state has been involved in the pathogenesis of schizophrenia, depression, and bipolar disorder. There are many factors that contribute to this type of inflammation, including stress, smoking, obesity, and sleep problems, but an unhealthy diet is especially influential. (Berk, Williams, et al., 2013) Recent research has shown that healthy eating templates, such as the Mediterranean diettary pattern, which is rich in vegetables, fruits, fiber, and polyunsaturated fatty acids (PUFAs), are is linked to reduced levels of inflammatory markers. (Estruch, 2010) Besides, studies implementing intervention approaches have provided evidence that following the Mediterranean diet rigorously leads to a noteworthy reduction in elevated levels of inflammation markers. (Schwingshackl & Hoffmann, 2014)

2.2.2. Neuroplasticty Factor

Neurological adaptability, alternatively referred to as brain plasticity or neuroplasticity, denotes the brain's capacity to transform and adjust when confronted with novel encounters and obstacles. This process is essential for

learning, memory, and overall brain health. While many factors can influence brain plasticity, including exercise, sleep, and stress management, nutrition also plays a significant role. The brain is a highly complex organ that consists of billions of neurons, or nerve cells, that communicate with each other through synapses. Brain plasticity pertains to the brain's aptitude for establishing new synaptic connections, strengthen existing ones, and reorganize neural networks in response to new experiences and stimuli. This process is essential for learning and memory, as well as for recovering from brain injuries and adapting to age-related changes in brain function. In short, brain plasticity is what allows our brains to learn, grow, and adapt throughout our lives. Neurogenesis is also important for mood regulation, particularly within the hippocampus. Modifications in neurogenesis have the potential to exert notable effects on mental well-being. It is postulated that the involvement of brain-derived neurotrophic factor (BDNF) and various other neurotrophins, including vascular endothelial growth factor and bcl-2, is instrumental in the regulation of neurogenesis within the hippocampus. (B. S. Fernandes et al., 2015) While the available clinical research on the influence of dietary factors on neurogenesis is restricted, initial indications propose that diet has the potential to impact the levels of brain-derived neurotrophic factor (BDNF). As an illustration, a dietary intervention spanning four weeks, wherein the intake of carotenoid-rich fruits and vegetables was augmented to eight portions each day, yielded elevated serum levels of brain-derived neurotrophic factor (BDNF) among individuals diagnosed with schizophrenia, in comparison to the control group. (Guimarães et al., 2008) Malnutrition has also been linked to a decrease in hippocampal volume, according to an epidemiological study of older adults. (Jacka, Cherbuin, Anstey, Sachdev, & Butterworth, 2015) Particular nutrients, such as B vitamins and omega-3 fatty acids are essential for neurogenesis and can help support the growth and development of new neurons. Antioxidant and anti-inflammatory nutrients like polyphenols, l-theanine, and vitamin E are beneficial for stimulating neurogenesis as well as omega-3 fatty acids. On the contrary, diets abundant in fat and sugar have the potential to disrupt this mechanism. It is advisable to minimize the consumption of processed and high-fat foods, as they have been implicated in promoting brain inflammation and impeding brain plasticity. (Martire et al., 2014)

2.2.3 Oxidative Stress Factor

Oxidative stress is a state of physiological imbalance that emerges when the generation of reactive oxygen species (ROS) surpasses the body's capacity to effectively detoxify and restore the resulting harm. Prolonged oxidative stress has

been implicated in the development of various chronic diseases, including cardiovascular disease, neurodegenerative disorders, and cancer. While endogenous factors contribute to oxidative stress, emerging research highlights the significant role of nutrition in modulating oxidative stress levels. Oxidative and nitrosative stress is thought to be associated with mental health as well as playing a role in various chronic diseases. Notably, individuals affected by schizophrenia demonstrate a decrease in brain glutathione levels, a crucial antioxidant involved in the maintenance of cellular redox balance. This reduction in glutathione levels contributes to an impairment in the metabolism of glutamate, a key excitatory neurotransmitter in the brain. Consequently, disrupted glutamate metabolism disrupts the delicate balance of neurotransmission, potentially impacting cognitive function and synaptic plasticity. Furthermore, the diminished brain glutathione levels and impaired glutamate metabolism in patients with schizophrenia lead to an upsurge in oxidative stress. Compared with the healthy control group; In populations affected by depression, comparable findings have emerged, indicating elevated levels of indicators associated with oxidative stress alongside diminished levels of vital antioxidants, including coenzyme Q10, vitamin C and E, glutathione. (Moylan et al., 2014) In addition, an extensive meta-analysis examining 115 researches revealed a discernible decrease in antioxidant capacity among individuals experiencing acute episodes of depression. (Liu et al., 2015) This result may be modified through diet, given the wealth of antioxidant compounds found in healthy foods likewise fruits, vegetables.

2.2.4 Mitochondrial Dysfunction Factor

Mitochondrial dysfunction, characterized by impaired mitochondrial structure and function, has been associated with the development of diverse ailments, encompassing metabolic dysfunctions, neurodegenerative diseases, and cardiovascular diseases. Emerging indications propose that nutrition plays a crucial role in modulating mitochondrial health and function. Particularly depression, schizophrenia, and bipolar disorder may be linked to impaired mitochondrial energy production, size, and distribution. The observed alterations might stem from a decline in antioxidant capacity and an elevation in mitochondrial-derived proinflammatory cytokine-mediated nitrogen-free and oxygen-free radicals. This suggests that inflammation and oxidative stress trigger mitochondrial dysfunction. (Morris & Berk, 2015) Animal studies have shown that certain dietary components and nutraceutical composites, for instance α-lipoic acid, coenzyme Q10, creatine, carnitine, NAC, resveratrol, as well as some

antidepressants can enhance mitochondrial respiratory function. (Wright et al., 2015)

2.2.5. Microbiota Brain-Intestinal Pathway

The gastrointestinal microbiota has many effects on mental health, including modulation of serotonin neurotransmission, BDNF, the hypothalamic-pituitary-adrenal axis-mediated stress response and immune function. For instance, in the context of rodent studies, it has been observed that germ-free rats without gut microbiota exhibit an intensified stress response, alongside reduced grades of brain-derived neurotrophic factor and serotonin receptors in both the cortex and hippocampus regions of the brain. Conversely, rat with intact intestinal colonization display contrasting patterns in these neurochemical parameters. (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015) Certain pathways are known to possess a reciprocal association with stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis, a neuroendocrine system liable for regulating the body's stress response. Intriguingly, investigations have unveiled that the modulation of microbiota in rats can be influenced by the functioning of the HPA axis. (O'Mahony et al., 2009) A reduction in the abundance and variety of gut microbiota was noted among individuals with depression in comparison to the control group of healthy individuals. Transferring microorganisms from individuals with depression to rats induces symptomatic behaviors related to depression. Adjustment of the intestinal microbiome through probiotic supplementation or consumption of probiotic-containing foods influences depression-related behaviors in animals. (Kelly et al., 2016; Zheng et al., 2016)

