Academic Studies in Health Sciences

EDITOR
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Dr. Nuray KILIC

DUVAR

ACADEMIC STUDIES IN HEALTH SCIENCES

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Academic Studies in Health Sciences
Editor: Assist Prof. Dilek ATIK Ph.D
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Editor in chief: Berkan Balpetek
Cover and Page Design: Duvar Design
Printing : First Edition-December 2021
Publisher Certificate No: 49837
ISBN: 978-625-7502-60-3

853 Sokak No:13 P.10 Kemeraltı-Konak/Izmir/ Turkey
Phone: 0 232 484 88 68
www.duvaryayinlari.com
duvarkitabevi@gmail.com

Printing and Binding: REPRO BİR
Repro Bir Mat Kağ. Rek. Tas. Tic. Ltd. Şti.
İvogsan 1518. Sokak 2/30 Mat-Sit iş Merkezi Ostim
Yenimahalle/Ankara
Certificate No: 47381

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CHAPTER 1

PERCEIVED STRESS IN 8-11 YEARS OLD AGE CHILDREN DURING THE COVID-19 PANDEMIC

Berna GOKKAYA[1]

Betul KARGUL[2]

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1 Corresponding Author, Dr. ,Bahcelievler Oral and Dental Health Hospital, Clinic of Pediatric Dentistry Adnan Kahveci Bulvarı 141/2 Bahcelievler/Istanbul/Turkey
E-mail:bernagokkaya78@hotmail.com Phone: 90.505.256.27.31 ORCID: 0000-0001-7904-9832

2 Prof. Dr., Marmara University, Department of Pediatric Dentistry Basibuyuk Saglik Yerleskesi 9/3 Maltepe/Istanbul/Turkey E-mail: bkargul@marmara.edu.tr Phone: 90.532.326.72.07 ORCID: 0000-0002-3294-8846

INTRODUCTION

Novel coronavirus pneumonia (NCP) emerged in Wuhan, China, in December 2019, and the pandemic has since spread rapidly all around the world [1,2]. On February 12, 2020, the World Health Organization officially named the disease caused by this novel coronavirus as "Coronavirus Disease 2019" (COVID-19) [3]. As of 10 am on February 29, 2020, a total of 53 countries had reported outbreaks of COVID-19, with the number of cases rising to 85,403, indicating a serious situation for prevention of the disease [4]. At the time of writing (Nowember, 2021), after the mutasions, it is hardly spread all around the Europe and especially in Turkey.

The life style of families suddenly and deeply changed. In the home environment, the educational role of parents for children has become even much crucial than before. Children have only their parents around them, to provide support with homework/homeschooling when necessary and promote a positive development and new learning experiences for toddlers and preschoolers [2]. Parents have been left alone not only in taking care of home-schooling their children, but also in general in the management of their children and of the home environment. All other educational services are closed, babysitters and grandparents are not available, and contact with peers is not allowed. Many parents also must do home office working, and handling time and spaces to work with children around may be very problematic. Though quarantine means that time that can be shared with loved ones has increased, it also poses a major burden on parents' shoulders, as they are called to take an educational role while also trying to live their own lives and get on with their everyday job commitments. This situation has significantly increased the risk of experiencing stress and negative emotions in parents, with a potentially cascading effect on children's wellbeing [5].

There is no evidence available in the literature for how quarantine and social isolation in the midst of this pandemic would affect daily life of children. Therefore, the purpose of this study was to assess the effect of the COVID-19 pandemic on PS in children who are 8-11 years old age children as measured through a PSS-C as user-friendly format.

METHOD

Ethics: The study protocol was approved by the ethics committee of the corresponding research institute Bakırköy Sadi Konuk Research and Training Hospital [2020/200]. The online questionnaires involved in this study were anonymous and informed consent of the parents of children were obtained before starting.

Study design: A cross-sectional, web-based survey to assess the impact of COVID-19 was conducted following structured. Parents implied consent after agreeing to proceed with the survey, which required an answer to all questions by their children. Demographic and practice details were acquired and presented in a descriptive manner, but were kept anonymous (Google Forms). Province to take online electronic questionnaire surveys. All participants in the survey were patients of Bahcelievler Oral and Dental Health Hospital's Pediatric Dentistry Clinic's during the dates between 01.01.2019 to 31.12.2019. Children who were 8–11 years old age were invited to participate in the online survey through the google docs platform on May, 2020. The parents of children were reached using the social media. After removing the data of participants with incomplete questionnaires, 303 children were included in the analysis. Parents were recruited for the study through personal networks and referrals. Parents asked their children to complete PSS-C independently; all assessments were completed in the child's home with parents in the room but not assisting. Children were also asked whether they thought the scale was "easy" or "tricky/hard". Each PSS-C was scored and entered into a database by BG, from May 7, 2020, to May 31, 2020, 310 children were randomly selected from.

Inclusion criteria were: (1) age 8 years or older; (2) healthy children; (3) being informed parents about the study and willing to participate in the survey. Exclusion criteria were: a history of mental illness; or suffering from other brain organic lesions or serious physical diseases. Elimination criteria were: (1) filling out the electronic questionnaire in too short a period of time; and (2) submission of a questionnaire that was obviously inconsistent with the actual situation.

The scale was designed by the researchers themselves and included gender, age, parents job category, whether their family members had confirmed/suspected cases and whether their knowledge of COVID-19.

PSS-C (8-11 years) was used in the study. PSS-C, developed by Snoeren and Hoefnagels [6], adapted to Turkish by Oral and Ersan [7], was used to determine the perceived stresses of children. PSS-C is a questionnare designed for children to measure PS due to subjective experiences independent of a specific context. The adaptation study was conducted with 380 students ranging in age from 8 to 11. The scale is one dimensional and consists of 9 items. Children were asked to rate how often they had experienced various stressors or reactions to stress over the last week, on a 4-point Likert scale (1=never, 2=sometimes, 3=often, 4=very often). The total score that can be obtained by the participants on the scale, which has no reverse item, ranges from 0 to 27 points. A higher score on the questionnaire corresponds with greater PS [6].

Statistics: The members of the study group analyzed and screened the questionnaire responses filled in by the included subjects in strict accordance with the rejection criteria. After double-checking, the data obtained were transcribed to the SPSS database. All analyses were performed using the IBM Statistical Package for Social Sciences (SPSS) version 19.0. Means (M) and measures of standard deviations (SD) were calculated for PSS-C sum scores. Descriptive statistics (frequency, mean, standard deviation, range) were used to describe demographic data and the PS score and relationship with some factors. For demographic data, Chi-squared tests were used to analyze significant differences between categorical variables otherwise descriptive statistics were used. One-way Analyses of Variance (ANOVA) were used to examine differences in perceived stress among the children of relationship with the COVID-19 pandemic. Spearman correlation analysis was used for correlation analysis, and multiple linear regression analysis was used for multivariate analysis. The total test level was set at $P<0.05$.