Changes in diet that affect the permeability of the intestine, such as those caused by a diet rich in fat, can have an impact on mental health. The intestinal epithelial barrier is responsible for regulating the flow of substances from the intestinal tract into the bloodstream, and when it becomes restricted, it can lead to depression. The rationale behind this phenomenon lies in the escalation of intestinal permeability, a condition commonly referred to as "leaky gut." When intestinal permeability is increased, it allows for the passage of substances, such as bacterial components or toxins, from the gut lumen into the underlying tissues. This breach in the intestinal barrier activates immune cells residing in the intestinal wall, leading to an immune response characterized by the generation of pro-inflammatory cytokines and the initiation of nitro-oxidative stress pathways. The production of inflammatory cytokines, which are signaling molecules involved in the immune response, contributes to the systemic inflammation observed in this context. These cytokines, including interleukins and tumor necrosis factor-alpha (TNF-α), trigger a cascade of pro-inflammatory reactions

throughout the body. The elevated levels of inflammatory cytokines propagate the inflammatory response, resulting in a state of chronic inflammation. Simultaneously, the activation of nitro-oxidative stress pathways serve as a vital factor in the development of systemic inflammation's pathogenesis. Nitro-oxidative stress characterizes a state of disharmony between the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the body's ability to counteract their detrimental effects. Under conditions of increased intestinal permeability, the passage of bacterial components and toxins stimulates the production of RNS and ROS in immune cells and other cells of the intestinal wall. These reactive species, in turn, trigger oxidative damage and further perpetuate the inflammatory response. The combination of nitro-oxidative stress pathways and inflammatory cytokines contributes to the systemic inflammation observed in the body. This state of chronic inflammation can have far-reaching effects, impacting various organs and systems beyond the intestines. It has been associated with a range of health issues, including metabolic disorders, cardiovascular diseases, neurodegenerative conditions, and mental health disorders. (Maes et al., 2013)

The regulation of serotonin metabolism by a healthy gut microbiota has also been noted, and a high-quality and nutritious nutritional choices have the potential to alleviate brain-related stress and diminish inflammatory responses, regulate the gut microbiota, and maintain healthy mental acuity across the lifespan. (Codagnone et al., 2019; Mörkl et al., 2020) In a study on stressed adolescent mice, a diet enriched with vitamin A, omega-3 polyunsaturated fatty acids including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) helped reduce harmful behavioral, cognitive, and neurochemical effects. This diet also led to alterations in the microbial community structure. (Provensi et al., 2019)

Recent research has highlighted the crucial connection between the gut microbiome and brain function and development. (Dinan et al., 2019) The configuration of the intestinal microbial population, both during the prenatal and postnatal periods, assumes a pivotal role in determining the susceptibility to psychiatric disorders in later stages of life. Moreover, the complex interaction connecting the gut microbiome and the host organism impacts a wide array of psychological factors, encompassing stress reactivity, anxiety levels, depressive symptoms, and the development of various psychiatric disorders, as like attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASD), and anorexia nervosa. (Mörkl et al., 2020)

The intricate configuration of the gut microbiota is shaped by a combination of elements, including the host's genetic makeup, external influences, and lifestyle choices, wherein the most influential determinant appears to be dietary patterns. Studies have demonstrated that nutritional elements can directly influence the

composition of the microbial community in both animal models and human subjects. Nutrition and diet is therefore a modifiable factor for the structure of the intestinal microbial community. (Xu & Knight, 2015) Research has indicated that diets rich in dietary fiber and following a Mediterranean eating pattern facilitate expansion of the intestinal microbial community and correlate with a diminished probability of experiencing depressive symptoms. (Gopinath, Flood, Kifley, Louie, & Mitchell, 2016) Additionally, fermented foods may also have the prospect to improve mental health by altering gut microbiota and physiology. (Aslam et al., 2020)

2.2.6. Genetic and Epigenetic Factors

Cofactors from micronutrients are important in guiding DNA methylation steps. In addition, these cofactors are prominent for brain development, epigenetic regulation, and mental health. Folate, vitamin B12, and SAM are key compounds in progress of DNA methylation. Thus, proofs that neuropsychiatric disorders are effectively treated with dietary supplementation indicates a probable connection between diet, DNA methylation, and behavior; however, more research is needed in this field. (Gaudio, Wiemerslage, Brooks, & Schiöth, 2016; Sarris, Logan, Akbaraly, Amminger, et al., 2015)

Looking at the available evidence, researchers in the fields of mental health and nutrition have gathered independent proof that put foward a link between mental well-being and nutritional quality through epigenetic processes. (Sarris, Logan, Akbaraly, Amminger, et al., 2015) Inadequacies in both micronutrients and macronutrients have been linked to elevated behavioral challenges, while dietary supplementation has shown to be effective in treating some neuropsychiatric disorders. (Neugebauer, 2006) (J. J. Rucklidge, Frampton, Gorman, & Boggis, 2014) Unhealthy diets during pregnancy have also been linked to a number of adverse psychiatric outcomes. (Barouki, Gluckman, Grandjean, Hanson, & Heindel, 2012) Research findings indicate that diminished methylation, SAM, and folate levels exhibit connections with exhaustion, depressive symptoms, autism spectrum disorders, and various neurologic ailments. (Kato & Iwamoto, 2014) Additionally, unhealthy, and inappropriate methylation can lead to a variety of health issues, such as autism and schizophrenia. (Schneider et al., 2016)

2.3. Studies of Diet Patterns and Mental Illness

Substantial epidemiological and empirical evidence consistently supports the association between the quality of consumption of food and beverages and mental well-being, as observed across diverse populations, thereby ruling out the possibility of alternative explanations stemming from demographic variations. (B. S. Fernandes et al., 2016)

2.3.1. Childhood, Maternal and Perinatal Findings

The newborn's body comprises about 13% of the overall weight attributed to the brain, and proper growth and development depend on both energy intake and nutritional status. (Cunnane & Crawford, 2014) It is crucial to ensure sufficient nutritional consumption amidst this phase of swift development, as development in early childhood can impact susceptibility to diseases later in life. Nutritional intervention during the first 1000 days, from pregnancy to two years of age, might exert a more pronounced influence on subsequent well-being compared to interventions in subsequent stages of life. (Dimov et al., 2021) In addition, premature infants or infants small for gestational age, who are among the groups at high risk of neurological disorders, are affected by nutritional status. (Castanys-Muñoz et al., 2017) Nutrition during early life also affects cognitive function later in life, and further research should explore the manner in which nutrients influence signaling mechanisms crucial for cognitive performance. (Dinan et al., 2019)

Investigations have explored the correlation between dietary patterns and mental well-being among individuals during the perinatal stage, adolescence, and among women. (Mühlig, Antel, Föcker, & Hebebrand, 2016; Sparling, Henschke, Nesbitt, & Gabrysch, 2017) High-quality diets have been linked to better mental well-being outcomes in children and adolescents, while unfavorable diets exhibit connections to worse mental well-being outcomes. (O'Neil et al., 2014) Mental symptomatology, like internalization and externalization difficulty in children 5 to 7 years old, has been reported to be independently associated with nutrition. (Steenweg-de Graaff et al., 2014)

During the course of pregnancy, women face an exceptional period characterized by intricate physiological changes and increased nutritional requirements due to the growing demands placed upon them by the developing fetus. This pivotal stage necessitates a heightened attention to dietary adequacy to ensure optimal maternal and fetal well-being. The unique demands of pregnancy result in an increased susceptibility to nutrient deficiencies among expectant mothers. The growing fetus relies on the maternal nutrient supply to fuel its growth and development, thereby placing an additional burden on the mother's nutritional status. Of particular concern is the potential link between nutrient deficiencies while pregnancy and the risk of perinatal depression. Research has shed light on the correlation linking particular nutrient deficiencies and an elevated vulnerability to depression during the perinatal period, a condition of emotional disturbance that impacts women throughout gestation and in the postpartum period. Diet is important for mental health at all stages of life. (Baskin, Hill, Jacka, O'Neil, & Skouteris, 2015)

2.3.2. Findings of Adults and Elderly Individuals

Several research studies have indicated a connection between depression and nourishment in adults. (Li et al., 2017) Consuming a healthful diet has been connected with a reduced risk of developing depression, as shown in various systematic reviews and meta-analyses. (J. S. Lai et al., 2014; Li et al., 2017; Psaltopoulou et al., 2013) Extensive research findings have showcased the beneficial impact of following a Mediterranean-style diet, which entails embracing a dietary pattern abundant in low-fat dairy products, whole grains, fish, olive oil, fruits, and vegetables. Adherence to such a nourishing diet has been consistently linked with a reduced likelihood of experiencing depression. On the other hand, dietary habits marked by the excessive consumption of high-fat dairy products, sweets, refined grains, and red and processed meats have demonstrated links to an elevated risk of developing depressive signs. These investigations emphasize the detrimental implications of diets rich in such unhealthy components, underscoring the importance of adopting dietary patterns that prioritize whole, unprocessed foods for the preservation of mental well-being.