RESULTS

We employed a self-administered electronic tool to collect data using our validated questionnaire which consisted of 306 children's parents. From these, 3 (0.9%) were excluded from further analyses because of incomplete or insufficient information. The final sample consisted of 303 children with an age range of 8 to 11 years. Among the 303 valid children, 56.4% (n=171) were girls and 43.6% (n=132) were boys (**Table 1**).

No significant differences in age (F=0.906; p=0.597) although there was a trend 9-11 years old children and 9 years old children were seen the most stressfull age group (**Table 1**). Respondents who filled out a PSS-C demonstrated a moderate level of stress over the last month (Mean score 11.46±5.83; Range 0-26) (**Table 1**). Among the included children who took the survey, Scores on the PSS-C for the entire sample (N=303) showed a wide range of distribution, with scores ranging from 0-26 (highest possible score is 26). The mean (11.46±5.83), median (11) were consistent in values. The scores for each dimension are shown in **Table 1.** There were no statistically significant differences in terms of PSS-C scale (p=0.597) in age group. The only difference in gender being more affected by pandemic restrictions (p=0.029) (**Table 1**).

The most negative scoring response in the scale was for "In the last week how often did you lost your patience?" (Mean score 1.92; Range 0-3), whereas the response with the most positive response was for "In the last week How often did you have fights with your friends?" (Mean score 0.49; Range 0-3) (**Table 2**).

30 (9.9 %) children's scored above 20 in the PSS-C indicative of high

stress levels. Through the one-way ANOVA, factors were not statistically significant (P>0.05), mother job (F=0.847, P=0.693; father job (F=1.113, p=0.326**)** confirmed/ suspected cases in family(t=1.022, p=0.308); Knowledge of COVID-10 (t=0.466,p=0.327)(**Table 3).**

Multiple linear regression analysis was carried out with the factors as age, gender, mother job, father job confirmed/suspected cases in family and Knowledge of COVID-10. High total PS in female (B= -0,148, P=0.011) were important related factors for PSS-C score in children (**Table 4**).

DISCUSSION

In the literature, only a few studies have examine how pandemics affect specifically smaller years old age. Children seem more likely to develop symptoms of depression and anxiety [8], and are also more likely to experience regression, irritability, fear, and changes in mood than they were before the COVID-19 pandemic [9]. Besides, physiological effects include a weakened or compromised immune system leading to increased susceptibility to other diseases [10].

Children at different ages express distress and the importance of sharing and talking about fears and negative emotions [11]. People younger than 20 years had the lowest PSS scores in China (12). Concerns over a child younger than 16 years getting COVID-19 have been reported as very common among parents in China [2].

A recent study [13] in 25 countries on susceptibility to stress during the COVID-19 pandemic, indicated that women report greater levels of stress. Similar gender differences for stress, anxiety and depression symptoms were found by different study [2] in a Chinese sample during the initial stage of the COVID-19 pandemic. We found that girls had a high level PS than boys, but no significant differences. On the other hand, gender was effect PSS-C score when mother's or father's HCW's. A study in China [14] declared that no significant difference in gender during COVID-19 pandemic among college students.

During the equine influenza outbreak in Australia, they [15] found that individuals having one child had a 1.2 times higher risk of experiencing distress than those with no children. Similarly, our study results showed that children's stress level had increased during pandemic.

One study [16] said that just 0.34% of the sample tested positive for the virus, history of contact with COVID-19 wasn't found to be significantly correlated with PSS-10 score. Another study [17] found that sociodemographic variables, such as gender, living circumstances, and personal COVID-19 exposure, were related to increased risk of depression and anxiety symptoms. In the present study, we found

that children didn't feel PS about their's COVID-19 (+) family members. The stress level of parents displays a negative impact on the wellbeing of their children [18].

Prolonged school closure and home qurantine during pandemic might have negative effects on children's physical and mental health [19, 20]. Studies said that when children are out of school (eg, weekends and summer holidays), they are physically less active, have much longer screen time, irregular sleep patterns, and less favourable diets: besides closure and home qurantine during pandemic might have negative effects on children's physical and mental health [19; 21]. Stressors such as prolonged duration, fears of infection, inadequate information, lack of in-person contact with classmates, friends, and teachers, lack of personal space at home, and family financial loss can have even more problematic and enduring effects on children and adolescents [20]. For example, a study [5] showed that the mean post-traumatic stress scores were four times higher in children who had been quarantined than in those who were not quarantined. We know that children have lower personal resources to deal with the many changes the pandemic is imposing on their life [22] and guidelines suggest parents should discuss and explain the situation with them, since correct information about what is happening and the reasons for the restrictions children have to face is crucial to prevent negative psychological consequences [11].

In the present study, multiple linear regression analysis showed that demographic variables were effect PSS-C score. Other study said that multivariate logistic regression analysis revealed that adolescents with low social support showed 4.2 times greater risk of depression symptoms and 3.2 times greater risk of anxiety symptoms than those with high social support [17]. These findings indicate that social support is a significant important protective factor for mental health among adolescents and children [23; 24]. Children are constantly exposed to epidemic-related news, so having direct conversations with children about these issues could alleviate their anxiety and avoid panic. With the right parenting approaches, family bonds can be strengthened, and child psychological needs met [25]. For this reason, listening to what children believe about COVID-19 transmission is important; providing children with an proper explanation that is meaningful to them will ensure that they do not feel unnecessarily frightened or guilty [26].

Limitations: There are some limitations to the present study. The first is that the study focused on children in specific areas of Turkey and include aged <12 years, which may impact sample representativeness. Second limitted our ability to make statements about causal relationships. It is necessary to conduct further prospective and longitudinal studies to assess the levels of mental health and social support at different points in the future within the context of COVID-19.

CONCLUSION

To our knowledge, this study is the first study that was to assess the effect of the COVID-19 pandemic on perceived stress in children as measured through a PSS-C. The study has several key findings. First, the data showed that COVID-19 was generally a moderate level of stress in 8-11 years age old children. Second, there was no statistically significant differences in terms of PSS-C scale in age group. But the only difference in gender being more affected by pandemic restrictions. Besides, the study found that high total PS in female were important related factors for PSS-C score in children. Third, PSS-C scale is the user-friendly scale for children especially 8-11 years age old children. Because it is include only 9 short questions and it is understandble. Only 2-3 minutes is enough for answering.

With this study, we wanted to focus attention to 8-11 years old age children. This study confirms previous reports from Turkey suggesting that social and psychological support is a significant important protective factor for mental health among children. Children can't advocate for their needs, their feelings when they have smaller years age old. They fear unknown situation and things. It is necessary to give the right information at the right time. For this reason, the observations of parents and teachers are very important factor for understanding their feelings.

Acknowledgement: None.