A 12-week randomized controlled trial conducted on 119 people with depression and/or anxiety reported improvement in depression and anxiety symptoms with personalized lifestyle interventions focusing on motivational interviewing, physical activity, and dietary changes. (Forsyth, Deane, & Williams, 2015)

In addition, higher diet quality and healthy diet consumption in adulthood have been linked with lower likelihood of cognitive deterioration. (Smyth et al., 2015) Dietary consumption of polyphenolic antioxidants has been linked with enhanced cognitive performance in older adults. (Anton et al., 2014) Mediterranean diet enriched with nuts and olive oil has also been linked with enhanced cognitive performance in older adults. (Valls-Pedret et al., 2012) These findings suggest that nutritional interventions can prevent cognitive deterioration, particularly during the aging process populations experiencing stress and anxiety. Hence, it is essential to focus on the positive impact of nutrition on mental well-being, especially with the increasing prevalence of stress and aging population. (Wu, Ying, & Gomez-Pinilla, 2004)

2.3.3. Specific Nutrients and Dietary Patterns

While scientists continue to delve into the intricate relation between nourishment and mental well-being, a plethora of research studies provide compelling evidence pointing towards a robust association between a nourishing diet and psychological well-being. (Dinan et al., 2019) For instance, eating more fresh vegetables and fruits has been linked with feelings of happiness and better mental health. (Moreno-Agostino et al., 2019)

A 2014 analysis linked a diet including fruits, leafy greens, vegetables, whole grains, and fish with lower risks of depression. (J. S. Lai et al., 2014) From that point onward, a surge can be observed in scholarly publications supporting the utilities of healthy diets, particularly the The Mediterranean eating pattern, known for its capacity to decrease the occurrence of indications related to depression, as evidenced by research findings in more than 50 studies. (Lassale et al., 2019)

Healthy diets usually consist of nutrient-rich plant foods and high-quality protein sources, while a Western diet typically includes processed foods, refined grains, and sugary snacks. (Rahe, Unrath, & Berger, 2014) (Jacka et al., 2010; Schwingshackl & Hoffmann, 2014)While there is much variation in defining a healthy diet due to cultural differences, a healthy diet should prioritize nutritious foods and sufficient intake of vitamins and minerals. According to one study, both the a healthy traditional diet and Mediterranean diet that includes moderate amounts of meat and dairy products and legumes can help protect against depression. (Rahe et al., 2014)

A multitude of research investigations have unveiled compelling evidence supporting the notion that adopting a "wholesome diet" marked by substantial consumption of whole grains, fruits, vegetables and fish, can substantially diminish the likelihood of experiencing depression. (J. S. Lai et al., 2014) Specifically, following the Mediterranean eating pattern has been associated with a reduced risk of depression in multiple studies of cohort and case-control. (Psaltopoulou et al., 2013) Dietary interventions have also been found to have potential in reducing the incidence of depression. (Joseph Firth et al., 2019; Lassale et al., 2019) On the other hand, the ingestion of energy-dense, fat-laden, sugar-laden, and sodium-laden food items has been linked to a subsequent surge in unfavorable mood states within a span of 48 hours post-consumption, according to a study of healthy college students. (Hendy, 2012)

Last meta-analyses of observational studies indicate that consuming fish and getting enough iron, magnesium and zinc in your diet may help lower the risk of depression. (Delgado-Lista et al., 2016) However, observational studies on the effects of varied diets, like the traditional Japanese and Norwegian diets, have produced limited and sometimes contradictory evidence. (Nanri et al., 2013) It is crucial to highlight that the link between healthfull foods and better mental well-being is not related to the link between unhealthy foods and worse mental well-being, suggesting that different physical pathways may be at work. (Jacka, 2017) These connections are also separate from the effects of weightiness, suggesting that diets be able to influence mental illness regardless of individual's weight. Although observational studies have accounted for variables that can influence mental health, like socioeconomic status, physical activity, and smoking, these factors can still impact the results. (Jacka, Cherbuin, Anstey, & Butterworth, 2014)

While a multitude of observational studies consistently establish a correlation linking the unhealthy of an individual's dietary choices and the prevalence of prevalent mental and mood disorders, the body of intervention studies investigating this intricate association remains relatively limited in scope. A systematic review from 2013 evaluated 17 dietary intervention studies for depression, anxiety, and mood disorders and found that approximately half of the studies reported improvement. Among the interventions that yielded the most favorable outcomes, a common thread emerged, as they encompassed components such as personalized or collective counseling sessions, oversight from a nutrition specialist, and dietary guidance centered around the incorporation of fiber-rich foods and/or an abundance of fruits and vegetables. It is worth noting, though, that these studies were comparatively less inclined to provide recommendations pertaining to weight management, curbing the intake of red meat, or adhering to a low-cholesterol dietary regimen. (Opie, O'Neil, Itsiopoulos, & Jacka, 2015)

The researchers did a post hoc analysis of the study, called PREDIMED. Their aim was to examine the potential effect of the Mediterranean diet on the incidence of de novo depression. The PREDIMED study is a large randomized controlled trial that investigates the impact of the Mediterranean diet on cardiovascular disease. The examination revealed that adherence to the Mediterranean eating pattern potentially diminishes the occurrence of de novo depression, particularly in people diagnosed with type 2 diabetes, but its effect was not significative for advanced depression. (Sánchez-Villegas et al., 2013) Moreover, an additional study focused on prevention and early intervention revealed that the provision of dietary counseling can be equally efficacious in averting the progression to depression among older individuals, comparable in effectiveness to traditional psychotherapeutic approaches. (Stahl, Albert, Dew, Lockovich, & Reynolds, 2014)

Studies conducted on animals consuming a cafeteria diet suggest that the abundance and assortment of calorie-rich food options that are palatable can bring about changes in gene expression within the reward center of the brain, which can lead to prolonged overconsumption of such foods. (Maniam & Morris, 2010); South, Holmes, Martire, Westbrook, and Morris (2014) These findings are supported by research in humans, which has established a positive association between psychological issues and increased consumption of comfort foods. (Weltens, Zhao, & Van Oudenhove, 2014)

The SMILES trial was a twelve-week study employing randomized control methods that examined the effects of a modified Mediterranean diet intervention on individuals with major depression. Individuals assigned to the experimental group were provided with personalized nutritional counseling and a diet program that followed the Australian Dietary Guidelines and the traditional Mediterranean diet,

while those in the group of comprasion received social support through "friendship" protocols. The results showed that participants in the dietary support group experienced a significantly enhanced progress in depression scores than those in the social support control group within twelve weeks, and their body weight or energy intake did not change significantly. These observations indicate that dietary approaches might be feasible and beneficial for individuals with depression. (Jacka et al., 2017)

2.3.4. Nutraceutical Interventions and Observational Literature

A vast array of nutraceutical interventions exist, each targeting distinct pathways implicated in mental disorders. These interventions encompass the realms of inflammation modulation, oxidative stress reduction, methylation cycle modulation, mitigation of cognitive decline associated with the hippocampus, amelioration of mitochondrial dysfunction, and inhibition of neurotransmitter pathways. (Lim et al., 2016) In light of the influence they exert on these biological pathways, clinical experiments have been undertaken to explore the potential impacts of specific nutrients and botanical formulations on mental well-being.