Conflict of interests: No financial or nonfinancial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

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Table 1. Demographic characteristics of participants (N=303) and Age and gender distrubition of PSS-C scores

Characteristic N=303	**n (%)**	**PSS-C Mean(SD)**	**F**	**p**
Age				
8	120 40%	10.25±5.52		
9	77 25%	13.52±5.58		
10	75 24%	10.92±5.32	**0.906**	**0.597**
11	31 11%	12.39±7.32		
gender				
girls	56.4% (171)	12.25±6.09		**0.029**
boys	43.6% (132)	10.42±5.31		
total		11.46±5.83		

Table 2: PSS-C scores for each question

PSS-C Questions	**PSS-C scores**
1. In the last week **How often did you feel scared and nervous**	0.96±1.03
2. In the last week **How often did you feel worried about being too busy**	1.45±1.17
3. In the last week **How often did you have some problem**	1.19±1.10
4. In the last week **How often did you have fights with your friends**	0.49±0.86
5. In the last week **how often did you feel rushed or hurried**	1.46±1.15
6. In the last week **how often did you feel angry**	1.33±1.15
7. In the last week **how often did you lost your patience**	1.92±1.12
8. In the last week **how often did you feel unhappy**	1.45±1.11
9. In the last week **how often did you feel worried**	1.21 ±1.16

Table 3: PSS-C Scores for confirmed/suspected cases in family and knowledge of COVID-19

confirmed/suspected cases in family	**Mean (SD)**	**t**	**p**
Yes	13±6.07	1.022	**0.308**
no	11.37±5.81		

Knowledge of COVID-19	**Mean(SD)**	**t**	**p**
Yes	11.53±5.82	0.466	**0.327**
no	10.56±5.68		

Table 4: Multiple linear regression of PSS-C scores (N=303)

	Unstandardized Coefficients		**Standardized Coefficients**	**t**	**p**	**95.0% Confidence Interval for B**	
	B	Std. Error	Beta			Lower Bound	Upper Bound
age	.335	.337	.059	.994	0.321	-.328	.997
gender	-1.732	.679	-.148	-2.552	**0.011***	-3.068	-.397
Mother's job	-.043	.238	-.012	-.182	0.855	-.512	.425
Father's job	-.153	.253	-.040	-.606	0.545	-.651	.345
Knowledge of COVID-19	.308	1.999	.009	.154	0.877	-3.625	4.242
confirmed/ suspected cases in family	1.321	1.509	.051	.875	.382	-1.649	4.291

CHAPTER 2

THE HISTOPATHOLOGICAL PITFALLS OF ATYPICAL APOCRINE ADENOSIS AND ADENOMYOEPITHELIOMA

Payam HACISALIHOGLU[1]

Elbrus ZARBALIYEV[2]

1 Md, Department of Pathology, Gaziosmanpaşa Hospital, Yeni Yüzyil University, İstanbul, Turkey

2 Md, Department of General Surgery, Gaziosmanpaşa Hospital, Yeni Yüzyil University, İstanbul, Turkey

Atypical apocrine adenosis

Apocrine metaplasia and sclerosing adenosis are changes that are associated with insignificantly increased risk of breast carcinoma. Apocrine adenosis is a term used when atypical apocrine metasplastic changes are overlapped with sclerosing adenosis (1).

Atypical apocrine adenosis, which is a very rare benign lesion of the breast has an incidence rate ranging between 0.3% and 0.4%. It is a highly cellular lesion which may cause diagnostic challenges for the pathologists because of the prominent cellular atypia it displays (2). Apocrine atypia is a term which is used for apocrine cells with significant atypia. The nuclei of the cells in apocrine atypia are 3 times larger than that of a normal ductal cell. These large nuclei are hyperchromatic and have prominent nucleoli (3). Observing cells with apocrine adenosis together with apocrine atypia in the pathology specimens are the key diagnostic features of atypical apocrine adenosis (Figüre 1) (4).

Apocrine atypia is not a single entity but it appears to be a part of a spectrum ranging from benign epithelial proliferations to in-situ and invasive breast carcinomas with apocrine differentiation. Since atypical apocrine adenosis is an uncommon entity, it may be misdiagnosed as apocrine ductal carcinoma in-situ. Large, hyperchromatic nuclei with large, apocrine cytoplasm are the histomorphological findings of apocrine atypia yet irregular nuclear membrane, increased mitotic activity and necrosis are the histomorphological features of in-situ ductal carcinoma with apocrine features (2).

Calcification is a frequent finding in atypical apocrine adenosis. In atypical apocrine adenosis, generally there is no necrosis and the mitotic rate is not increased. In a small percentage of cases, areas with atypical ductal or lobular hyperplasia accompany atypical apocrine adenosis (2). In a study of Hou et al, with 41 cases of apocrine adenosis and atypical apocrine adenosis, the authors detected coexisting atypical ductal hyperplasia or ductal carcinoma in 10 patients. Authors stated that apocrine adenosis or atypical apocrine adenosis without coexisting ductal carcinoma in situ or invasive carcinoma did not display any malignancy after short term follow up, hence atypical apocrine adenosis is a lesion which did not need further surgical therapy (5).

Likewise, in other studies, long term follow up of the patients with atypical apocrine adenosis concluded that atypical apocrine adenosis was not a precursor lesion for breast carcinoma (2, 4).

On the other hand, according to a study of Chang S et al, which was conducted with 24 patients with the diagnosis of atypical apocrine adenosis via trucut biopsy, 20% of the patients had concurrent invasive breast carcinoma in the excisional

biopsy specimens (6). In a study of Seidmann et al, with 37 atypical apocrine adenosis patients with an 8.7-year follow-up, elderly women with atypical apocrine adenosis were stated to have an increased risk for developing invasive breast carcinoma (7).

Atypical apocrine adenosis may even sometimes be misinterpretated as invasive ductal carcinoma with apocrine features. Demonstration of the myoepithelial cell layer with immunohistochemistry may help in identifying these two entities (6).

Adenomyoepithelioma

Adenomyoepithelioma is a rare biphasic benign breast tumor which is composed of the proliferation of epithelial and myoepithelial cells (1). Up to date, only 6 cases were reported in the English literature (8). Myoepithelial cells are normally located in the ductal system of the breast and proliferate in sclerosing adenosis, intraductal papilloma and ductal hyperplasia (8). Cellular atypia and increased mitotic rate are the necessary histomorphological features of adenomyoepithelioma (1). In a study of Firat et al, necrosis, epithelial and myoepithelial proliferation were observed in some of the adenomyoepithelioma cases (9).

Most of the adenomyoepitheliomas are the variants of intraductal papilloma. A lesser amount of the adenomyoepitheliomas originate from lobular proliferation and adenosis. Adenomyoepithelioma may display a combination of tubular, papillary and solid growth patterns (Figure *2*) (8).

Both benign lesions with abundant myoepithelial cells such as intraductal papilloma and invasive breast carcinomas such as adenoid cystic carcinoma are included in the differential diagnosis of adenomyoepithelioma. Some solid intraductal papillomas contain a great number of myoepithelial cells, mimicking adenomyoepithelioma. Intracystic papillary structures, fibrovascular core- like structures and the architecture of myoepithelial cells lacking a proliferation of myoepithelial cells in sheets and fibers may aid in the diagnosis of an intraductal papilloma (10). Most of the adenomyoepitheliomas have distinct borders; they can even havea capsule. However, some of the adenomyoepitheliomas may have irregular borders which can macroscopically mimic malignancy (11).

Exact diagnosis of the lesion is crucial to abstain misdiagnosis as invasive carcinoma, which may result in excessive aggressive treatment (12).

In the literature, it is stated that some adenomyoepithelioma cases were misdiagnosed as invasive carcinoma, especially on core needle biopsies (13). Because adenomyoepithelioma has various histopathological forms, there is always a diagnostic challenge in fine needle aspiration cytology, as well. In a

study of Ivengar et al, consisting of 12 adenomyoepithelioma cases, the authors stated that two cases had the cytological diagnosis of "adenoid cystic carcinoma and adenocarcinoma",two cases were diagnosed as "suspicious for malignancy" and six cases had the diagnosis of "atypical ductal proliferation". Only one case had benign diagnosis (12).

Hikino et al reported an adenomyoepithelioma misdiagnosed as intracystic carcinoma both radiologically and cytopathologically (13).

In a series of Rosen et al, half of the adenomyoepithelioma cases were misinterpreted as carcinoma (9). Also in a study of Tavasolli et al, it is reported that almost one third of the adenomyoepithelioma cases had undergone mastectomy and axillary dissection because of the initial misinterpretation (14). The authors stated that being unfamiliar with the characteristic features of adenomyoepithelioma and the limitations of core biopsies by means of tissue sampling may be the possible explanations of the misdiagnoses. In some adenomyoepithelioma cases, proliferation of myoepithelial cells with clear cytoplasm among the glandular structures may be difficult to interprete. It should be kept in mind that in adenomyoepithelioma, proliferating glandular structures stream in one direction. Immunohistochemistry may be used to highlight the myoepithelial cells (10). Myoepithelial stromal rich tumors such as phyllodes tumor and adenoid cyctic carcinoma are the cytological differential diagnosis of adenomyoepithelioma. Identitification of the myoepithelial cell layer by preparing a cell block and using immunohistochemical myoepithelial markers such as p63 may be useful (15).

Adenomyoepithelioma may commonly resemble invasive ductal carcinoma, histopathologically, too. Diagnosis may be challenging. Demonstration of the myoepithelial differentiation is crucial. In difficult cases, myoepithelial differentiation should be verified by immunohistochemistry (16). Adenomyoepithelioma may also be confused with adenoid cystic carcinoma especially in trucut biopsies, and occasionally these two entities may even ocur concurrently (17). Basaloid cells and luminal cells forming gland like structures, surrounding hyaline-like material are the characteristic features of adenoid cystic carcinoma (18). In problematic cases, especially in trucut biopsies, CD 117 immunohistochemical antibody may be useful in differentiating these two entities: addition adenoid cystic carcinomas may express CD117 immunohistochemical antibodywhereas there is no expression in adenomyoepithelioma (19).

The clinical course of adenomyoepithelioma is mostly benign yet rare local recurrences, malignant transformation and distant metastasis have been reported in the literature, proportional to the patient age (19-22). Less than 11 malignant adenomyoepithelioma cases have been reported in the literature, to date (23).

Presence of myoepithelial cell layer together with the epithelial cells does not help to differentiate between malignant and benign adenomyoepithelioma. Marked cytological atypia, increased mitotic rate, necrosis, desmoplasia, infiltrating borders are the histopathological features of malignant adenomyoepitheliomas. It has to be kept in mind that malignant adenomyoepitheliomas may have only one or two of these histopathological features and malignant transformation may affect only one cellular component (8, 11).

The imaging features of breast adenomyoepithelioma are not fully described. The range of mammographic and ultrasonographic appearances is wide and varies from benign to malignant features but in fact there is no definitive mammographic, sonorgaphic or MRIpattern to differentiate benign adenomyoepithelioma form its malignant counterpart (24).

In a study of Lee et al with benign adenomyoepithelioma cases, two cases had a BIRADS category 4A and three cases had BI-RADS category 4B (25). In our study, one adenomyoepithelioma case was categorised as BIRADS 3: All other cases were categorized as BIRADS 4A, ultrasonographically.

Even benign adenomyoepithelioma s have a potential for recurrence, excision with wide surgical marginsis necessary (11).

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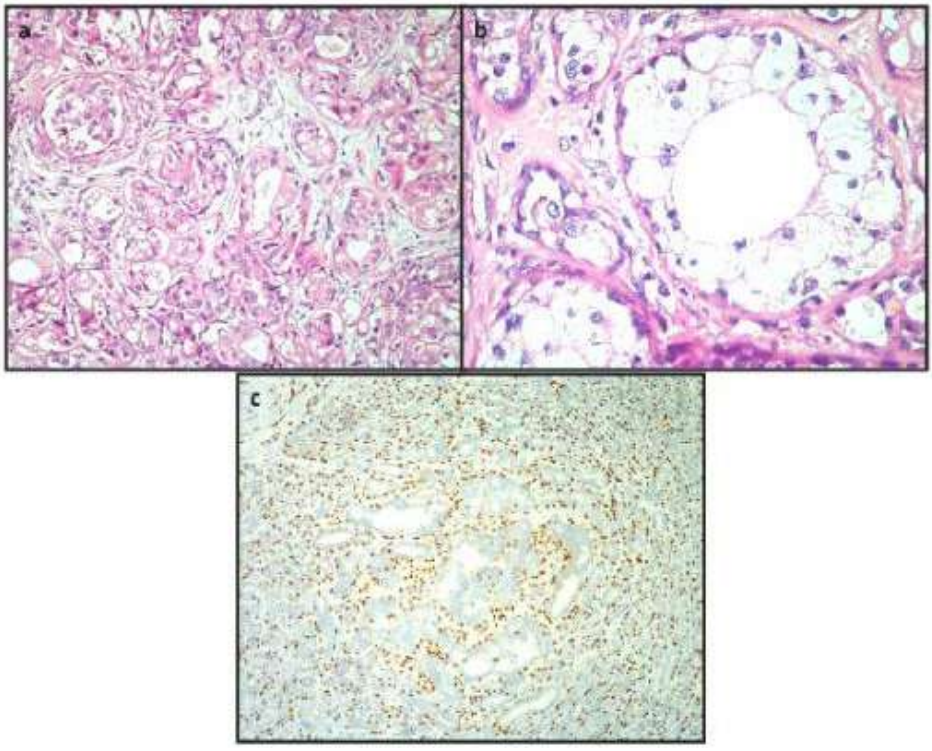

Figure I a,b: Atypical apocrine glandular cells in AAA with prominent pleomorphism (H&E, 200X, 400X). c: Intact myoepithelial cells highlighted with p63 immunohistochemical antibody. (p63, 200X).