There is a wide range of research investigating the potential utilities of various nutraceuticals for mental well-being. St John's Wort, for instance, was found in a meta-analysis to have almost identical antidepressant effects to medications known as selective serotonin reuptake inhibitors (SSRIs). (Cui & Zheng, 2016) Other substances like magnesium, L-tryptophan, branched-chain amino acids and folic acid have been examined to assess their prospective benefits for bipolar disorder-related mania. Certain chelated forms of minerals and vitamins have shown promise in betterment symptoms of both depressive and manic episodes associated with bipolar disorder. (Sarris et al., 2016) Furthermore, a combination of kava, passionflower, and L-arginine and L-lysine may be effective approaches targeting anxiety, according to a 2010 systematic review. Furthermore, it is worth noting that within the realm of nutraceuticals, there exist a variety of compounds that have demonstrated favorable outcomes in their early stages of investigation when employed in isolation. Notable examples encompass creatine, folinic acid, as well as meticulously crafted formulations integrating an array of amino acids. These particular substances, upon independent utilization, have exhibited encouraging preliminary data, paving the way for potential therapeutic applications in the field of nutrition and wellness. (Murakami & Sasaki, 2010)

Omega-3 PUFA is a widely studied supplement, with mixed findings reported in various meta-analyses. However, high EPA:DHA formulations of omega-3 have been shown to be beneficial for individuals experiencing depressive symptoms when used in addition to antidepressant medication. (Sarris et al., 2016) The synthesis of

three meta-analytic studies has ascertained that the augmentation of omega-3 intake exhibits potential benefits in addressing depressive symptoms, encompassing both unipolar and bipolar forms of the condition. (Grosso et al., 2014; Sarris, Mischoulon, & Schweitzer, 2012; Sarris et al., 2016) It should be underlined that even small dietary changes can affect early brain development, as lipids such as omega-3 and omega-6 polyunsaturated fatty acids, specifically DHA and arachidonic acid, are found in breast milk and experience an impact from maternal diet consumption. (Algarin et al., 2017) (Oosting, Verkade, Kegler, van de Heijning, & van der Beek, 2015)

While every nutrient plays a crucial role in the development of the brain, the nutrients that have been found to particularly aid neurodevelopment include vitamin A, protein, iron, iodine, choline, folate, vitamins B6 and B12, vitamin D, and long-chain polyunsaturated fatty acids, according to research by Georgieff, Ramel, and Cusick in 2018. (Georgieff, Ramel, & Cusick, 2018) Studies have shown that iodine deficiency during fetal development can lead to irreversible impairment of the cerebral cortex and deficits in cognitive function among children. Anemia linked to insufficient iron levels in infants has also been linked to changes in brain connectivity, as noted in research by Blasco and colleagues in 2017. (Blasco et al., 2017)

The dietary manipulation of the intricate connection between the microbiota, the gut, and the brain presents a hopeful avenue for intervention, holding promise as a target for modification. This can be achieved through the implementation of dietary strategies and the utilization of nutraceutical interventions, such as the incorporation of prebiotics (including high fiber foods and supplementary sources) and probiotics (which can be found in fermented foods or obtained through supplements). These approaches exert a direct influence on microbial populations, thereby exerting potential positive impacts on the microbiota-gut-brain axis. In a recent meta-analytical examination encompassing five distinct randomized controlled trials, it was observed that the administration of probiotic supplements exhibited a discernible reduction in the manifestation of depressive symptoms. (Huang, Wang, & Hu, 2016) In addition, A comprehensive analysis of ten randomized controlled studies determined that supplementary probiotics could potentially yield advantageous outcomes for cognitive function, emotional state, and anxiety. (Wallace & Milev, 2017) However, all studies presented the limitations of the study as the dose and strains of probiotics, and the optimal intervention time. Future interventions of higher quality are needed to improve the current the body of proofs sustaining the use of probiotic supplement and to find out the impact of dietary changes on mental health.

NAC, derived from the amino acid glutathione, possesses the capability to influence cerebral mechanisms encompassing glutamatergic and neurotrophic

transmission, antioxidative potential, mitochondrial operation, as well as the synthesis of glutathione. Through these multifaceted actions, NAC aids in diminishing inflammatory responses within the brain. (Berk, Malhi, Gray, & Dean, 2013) Recent research suggests that NAC may be beneficial for individuals with addiction, bipolar disorder, schizophrenia, and depression, although the current evidence is limited. A last meta-analysis supports the use of NAC for treating depression and its signs, but further randomized controlled trials are necessary to confirm these findings. (Deepmala et al., 2015; Brisa S Fernandes, Dean, Dodd, Malhi, & Berk, 2016)

S-adenosylmethionine (SAMe), an endogenous substance abundant in the human body and characterized by its sulfur content, assumes a pivotal function within the intricate machinery of the one-carbon cycle. This intricate process, facilitated by methylation, exerts profound regulatory influence over neurotransmitters involved in the modulation of state of mind. Clinical studies have found that SAMe is a potent antidepressant, and when used in combination with other antidepressants, it can enhance their effectiveness. (Papakostas, Mischoulon, Shyu, Alpert, & Fava, 2010; Sarris, Papakostas, Vitolo, Fava, & Mischoulon, 2014)

Zinc, a trace mineral, actively participates in the modulation of cytokines and facilitates the process of hippocampal neurogenesis. Its engagement in these processes is achieved through the upregulation of BDNF, a pivotal element in promoting the growth and development of neurons within the brain. Additionally, zinc exerts its influence by modifying the activity of N-methyl-D aspartate and glutamate, two important elements involved in neuronal signaling. Zinc deficiency is thought to correlate with a rise in indications of depression. There is emerging evidence that the use of zinc supplements, particularly as an adjunct to antidepressant cure, be able to improve depressive mood. (J. Lai et al., 2012)

Studies have shown that especially vitamin deficiency negatively affects cognitive performance. (Gaudio et al., 2016; Giannunzio et al., 2018) When we look at vitamin B12, it can be said that it has the strongest relationship in terms of cognitive performance. (Smith, Warren, & Refsum, 2018) Insufficient levels of vitamin B12 result in feelings of exhaustion, sluggishness, melancholy, impaired cognitive function, and are linked to episodes of heightened euphoria and loss of touch with reality. Similarly, various other B complex vitamins exhibit a robust interdependence. Inadequate amounts of thiamine, also known as vitamin B1, contribute to the onset of beriberi, characterized by sensations of numbness and the emergence of a neurological disorder recognized as Wernicke's encephalopathy. (Okafor, Nimmagadda, Soin, & Lanka, 2018)

Insufficient intake of folic acid, or vitamin B9, can have negative impacts on neurodevelopment in both the fetal and infant stages. (Enderami, Zarghami, &

Darvishi-Khezri, 2018) Additionally, inadequate intake in adulthood may elevate the likelihood of experiencing depressive tendencies. Pellagra with dementia is a condition that is caused by niacin (vitamin B3) deficiency. (Hegyi, Schwartz, & Hegyi, 2004) While the significance of subtle subclinical or various insufficiencies in vitamin B on the field of mental performance impairment is not clear, B vitamins are essential to facilitate adequate operation of neurons. Several studies have suggested that folate deficiency is common in individuals with depressive symptoms and individuals who do not exhibit favorable reactions to antidepressant medications. (Fava & Mischoulon, 2009) In fact, the use of folic acid supplements with antidepressants has been shown to increase the proportion of participants who respond to treatment or improve the onset of response. Furthermore, a meta-analysis has suggested that the supplementation of folate and other B vitamins, such as vitamin B6 and vitamin B12, may potentially benefit specific groups identified with schizophrenia. (J. Firth et al., 2017)