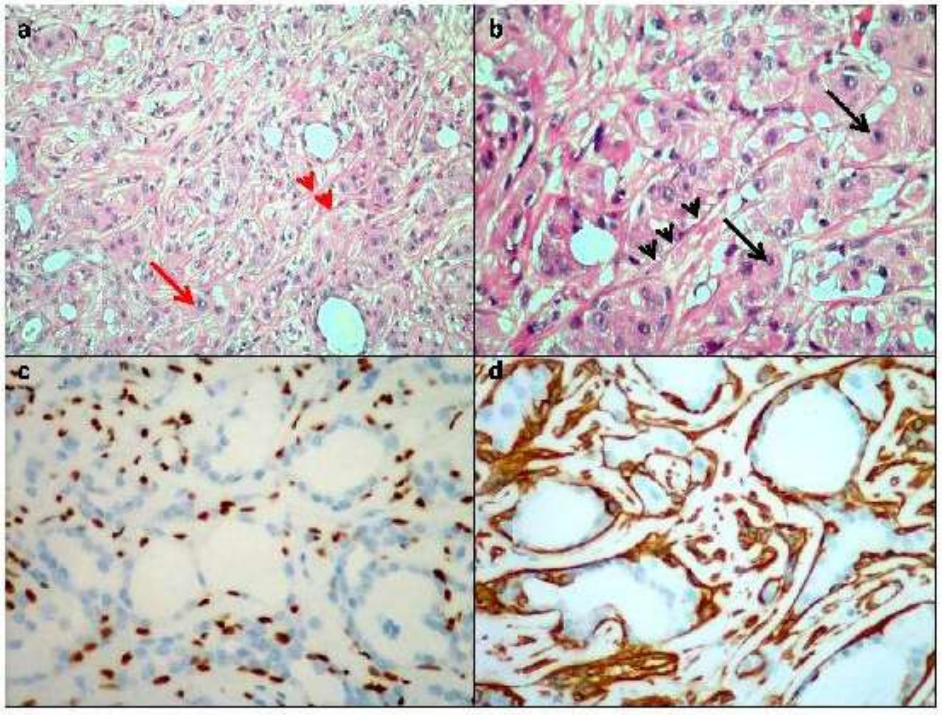

Figure II a,b: Tubular structures with cuboidal-collumnar epithelial lining (arrows) nested with fusiform myoepithelial cells with eosinophylic cytoplasm forming cordsand bundles (arrowheads). c: Myoepithelial cells showing positive immunoreactions with p63 antibody (p63, 400x). d: Intact basement membrane showing positive immunoreactions with cytokeratin 5/6 antibody (cytokeratin 5/6, 400x

CHAPTER 3

A GENERAL OVERVIEW OF CANCER AND MELANOMA CANCER IN TERMS OF EPIDEMIOLOGY, RISK FACTORS, DEVELOPMENT, SYMPTOMS AND DIAGNOSIS REGARDING

Esra BILICI[1]

Ömer Faruk LENGER[2]

1 Corresponding Author, Afyon Kocatepe Üniversitesi, Veteriner Fakültesi, Medikal Biyoloji ve Genetik ABD
e-mail: vet.hekimesrabilici@gmail.com ORCİD:0000-0001-6636-5975

2 Afyon Kocatepe Üniversitesi, Veteriner Fakültesi, Medikal Biyoloji ve Genetik ABD

Introduction

Cancer, which is a general term describing the uncontrolled and excessive proliferation and differentiation of body cells, occurs as a result of DNA damage caused by ultraviolet radiations, ionizing radiations, environmental agents and therapeutic agents (Saylor et al, 2009). Cancer, which is a serious chronic disease that negatively affects the quality of life and threatens life, continues to be the second leading disease after cardiovascular diseases. Although significant improvements have been made in its treatment and care, it is a frequently encountered disease that can cause death (Durna, 2013).

Cancer, which has more than 100 types, is known to adversely affect the human body (Fitzmaurice et al, 2015; Pavlopoulou et al, 2015). According to the cell, tissue type or organ they originate from, cancers; They are classified as lung cancer, breast cancer, prostate cancer, sarcoma, lymphoma, carcinoma and leukemia. They are examined in two parts according to their benign and malignant nature. Tumors consisting of cells that proliferate slowly and are limited to their initial sites are called benign tumors. Cysts and warts are examples of such tumors. Malignant tumors, on the other hand, consist of rapidly growing abnormal cells. These tumors spread (metastasize) to other tissues, disrupting the life of normal cells there (Hafaza et al, 2020). Cancer cells have many features that normal cells do not have (Figure 1).

Cancer cells, known as intelligent cells, have their own metabolism. Under normal conditions, when cells receive signals from the outer membrane, they grow and multiply by dividing. After the signals from the outside enter the cell and are transferred to the nucleus, the process begins. Before the cell divides, it checks its environment, after checking whether there is enough food and a place to grow, it starts to grow if the conditions are suitable (Baykara, 2016).

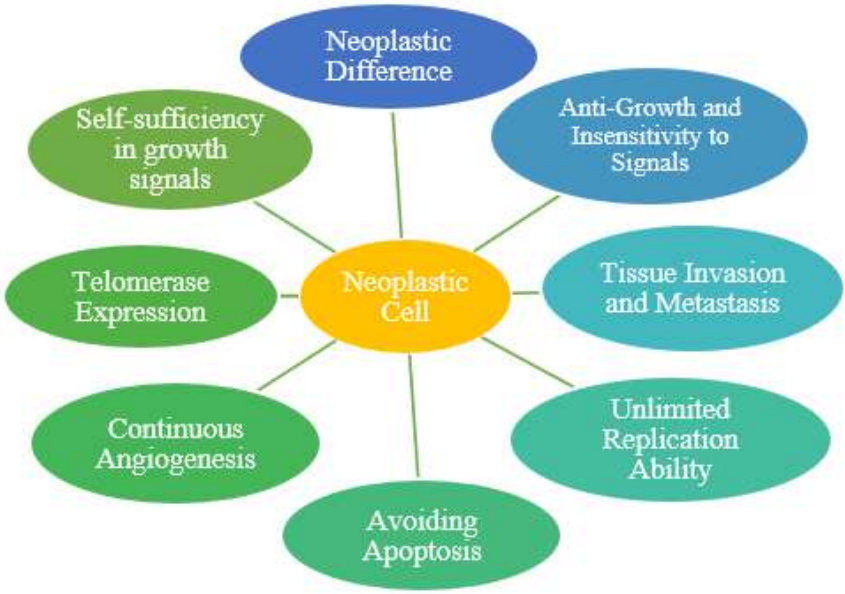

Fig. 1. General Characteristics of Cancer Cells (Hanahan ve Weinberg, 2000)

Cancer Formation Mechanism

Cells continue their life with control and management mechanisms such as growth, division and apoptosis or they terminate it. Carcinogenesis is observed with the failure of these mechanisms to function (Robson et al, 2010). Uncontrolled cell proliferation, spreading of proliferating cells to surrounding tissues and gaining the ability to metastasize to distant organs cover the entire process of carcinogenesis (Demirelli, 2003). Cancer is a multi-step process that is effective at genotypic and phenotypic levels (Yokuş and Çakır, 2012).

Mutations causing malignant transformation are classified as activation of proto-oncogenes that trigger cell proliferation and growth, tumor suppressor genes, inactivation of apoptosis and DNA repair enzymes (Hahn and Weinberg, 2002; Demirelli, 2003; Rieger, 2006).

Carcinogenesis consists of four steps; (Oner, 2013)

- ✓ Initiation (Beginning)
- ✓ Promotion (Increase)
- ✓ Progression (Progress)
- ✓ Invasion and Metastasis

Initially, the initiating agent mutates the DNA in a single cell, triggering the abnormal proliferation of the cell. The mutation that occurs here is irreversible (Dalay, 2006; Öner, 2013).

Then, the promoter factors that provide proliferation come into play and cause reversible mutations on the DNA. Changes in DNA are called epigenetic changes. If radicals act as promoters in DNA, these mutations become permanent (Tysnes and Bjerkvig, 2007).

Thus, the community of copying cells continues to grow rapidly (Dalay, 2006). The progression after the promotion phase is the phase in which genomic instability develops. Here, karyotypic changes and multiple mutations occur (Öner, 2013). Cells that undergo these changes become more advantageous in terms of faster proliferation, survival and metastasis, and they become completely malignant (Dalay, 2006; Öner, 2013). Cells, which have increased their proliferation rate, eventually begin to spread to the surrounding tissues. Apart from this, it can reach more distant regions by joining the blood and lymph circulation and form new tumor tissues. This process of spreading to other tissues and organs is called metastasis (Mazzarella et al, 2018).

It is not known exactly how the metastasis mechanism works. At the end of these stages, cells that have increased their ability to proliferate, survive, invade and metastasize are selected among others and separated (Cheng et al, 2013).

The Relationship Between Cancer Development and Cell Cycle

In the last two decades, researchers have revealed how important the regulation of the cell cycle is in the formation and progression of cancer (Malumbres and Barbacid, 2001).

Results of studies have shown that tumor cells combine mutations that contribute greatly to constitutive mitogenic signaling and unplanned proliferation, resulting in defective responses to anti-mitogenic signals (Massague, 2004). In addition, most tumor cells cause numerical changes in chromosomes and genomic instability through the occurrence of additional mutations (Kastan and Bartek, 2004).

All these changes, in addition to the advantage in reproduction, also cause an increased sensitivity to the accumulation of additional genetic changes. This situation contributes to the progression of the tumor and the emergence of more aggressive phenotypes (Kops et al, 2005). Studies conducted in the past years have emphasized the importance of the connections established between the proteins that regulate the cell cycle and oncogenesis (Kastan et al, 1991). Abnormalities in cell cycle regulation and checkpoints pave the way for cancer development (Sullivan et al, 2018).

Components involved in the overexpression and mutated cell cycle mechanism are identified in various human cancers. Some of these mutated cell cycle components are known as oncogenes and tumor suppressor genes (Pucci and Giordano, 1999). The cell cycle is programmed for cell growth and proliferation (Ho and Dowdy, 2002). The duration of the cell cycle, which is a program that makes a difference between cells and is carried out by the organism, varies from one minute to one year, but takes place in four phases (Figure 2).

1. G-1 stage: It is the stage in which the daughter cells formed in the first division are prepared for the S phase without entering the cell division again. DNA replication does not occur.
2. S phase (Synthesis): DNA replication, chromosome duplication, RNA and protein synthesis take place.
3. G-2 stage (G=Gap): DNA replication does not occur, RNA and protein synthesis continues.
4. M phase (Mitosis): Karyokinesis and cytokinesis occur (Weinberg, 1995; Ho and Dowdy, 2002).

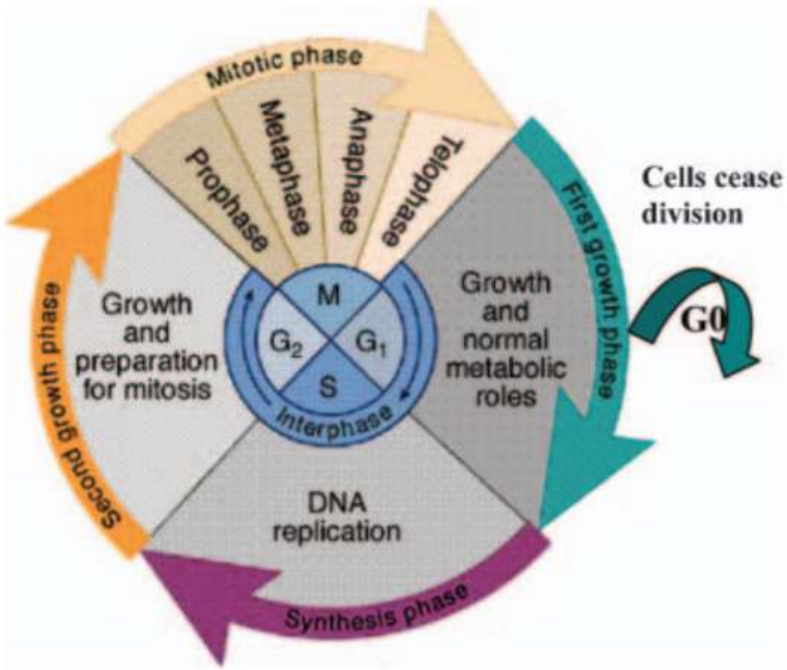

Fig. 2. Cell Cycle Stages (Anonym, 2006)

Cells that reach the G-1 stage after mitosis continue to divide or stop. The G-0 stage (stage between G-1 and S) is known as the resting stage of cells that have completed their final differentiation (Ho and Dowdy, 2002). When all biochemical events continue actively and cells that stop dividing pass, and the cell in the G-0 stage will divide again, it joins the cycle from the G-1 stage (Kuerbitz et al., 1992).

Mitogenic messages such as growth factors, tumor viruses and cytokines ensure that the cell enters the G-1 stage, where it prepares for the transition to the S stage. In the last stages of the M stage, there are points where the cell cycle is under control in the G-1 and G-2 stages (Weinberg, 1995).