Low levels of vitamin D, which is a neurosteroid, have been associated with an elevated likelihood of schizophrenia and heightened indications of depression. (Eyles, Burne, & McGrath, 2013) A blend of essential elements that align with the body's inherent requirements and more accurately reflect the spectrum of nourishment present in food demonstrates superior efficacy compared to solitary nutrients in isolation. (Julia J. Rucklidge, Johnstone, & Kaplan, 2013) Vitamin D supplements have been found to have positive effects on caution, cognitive recall, and depression in individuals 65 years of age and older, and to exert a positive impact on attention deficit and hyperactivity disorder. (Föcker et al., 2017; Mohammadpour et al., 2018) Vitamin D deficiency is widespread worldwide, which may have implications for neuropsychiatric disorders. (Brouwer-Brolsma et al., 2015)

CONCLUSION

The realm of nutritional psychiatry stands as a burgeoning frontier within scientific investigation, experiencing rapid growth and fostering great potential for revolutionizing the landscape of mental well-being. This interdisciplinary field, at the intersection of nutrition and psychiatry, offers a wealth of opportunities to unveil effective interventions capable of not only preventing but also treating various forms of mental illness. By comprehensively exploring the intricate connections between diet, nutrient intake, and psychological health, nutritional psychiatry strives to unravel the profound impact of nutrition on the brain and its intricate networks. The burgeoning body of research within this field holds the promise of unlocking novel strategies that can positively influence mental well-being, paving the way for innovative approaches to promote psychological resilience and enhance general well-being. This is achieved through the regulation of biological pathways, such as

inflammation, oxidative stress, gut microbiota, and neurotrophic factors. Observational studies have shown that diet quality is consistently related to common mental illnesses. Preliminary clinical evidence supports the feasibility and effectiveness of some nutraceutical and dietary interventions. However, further investigation is warranted to delve into the effectiveness and safety of these interventions within expansive and clinically significant cohorts, particularly among individuals grappling with conditions such as depression, schizophrenia, bipolar disorder, and anxiety disorders.

Scientific studies have examined multiple nutraceutical approaches in clinical trials to date; however, further research is needed to assess their effectiveness and safety in clinical settings, in populations with clinically diagnosed mental disorders. Nutritional psychiatry has provided substantial evidence showing that dietary patterns are linked to extensive mental illness. Diet patterns have been found to arrange multiple biological pathways that demonstrate a pivotal influence in the formation and advancement of psychiatric conditions. These pathways encompass mechanisms such as inflammation, oxidative stress, the intricate interplay between the gut and the brain, as well as the process of neurogenesis. So, factors such as diet, inflammatory status, and gut microbiome composition should be included in future interventions. Additionally, exploration is warranted to shed light on the influence of supplementary pathways on the intricacies of mental well-being, necessitating further research in this domain, including the influence of oxidative stress, mitochondrial dysfunction, epigenetics and to develop optimal strategies for interventions.

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Chapter 22

Antioxidants

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ABSTRACT

Free radicals and antioxidants, one of the most studied debates in recent years, are gaining more and more importance day by day. Under normal conditions, when the living metabolism is healthy, antioxidants and free radicals are in balance. However, when this balance becomes evident, the susceptibility to lesions caused by oxidative stress increases. Environmental pollution, alcohol and tobacco consumption, forest fires, increased exogenous free radicals such as X-rays and UV rays can cause damage to the carbohydrates, fat, protein and proteins of the human body, causing the living. With the groups of free radicals, endogenous antioxidants may not be sufficient and this may require exogenous antioxidant support. In this thesis, it is aimed to eliminate the general information about natural antioxidants and synthetic antioxidants by classifying natural and synthetic antioxidants.

Keywords: Natural Antioxidants, Synthetic Antioxidants

INTRODUCTION

An antioxidant is a substance that inhibits or delays undesirable oxidation reactions. It refers to substances or molecules that can delay or even prevent the irreversible damage of other substances due to the imbalance of some metabolites present in a living system. Generally, antioxidants are phenolic in nature. Antioxidants strongly inhibit or slow down the oxidation of reactive oxygen species and free radicals.

Reactive Oxygen Species and Free Radicals can be defined as prooxidants and these prooxidants cause oxidative damage to lipids, proteins and nucleic acids, which are the building blocks of the cell. As a result of this damage, they cause many various pathological reactions and diseases, so they are highly toxic substances. Although the presence of prooxidants negatively affects human health, it makes antioxidants important for a healthier life because antioxidants break down these toxic compounds into low toxic or nanotoxic products with different reactions.

The main factors that determine the importance of antioxidants in human life are their chemical structures, solubility, structure/activity relationships and accessibility to natural resources (Kaur and Kapoor, 2001).

Antioxidants are produced through the cells of our body and can also be obtained from essential nutrients.

Since antioxidants have oxidizing properties, they are broken down by reactions that break oxidation. Therefore, antioxidants can retain the oxidizing agent for a limited period of time, and after a certain period of time, the oxidizing agent continues to oxidize as if there was no antioxidant in the field before. Chemical activity of antioxidants and their reduction potential as hydrogen or electron donor are generally expressed as free radical scavengers (Güçlü et al, 2009).

1.1. Free Radicals

Electrons in an atom are spatially called orbitals. Atoms are found in pairs in this region. The interactions between these atoms result in the formation of bonds and the formation of molecular structures. Free radicals are the name given to a single unpaired electron segment in an atomic or molecular structure. These molecules easily exchange electrons with other molecules. Also known as "Oxidant Molecules" or "Reactive Oxygen Particles" (ROP)" (Halliwell B, 1991).

Free radicals are atoms or molecules that dominate many conjugate electrons in their orbitals. These unshared electrons confer a high degree of activity to free radicals. Free radicals are small molecules, have low activation energies

and have a short lifespan. Due to their small size, they can easily pass through cell membranes (Jensen, 2003).

1. Reactive Oxygen Species

Reactive oxygen species (ROS) are superoxide radical (O2·−), hydrogen peroxide (H2O2) and hydroxyl radical (OH•), which occur to a lesser extent during normal oxygen metabolism. Molecular oxygen has mainly 3 reduction products and 1 excitation product; Radicals formed as a result of the action of free O2 radicals:

1. (R•) (Carbon-centered radicals)
2. (ROO•) (Peroxyl = Carboxyl)
3. (RO•) (Alkoxyl)
4. (RS•) Thiol radicals are formed.
5. Thiol radicals react with O2 to form radicals such as sulfonyl (RSO•) and thiol peroxyl (RSO2•).

1.1. Reduction Products

1.1.1. Superoxide Radical (O2 -)

As a result of reduction of O2 by gaining an electron in all cells , free superoxide radical anion is formed.

O2 (molecular oxygen) + e- (electron) = O2 - (superoxide).

It does not directly cause damage. Its main importance is as a source of hydrogen peroxide and being the reductants of transition metal ions (Fe3, Cu2, Mn2, Mo5).

1.1.2. Hydrogen Peroxide (H2O2)

Peroxide formed as a result of molecular O2 taking 2 e- (electrons) or superoxide; as a result of its association with 2 hydrogen atoms, hydrogen peroxide is formed.

Superoxide hydrogen peroxide is synthesized by the catalysis of the enzyme superoxide dismutase.

H2O2 is a long-lasting oxidant that easily passes through membranes.

Hydrogen peroxide is included in the reactive O2 species, although it is not mentioned as a free radical. Because it reacts with superoxide and forms (OH.) (hydroxyl radical).

• H2O2 + O2•- → (OH•) (hydroxyl radical) + OH- (hydroxyl ion) +O2 (Haber-Weiss reaction)

1.1.3. Hydroxyl Radical (OH•)

It is formed by the reduction of H2O2 in the presence of transition metal ions or by the exposure of water to high-energy ionizing radiation.

Hydroxyl Radical (OH•) is highly reactive. It has a quite short half-life and causes great damage where it occurs.

1.1.1.2. Excitation Products

1.1.1.2.1. Singlet Oxygen O- (1O2)

It is a non-radical O2 molecule with no unpaired electrons. It happens when one of the electrons of oxygen is loaded with energy and displaced to another orbital opposite its own orbital radicals formed as a result of the action of free O2 radicals:

- (R•) (Carbon-centered radicals)
- (ROO•) (Peroxyl = Carboxyl)
- (RO•) (Alkoxyl)
- (RS•) Thiol radicals are formed.
- Thiol radicals react with O2 to form radicals such as sulfonyl (RSO•) and thiol peroxyl (RSO2•).

2.Oxidative Stress

The cellular redox regulation system manage for modulating redox homeostasis, which maintains the regulatory balance between oxidants and antioxidants. The progression of the cellular redox reaction in the direction of the reaction with oxidants is called oxidative stress/damage. Basically, the oxygen molecule is considered a risky gas in terms of toxicity, as it can cause oxidation of cellular macromolecules.

The imbalance between reactive oxygen particles or other free radicals and the antioxidant system is called oxidative stress, and this imbalance can cause irreversible damage to important parts of the cell.

Free radicals are constantly formed as intermediates at the active site of enzymes in enzymatic reactions in the normal metabolic system in the cell. From time to time, these free radical intermediates leak into the active sites of enzymes, randomly interact with molecular oxygen to form free oxygen radicals. Reactive oxygen species (ROS) occurring in the cell are destroyed by systems called "antioxidant defense systems" or simply "antioxidants". At times, more reactive oxygen species (ROS) may occur, which are eliminated through the cellular defense mechanism.

Oxidative stress is defined as the formation of more reactive oxygen species (ROS) that are destroyed by the cellular defense mechanism in the organism.

It is thought to benefit the complications of more than one chronic disease, thanks to cell damage caused by free oxygen radicals by oxidative stress. Such as atherogenesis, emphysema/bronchitis, Alzheimer disease, Duchenne type muscular dystrophy, pregnancy preeclampsia, alcohol-induced liver cirrhosis, hemodialysis patients, diabetes mellitus, acute renal failure, spinal muscular atrophy, aging, retrolental fibroplasia, cerebrovascular disorders, atherosclerosis. The role of oxidative stress in the pathogenesis of these conditions is mentioned.

The harmful effects of oxidative stress on human health is an important branch of research. The instability between reactive oxygen particles formed under the influence of metabolism or external factors such as superoxide anion ($O2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H2O2) and enzymatic or non-enzymatic antioxidant compounds in the body causes oxidative stress. Because antioxidants react with reactive oxygen species and free radicals, they are the most efficient way to combat such undesirable changes and health risks.

2.1. Nitric Oxide (NO•)

Nitric oxide (NO•) is a free radical that has an major role in both physiological and pathophysiological processes.

NO• synthesis occurs in some cells in response to the binding of a stimulator to a receptor or in neurons in response to a nerve impulse. Nitric oxide (NO•) is synthesized from L-arginine and oxygen by the action of nitric oxide synthase (NOS, EC 1.14.13.39) as a result of the activation of different receptors such as muscarinic or histamine receptors.

It is known that nitric oxide (NO•) production has an important place in the codification of vascular tone in humans and has a specific role in the control of blood pressure and kidney function. Nitric oxide (NO•) is a very significant vasodilator occurring in vascular endothelial cells. NO• enters smooth muscle cells and suppresses soluble guanylate cyclase to form 3',5'-cyclic GMP (cGMP). Thus, the concentration of cGMP in the cell begins to increase. In smooth muscle cells, cGMP activates many protein kinases like cAMP. Activated protein kinases cause smooth muscle relaxation followed by vasodilation.

Oxidative effects of nitric oxide (NO•): Nitric oxide (NO•) removes iron from Fe-S proteins and binds to itself, thus suppressing the Fenton reaction and taking a role in carcinogenesis with this mechanism.

Peroxynitrite (ONOO−) is formed when nitric oxide competes with the enzyme superoxide dismutase (SOD) and interacts with the superoxide ($O2\cdot-$) radical. Thus, the physiological effect of nitric oxide is suppressed, and its

oxidative effect arises. It is thought that the physiological balance between superoxide (O2·−) and nitric oxide (NO•) is important for the regulation of vascular tone.

Peroxynitrite is primarily responsible for the harmful effect of nitric oxide. Peroxynitrite has direct harmful effects on proteins and turns into toxic particles such as nitrogen dioxide (NO2•), hydroxyl radical (OH•), nitronium ion (NO2+). Peroxynitrite undergoes chemical reactions to form nitrite (NO2−) and nitrate (NO3−). The fixed end products of the NO• radical are nitrite and nitrate.

2.2. Conditions Caused By Oxidative Stress

Oxygen is necessary for the conversion of lipids, proteins and fats, which are essential nutrients and cell sources, into energy. But oxygen can also turn into highly reactive and harmful substances called "free radicals". Free Radicals can react with healthy cells and cause deterioration of the function and structure of healthy cells. Free radicals carry a free electron. Therefore, they try to take an electron from the surrounding matter. Thus, while a radical is neutralized, a new radical is formed, and the successive reactions follow each other. Antioxidants stabilize these molecules before attacking surrounding tissues. Antioxidants are essential for optimal cellular and systemic stability.

The antioxidant system may not always be at a sufficient level. Oxidative stress indicates that the oxidant/antioxidant balance is disrupted by oxidative metabolism to high levels. It is known that these damages caused by Free Radicals have an important share in aging, degenerative diseases, cancer, cardiovascular diseases, immune disorders and disorders in brain functions. However, the formation of Free Radicals is controlled by substances called antioxidants. Problems occur when the activity of antioxidants decreases or if Free Radical formation increases.

Aging is a biological process that cannot be prevented for all living things. Despite being emphasized, wondered and researched, the issue of aging is still not fully explained. Currently, the only successful application in the field of anti-aging applications is dietary restriction. It increases the lifespan of animals by 30-40%, and it is thought that the reason for this gain is related to the antioxidant activity that occurs with dietary restriction.

Studies have been carried out on the prevention of free oxygen damage in aging and its relationship with antioxidants, and the most studied study has been the prevention of aging by improving antioxidant systems. Although only a single antioxidant agent has been used in more than one study, it is correct to use different antioxidants and antioxidant cofactors simultaneously against antioxidant stress. The antioxidant system in the human body resembles a tree

with many branches. Only when surrounded by all branches can it be successful. For example, a single vitamin deficiency may increase the risk of various diseases, and suboptimal levels of various factors may increase the risk synergistically. Similarly, in different situations, high doses of these antioxidants that disrupt the balance with other antioxidants can have the opposite effect. Antioxidants may have other effects in other diseases. However, studies on antioxidant use and the state of aging are mostly animal studies. Results are lacking due to the lack of a known biomarker of aging and the short duration of human studies. Long-term studies with competent planning are needed.

Gene therapies that will provide more antioxidant production in the body, which are mentioned on the agenda, are genetically produced plants that contain more antioxidants, synthetic antioxidant enzymes (SOD mimics), foods added from antioxidants and new foods.

Demand is to slow down aging as much as possible and aim for a balanced and healthy aging of the body as a whole. Just as old age is not considered a disease, it cannot be done to resist aging. Eating right, exercising, staying away from environmental and psychological stresses (environmental conditions, U-V light, alcohol, smoking, irregular life) are the best known anti-aging factors.

3.Antioxidants

The body has developed a series of defense mechanisms to prevent the formation of reactive oxygen species (ROS) and the damage they cause (Byung, 1994). Antioxidants are also a defense mechanism that inhibits oxidation products (Niki, 2010) and effectively prevents the damage they cause (Keser, 2012). In summary, the defense systems that work in the body to prevent the formation of reactive oxygen species, to prevent the damage caused by these substances and to perform detoxification are named "antioxidant defense systems" or "antioxidants" (Şener G, 2009).