The cell cycle is an organized process aimed at cell division through duplication of genetic information, and its activity is abnormal in tumor cells and is the hallmark of human cancer (Otto and Sicinski, 2017). Among the relevant molecular machinery, the cyclin-dependent kinase (CDK4/6)-Retinoblastoma (RB) pathway plays the most important role by regulating the cascade between G0-1 and S phase, determining the duplication of the genome under physiological conditions (Sherr et al, 2016). It is observed in 90% of both preclinical and clinical models (Xu and McArthur, 2016). Regarding BRAF mutant melanoma, an overactivated MAPK pathway is responsible for increased cellular proliferation by enhancing CDK4/6 functions (Scheiblecker et al, 2020).

Cell cycle damage occurs directly or indirectly as a result of misregulation of cyclin-dependent kinases (CDKs) (Malumbres and Barbacid, 2005; Malumbres and Barbacid, 2009). For CDKs to show activity, regulatory subunits known as cyclins must bind to these structures. Cyclins are synthesized and degraded for specific time periods during the cell cycle. These behaviors of cyclins during the cell cycle provide regulation of the activity of kinases. Human cells have multiple regions encoding CDKs and cyclins (regions 13 and 25, respectively) (Malumbres and Barbacid, 2005). However, only a specific subset of the CDK-cyclin complex directly drives the cell cycle. These; three interphase CDKs (CDK2, CDK4 and CDK6) belong to a mitotic CDK [CDK1, also known as cell division control protein 2 (CDC2)] and four different classes (types A, B, D and E) They consist of ten cyclins. Tumor-associated mutations usually deregulate a particular CDK-cyclin complex. Dysregulation of the CDK-cyclin complex results in its unplanned re-entry into the cell cycle and maintenance of proliferation. They are among the characteristic features of many human tumor cells (Malumbres and Barbacid, 2001). Although the cell cycle is a process that aims cell division through the duplication of genetic information, its activity is abnormal in tumor cells (Otto and Sicinski, 2017). The molecular mechanisms involved, the cyclin-dependent kinase (CDK4/6)-Retinoblastoma (RB) pathway regulates the cascade

between G0-1 and S phase. Its most important role is to determine the duplication of the genome under physiological conditions (Sherr ve ark, 2016).

Melanoma Epidemiology

Skin cancer types are examined under three main headings as squamous cell carcinoma, basal cell carcinoma and melanoma. Constituting only 4% of skin cancers, melanoma is responsible for 80% of skin cancer deaths worldwide. The incidence of melanoma has increased rapidly in the last 30 years. 62,000 new cases are detected each year in Europe. According to the American Cancer Society's 2016 report, it is estimated that 76,380 cases will be diagnosed and 10,130 people will die of melanoma in the United States in 2016. However, when melanoma is diagnosed early, the survival rate is approximately 90% (Cancer facts and figures, 2016; Öztürk et al, 2010).

Although the prognosis of melanoma with a high metastatic potential was very poor until recently, it would not be wrong to say that the treatment of advanced melanoma has undergone a real revolution recently.

Risk factors

The main environmental risk factor for melanoma is known to be sun exposure in general (Gandini et al, 2005). Intermittent sun exposure and a history of sunburn are also among the risk factors (Dennis et al, 2008). Exposure to UV radiation is a major risk factor for human melanoma and is the cause of genome-wide mutations Cancer Genome Atlas Network, 2015). Using artificial UV lamps (tanning booth) has also been shown to increase the risk (Lazovic et al, 2010). Genetic factors such as family history, number of nevi or clear phototype also play a role in the development of melanoma (Gandini et al, 2005). Thus, the risk of developing melanoma doubles in those with a family history of melanoma (Gandini et al, 2005).

Diagnosis

The mean age at diagnosis is 64 for men and 61 for women (Les cancers en france). Most melanomas are born novo in healthy skin. About 25% of melanomas arise in a pre-existing nevus (Bevona et al, 2003). A: asymmetry, B: irregular border, C: inhomogeneous color (brown, black, pink, brown, depigmented), D: melanoma diagnosis is clinically suspected when a pigmented lesion greater than 6 mm in diameter has the classic ABCDE criteria. And E: final scalability (size, shape, color change). However, some forms, such as nodular or acromic melanomas, do not have these clinical criteria. Dermoscopic examination

(light source combined with a magnifying glass) complements the clinical examination of pigmented lesions and significantly improves the performance of melanoma diagnosis (Bafounta et al, 2001; Vestergraad et al, 2008). The diagnosis should be confirmed by histological examination of the lesion after resection. This analysis will confirm the melanocytic nature and malignancy of the lesion based on architectural criteria. This examination also allows to deeply evaluate the level of invasion of the tumor (Clark level), measure its thickness (Breslow index), determine whether there is ulceration and evaluate the mitotic index. These morphological elements represent part of the prognostic factors of the AJCC (Joint American Committee on Cancer) classification and are used to guide therapeutic management.

Classification

One of the tumors with the highest mutation burden is melanoma (Alexandrow et al, 2013; cancer genome atlas network, 2015; Hayward et al, 2017). The most common somatic mutations are related to BRAF, NRAS, NF1, PTEN, KIT, which are genes involved in the MAPK (mitogen-activated protein kinase) and PI3K (phosphoinositol-3-kinase) pathways that control proliferation, differentiation and even survival (Leonardi et al, 2018). The MAPK pathway is the most frequently activated pathway in melanoma. For the BRAF oncogene, the activating mutation on codon 600 is the most common, found in 50% of melanomas: 80% to 90% of BRAF V600E mutations (substitution of valine for glutamic acid), 5% to 12% of BRAF V600K (substitution of valine for lysine) (Lovly et al, 2012). These mutations lead to abnormal activation of the MAPK pathway. Other mutations occur less frequently: 15 to 30% of NRAS mutations (excluding BRAF mutation), 10 to 15% of NF1 mutations, and less than 10% of KIT mutations (Curtin et al, 2005; Handolias et al, 2010; Maerten et al, 2013).

Thus, taking into account these molecular data and its relationship to the mode of UV exposure, a molecular classification of melanomas was established. Melanomas that occur in areas with intermittent sun exposure, such as the proximal areas of the trunk or limbs, tend to affect people under 55 years of age and are mostly mutated for BRAF V600E. Melanomas in chronic photo-exposure areas tend to occur in patients over 55 years of age in areas such as the face and neck and are primarily mutated for NRAS, NF1 or non-BRAF V600E (V600K) (cancer genome atlas network, 2015; Menzies et al, 2012).