Oxidative stress and, as a result, free oxygen radicals increase to a large extent with effects such as hereditary disorders, damages, heavy physical activities, exposure to physical and chemical effects (ozone, cigarette smoke and sunlight) in the environment and unbalanced nutrition (Aslan et al., 1997). Recently, the identification of free radicals as one of the strongest factors in many diseases has increased the interest in antioxidants (Diplock, 1991). Antioxidants play an important role in preventing diseases and protecting human health (Niki, 2010). The main factors that determine the position of antioxidants in human health are their chemical structures, solubility,

structure/activity relationships and easy accessibility with natural resources (Kaur and Kapoor, 2001).

Antioxidants are produced by body cells and can also be obtained from food. The main natural antioxidants found in foods that protect the human body from harmful free radicals are mainly vitamins (vitamins C, E and A), flavonoids, carotenoids and polyphenols. Many studies have found an inverse relationship between fruit and vegetable consumption and the occurrence of certain cancers and heart diseases (Güçlü et al., 2009). The most important antioxidants are polyphenols and their derivatives. These compounds may behave differently in the oxidation system. For example; They can reduce oxygen concentration by absorbing singlet oxygen. They use their ability to capture primary radicals such as hydroxyl radicals and bind metal ion catalysts, preventing chain reactions from starting. They bind metal ion catalysts (Shahidi, 1996).

Since antioxidants are oxidizing agents, they are broken down by oxidation, breaking chain reactions (for example, radical chain reaction leading to oxidative breakdown of lipids). Therefore, antioxidants can only protect an oxidizing substance (for example, biological macromolecules) for a limited time, and after a certain time, the substance continues to oxidize as if there were no antioxidants in the environment. The chemical activities of antioxidants, in other words, their reduction potential as hydrogen or electron donor vehicles are generally expressed by their potential as free radical scavengers (Güçlü et al., 2009).

In the interpretation of chain-breaking antioxidant activity, both the number of electrons that the antioxidant can donate to each molecule or the number of free radicals it can remove (i.e. reaction stoichiometry) and the reaction rate (kinetics) are important (Rice-Evansvd, 1997).

The initiation or prolongation of the radical reaction is inhibited by the antioxidant molecule (AH). Here, L˙ stands for lipid, LO˙ for alkoxyl, and LOO˙ for peroxyl radicals. Antioxidants that act through this mechanism are called 'primary antioxidants'.

On the other hand, compounds called 'secondary antioxidants' reduce the oxidation rate and generally try to inhibit Fenton-type reactions (Apak et al., 2007). The Fenton reaction is a reaction that causes the formation of hydroxyl radicals (Graf et al., 1984).

The activity of an antioxidant is determined by the following factors:

- Reactivity as a hydrogen or electron donor medium (Generally dependent on reduction potential).
- The fate of the radical derived from the antioxidant.
- Ability to interact with other antioxidants.
- Transition metal chelating potential (Rice-Evans et al., 1997).

3.1. Classification of Antioxidants

Under normal physiological conditions, antioxidant defense systems protect cells against oxidative damage caused by molecules such as free radicals and peroxides (Rice-Evans et al., 1997). These systems are divided into endogenous and exogenous.

3.1.1. Endogenous Antioxidants

3.1.1.1. Enzymatic Natural Antioxidants

Free radicals, which can occur as a result of different reaction mechanisms in cells, are removed by some enzymes. Although many enzymes are directly or indirectly involved in free radical scavenging mechanisms, the most important enzymes work as follows:

Superoxide Dismutase (SOD): The enzyme superoxide dismutase (E.C.1.15.1.1) uses the superoxide radical as a reducing agent and catalyzes the reduction of the other to less reactive hydrogen peroxide (Caudiere and Iliou, 1999).

SOD has two isozymes in humans, specifically Cu-Zn-SOD, which also contains copper and zinc ions in the cytosol, and the mitochondrial manganese superoxide dismutase enzyme, which contains manganese ions.

Cytosolic dimeric Cu/Zn SOD, one of the isoenzymes of superoxide dismutase, has two equal subunits containing one Cu and one Zn atom and a molecular weight of 32 kDa.

It is the most abundant form of SOD in cells (Sen S, 2011; Young IS, 2001; Valko M, 2007).

Another SOD isoenzyme, Mn SOD, has a molecular weight of 80 kDa. It is a mitochondrial enzyme with four identical subunits. Its active site contains Mn+3. Despite its unique properties, Cu/Zn catalyzes the same reaction as SOD. (Fridovich I. 1995; Halliwell B, 1999; Orbea A, 2000).

The molecular weight of extracellular superoxide dismutase (EC SOD) is 135,000 kDa. Although it is most commonly found in homotetramer form in organisms, it is also known to exist in tetramer, dimer or multimer forms. Each monomer of extracellular superoxide dismutase contains one Cu and one Zn atom. Enzymatic activity requires copper and zinc. The extracellular matrix and cell surfaces are major sites of extracellular superoxide dismutase. It is found in higher concentrations in these areas than in plasma. Fibroblast, glia, and endothelial cells produce and secrete extracellular superoxide dismutase. EC SOD levels are high due to the number of type II epithelial cells and smooth

muscle cells surrounding the airways and blood vessels in the lung tissue. O2.- enzymatically at the extracellular level.

EC SOD plays a critical role in the prevention of many lung diseases such as oxidant damage, inflammation and fibrosis, as it is the only antioxidant that can inactivate it. (Gao F, 2008).

Catalase (CAT): Catalase (H2O2:H2O2 oxidoreductase, EC 1.11.1.6) is a hemoprotein containing four heme groups. Catalase is mainly active in liver, kidney, heart, skeletal muscles and erythrocytes. CAT is found in 80% peroxisomes and 20% in the cytosol (Özkan et al., 2000).

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen. Although the H2O2 produced by SOD is not a radical, it can cause oxidative damage as it is the precursor to the most reactive species, the OH radical. Catalase attempts to lower hydrogen peroxide concentrations by catalyzing the dismutation of two hydrogen peroxide electrons to water and oxygen.

At low hydrogen peroxide concentrations, it also works as a peroxidase using reducing co-substrates such as ascorbate and phenol.

Glutathione Peroxidase (GPx): Glutathione peroxidase resides in the cytoplasm of cells and protects them from oxidative damage produced by H2O2. As a result, it inhibits the production of OH from H2O2. Glutathione peroxidase consists of four different protein components. Each subunit contains a selenium atom (Sen S, 2011).

In the presence of H2O2, GPx is responsible for hydroperoxide reduction, while hydrogen peroxide is reduced to water and organic hydroperoxides to alcohol in this reaction, glutathione (GSH) is oxidized to oxidized glutathione (GSSG) (Chaudiere and Iliou, 1999).

Glutathione Reductase (GR): Glutathione reductase acts as a catalysis in the conversion of oxidized glutathione (GSSG), which occurs as a result of the reduction of hydroperoxides through GPx, to glutathione (GSH), which is its reduced form (Pektaş, 2009).

Glutathione-S-Transferase (GST): Glutathione-S-transferases (E.C. 2.5.1.18) act as an antioxidant defense mechanism through selenium-independent GSH-Px activity against lipid peroxides, particularly arachidonic acid and lineolate hydroperoxides. They perform a wide variety of catalytic and

non-catalytic actions. In addition to their intracellular binding and transport functions, they also serve as detoxifiers (Pektaş, 2009).

Glutathione: Glutathione (GSH) is a tripeptide that can be synthesized in the liver without genetic information. Glutathione (GSH) is an antioxidant that reacts with free radicals and peroxides to protect cells against oxidative stress. It plays a role in preventing the oxidation of hemoglobin to methemoglobin. It also keeps the protein sulfhydryl groups (-SH) in a reduced state and prevents the inactivation of functional proteins and enzymes by protecting the sulfhydryl group against oxidation. Glutathione (GSH) is also involved in foreign chemical detoxification and amino acid transport across membranes.

Glutathione (GSH) is responsible for the protection of blood cells, leukocytes and the lens of the eye against oxidative stress.

Melatonin: Melatonin is a powerful antioxidant that scavenges hydroxyl (OH•), the most dangerous type of free radical. In all intracellular compartments, it has the task of protecting macromolecules against oxidative damage. Melatonin protects nuclear and mitochondrial DNA, as well as proteins and lipids. Melatonin provides broad protection due to its widespread activity as a direct free radical scavenger and an indirect antioxidant. Thus, melatonin removes harmful reactive species and free radicals such as hydroxyl radicals, hydrogen peroxide, singlet oxygen, nitric oxide, peroxynitrite anion and peroxynitric acid from the body.

Uric Acid: When the uric acid concentration, known as the waste product, is high, it can crystallize, resulting in kidney stones and gouty arthritis. Uric acid is thought to fill nearly half of the overall antioxidant capacity of the blood. Hydroxyl, singlet oxygen, superoxide, peroxynitrite anion and peroxynitric acid are inactivated and the transition metals are chelated by uric acid. It has the ability to act as a preservative by inhibiting lipid peroxidation. Apart from being a powerful free radical scavenger, uric acid also works as a metal ion chelator like Fe and Cu. (Kumar AN, 2015; Waring WS. 2002).

Bilirubin: Bilirubin, a powerful antioxidant product, is essentially a substance formed by the destruction of the protein structure called heme, thanks to the breakdown of expired erythrocytes. It is a substance that is eliminated from the circulation by liver cells, metabolized and excreted from the body by different excretion routes (bile, urine). It realizes its antioxidant feature thanks to the bond breaking activity between the peroxyl structure.

Albumin: This protein, which contains about 585 aminoaist types and known as 66 kDa molecular weight, is highly decomposed into its components in water. It can be found in a concentration of approximately 35-50 mg/ml in a healthy person. Healthy individual serum albumin is known for its important functions such as regulation of colloidal osmotic pressure, acid-base buffering, a wide variety of endogenous ligands and drug transport, and free radical scavenging activities.

Especially hypochlorous acid, which has high oxidant activity, encounters the radical scavenging effect of albumin. Some active phagocyte molecules inhibit the formation mechanism of HOCl by releasing their enzymes into the environment. In this way, the albumin scavenges the HOCl already present in the environment.

It does not allow the protease inhibitor of ATT, also known as the molecule, to undergo changes.

Co-Enzym Q10: Ubiquinone, which is mentioned in every cell of the human body, is a vitamin-like benzoquinone that acts as a cofactor in mitochondrial respiration reactions. Its necessity in the ATP production mechanism is based on the formation of cofactors in all three mitochondria (I, II, III) complexes. Its facilitating factor in heart contraction improves blood flow. While animals can synthesize coenzyme Q10 themselves, it is possible to meet the daily requirement of this lipophilic substance in the body with the foods taken from outside.

The electron-reducing form of coenzyme Q10, CoQH2 (ubiquinol), is an antioxidant that is prone to soluble in non-polar solvents, and acts as an antioxidant in ETS by neutralizing oxidized free radicals. In this way, coenzyme Q10 fights against ROS with high toxicity rate in the body.

α-Lipoic acid: This substance, also known as thioxide, is based on caprylic acid and is produced in animals. ALA, soluble in water and ethanol, plays a role in oxygen-induced respiration.

It is ALA itself and its reduced formulation, dihydrlipoate, which reacts with reactive oxygen species. At the same time, it has the function of protecting the cell membrane by combining with glutathione, which has the ability to recycle vitamins C and E.

Ceruloplasmin: They are proteins produced in many organs in humans, including the brain. The ceruloplasmin protein is known as the α2 serum glycoprotein, which assumes the function of carrying high levels of copper in

the blood in a healthy individual. Ceruloplasmin is effective in the biotransformation of Cu(II) and can form bonds reversibly.

Transferrin: Transferrin, which is produced in the brain like ceruloplasmin, is a carrier protein that provides the transfer of Fe(III) and reduces the density of free-circulating iron in the blood. While it is not found in the serum of healthy people, it is present in low concentrations in the remaining fluids of the body. Although the main task of transfer is known as Fe(III) transport, it also acts as a growth factor.

Selenium: Selenium, which is originally a trace element, is an antioxidant substance that provides regulation. This micronutrient, which plays a role in body protection against peroxides, is necessary to increase the effectiveness of glutathione peroxidase. Because glutathione binds to selenium and shows strong antioxidant properties against peroxides in the form of Se-GPx.

2.1.2. Exogenous Antioxidants

2.1.2.1. Vitamin Exogenous Antioxidants

Vitamin E: Vitamin E, which has the ability to dissolve in non-polar solvents, is the most active stereoisomer structure in humans, α-tocopherol. Its antioxidant property is the protection it undertakes against lipid peroxidation. Vitamin E, working simultaneously with glutathione peroxidase, take on an integral task against each other. In other words, vitamin E uses its ability to inhibit the peroxide formation mechanism in order to have an antioxidant effect, and glutathione peroxidase provides the destruction of existing peroxides.

Ascorbic acid (Vitamin C): Ascorbic acid, which is soluble in water, acts as a co-antioxidant. It performs its antioxidant function by converting α-tocopheroxy to vitamin E using a precursor.

β-carotene: It is a member of the carotenoid group that is prone to fat-soluble and is known as a provitamin. Its function as an antioxidant is to scavenge oxygen radicals in the body.

Folic Acid (Vitamin M): Folic acid, also known as Vitamin B9 and pteroylglutamic acid, has a high water solubility and is used in the production of DNA. It takes on the task of protecting the cell against reactive oxygen species that can damage DNA.

2.2. Comparison of Endogenous and Exogenous Antioxidants

Antioxidants have become one of the most important issues in human nutrition due to the formation of lipid free radicals at high concentrations during and after food intake.

- It should not harm human health,
- It should be used in small amounts so as not to increase the cost,
- It should not interfere with the natural smell, appearance or taste of the food,
- It must be dissolved in the substance it will protect or mixed with the substance,
- The effect should not change during normal manufacturing (especially in high temperature applications) (Sezgin, 2006).

Storage, heating and digestion increase the amount of free lipid radicals. Antioxidants prevent or slow down the oxidation of lipids. This preserves food quality and extends shelf life. However, recent data show that exogenous antioxidants are toxic, costly, and less effective than endogenous antioxidants. For this reason, the search for these compounds has increased significantly due to the fact that antioxidants that can be taken with food are economical and have high antioxidant activity. The use of synthetic antioxidants is limited by laws due to their harmful effects and natural antioxidants are found to be safer.

In addition, synthetic antioxidants have increased the interest in natural antioxidants due to the possibility of causing internal organ damage (Wanasundara and Shahidi, 1998) and the observation that they have carcinogenic effects. The increase in interest in the use of natural products has led to an increase in research on plants.

RESULTS AND DISCUSSION

Although free radicals are already present in the metabolism of living cells, the presence of these reactive oxygen species may increase numerically through various enzymes and in line with some external factors. While these living cells, which are under oxidative stress, can lead to various diseases, they can also trigger existing diseases. At the same time, a healthy cell produces antioxidants in order to keep its own free radicals in balance, which reduces oxidative pressure in line with these antioxidants and somehow prevents diseases.

In the case of a decrease in the production of antioxidants due to various reasons, free radical levels are kept at normal levels by taking antioxidants from a balanced diet plan and adequate amounts of natural foods such as fruits/vegetables and supplements. From this point of view, antioxidants can be considered as important fighters in order to reduce oxidation-related diseases or risks.

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