Current Therapeutic Recommendations

A prime example of how basic and translational research has improved cancer

prognosis is melanoma. Considerable progress has been made in the last 10 years, with 13 new melanoma treatments approved in the United States, including targeted and immune-based therapies (Luke et al, 2017). Most human tumors have been shown to be immunogenic. Treatment with immune checkpoint inhibitors (ICI) includes BRAF V600E and MEK kinase inhibitors targeted at the mitogen-activated protein kinase (MAPK) pathway (Balch et al, 2001). The adaptive immune response to these tumors is mostly mediated by T cells. An immune response that overcomes the relative immune defense of tumors and destroys or limits the tumor has been observed in the aforementioned melanoma-associated vitiligo patients. Various immunological treatment tools are being developed that can trigger a similar anti-tumor response, and these agents have been shown to have positive effects on survival in the treatment of advanced melanoma (Desrichard et al, 2010). Today, immunotherapy is the primary systemic treatment method in metastatic melanoma (Diamantopoulos et al, 2016). One of the first agents used in melanoma to activate T cells and provide immune regulation is IL-2. It was approved by the FDA in 1988, but no phase 3 studies have been conducted. Cure has been observed in some patients with metastatic melanoma with IL-2. Although the overall response rates were low (10-15%) in the studies performed, long-term well-being was observed in the patients who responded (median: 8.9 months). The median response time could not be reached, especially in a small number of patients who achieved a complete response. Since it is a highly toxic treatment, it can only be considered in young patients without comorbidity, good performance status, good tumor biology and low tumor burden, and in intensive care conditions. The combination of IL-2 and INF alpha is not recommended as it is highly toxic (Diamantopoulos et al, 2016; Jiang et al, 2016).

In recent years, a new era has been opened in the treatment of melanoma, with the success of effective treatments through immune pathways, which enable us to better understand the role of the immune system in tumor control. Cancer cells neutralize immune mechanisms with escape mechanisms from the immune system. Under normal conditions, immune checkpoints, which were developed to protect the body from autoimmunity and prevent the inflammatory response from prolonging unnecessarily, act as negative controllers on T cells (Güngör and Akay, 2016).

Braf Gene Biology and Braf Mutations in Melanoma

The BRAF gene located on chromosome 7 (7q34) and located in the MAPK pathway encodes the BRAF protein, which is a 94 kDa intracellular enzyme of 766 amino acids (Peyssonnaux and Eychene, 2001). Consisting of a chain of

intracellular proteins, the MAPK pathway regulates physiological cell functions such as growth, proliferation, differentiation and apoptosis (Peyssonnaux and Eychene, 2001). The MAPK pathway consists of a chain of intracellular proteins that regulate physiological cell functions such as growth, differentiation, proliferation and apoptosis (Morrison, 2002). Besides BRAF, ARAF and CRAF are a MAPK kinase (MAPKKK)and are typically activated by GTPase proteins (i.e. RAS proteins) downstream from cell surface receptors such as EGFR (Epidermal Growth Factor Receptor) or KIT. Even though several more types of stimuli can lead to its activation (Cuevas et al, 2007). When activated, BRAF phosphorylates the mitogen-activated protein kinase/extracellular signal-regulated kinase ERK kinase (MEK), which in turn phosphorylates extracellular signal-related kinases 1 and 2 (ERK1/2) (McCubrey et al, 2007) . ERK proteins are the final effectors of the pathway: after being phosphorylated, they dimerize and migrate to the cell nucleus, thereby activating many transcription factors such as c-Jun and c-Myc through phosphorylation (Steelman et al, 2007). The ultimate targets of this signaling pathway in physiological conditions are the control of cell cycle progression and regulation of apoptosis (McCubrey et al, 2007).

Targeted Therapies

The BRAF V600 mutation is the most common genetic change in melanoma, affecting 50% of patients, making BRAF kinase a preferred therapeutic target (Davies et al, 2002). Vemurafenib is the first selective BRAF V600 inhibitor to receive Marketing Authorization in 2012, quickly followed by dabrafenib. These 2 molecules showed globally equivalent clinical benefits that were never observed in metastatic melanoma: 50% vs 5% response rate with chemotherapy, 6 to 7 month progression-free survival, and 13 median survival at 18 months (Chapman et al, 2011; Hauschild et al. , 2012). These inhibitors have specific toxicities such as photosensitivity for vemurafenib and fever for dabrafenib. In addition, 15-20% of patients develop squamous cell carcinoma due to paradoxical activation of the MAPK pathway in normal cells (Poulikakos et al, 2010; Chapman et al, 2011; Hauschild et al, 2012).

Examining the mechanisms of escape from monotherapy has identified the essential role of MEK in resistance to BRAF inhibitors and the potential synergy between BRAF inhibitors and MEK inhibitors, as well as a better understanding of how the MAPK pathway works. Phase II and subsequent phase III studies have shown that the combination of a BRAF inhibitor and a MEK inhibitor (vemurafenib with cobimetinib and dabrafenib with trametinib, respectively) is more effective than monotherapy with a BRAF inhibitor. Thus, response rates were 76% for the

combination, 54% for monotherapy, progression-free survival 8.8 months vs. 12 months, and median survival 18 months vs. 25 months. However, the toxicity of the combination is similar to that of monotherapy, although there is more febrile syndrome for the dabrafenib-trametinib combination and more photosensitivity for the vemurafenib-cobimetinib combination (Larkin et al, 2014; Robert et al, 2015; Long et al, 2015). On the other hand, the rate of cutaneous squamous cell carcinomas and side effects due to paradoxical activation of the MAPK pathway are significantly lower with the combination. MEK inhibitors have mainly cutaneous and digestive toxicity and also less than 10% risk of heart disease and retinopathy, warranting ophthalmologic alertness and monitoring. These treatments are generally well tolerated and less than 10% of patients discontinue treatment due to toxicity. A study comparing the two combinations indirectly found similar efficacy to dabrafenib plus trametinib but fewer side effects (Daud et al, 2017). Two combinations (V + C and D + T) are available since 2015. It is currently the reference therapy for patients mutated for BRAF V600, except as a contraindication to MEK inhibitors, which justifies monotherapy with anti-BRAF (Flaherty et al, 2012). Encorafenib and binimetinib, another not yet marketed combination, are promising in terms of efficacy and safety, with progression-free and overall survival outcomes that seem even better than first-generation combinations (Dummer et al, 2018).

Therapeutic Strategies and Perspectives

As is widely accepted, 40-50% of all melanoma patients harbor an activating BRAF mutation (mostly BRAF V600E). Identification and targeting of the RAS–RAF–MEK–ERK (MAP kinase) signaling pathway has been a valuable milestone for further and more recently the management of stage III and IV melanoma therapy (Ottaviano et al, 2021). Currently, only screening is done for the BRAF mutation. It is important in deciding on treatment. First-line therapy for metastatic patients with BRAF V600 mutated melanoma may be targeted therapy with a BRAF inhibitor and MEK or anti-PD-1 immunotherapy. Choosing between these 2 therapies is difficult. Indeed, the combination of BRAF inhibitor and MEK has exceptional, rapid and frequent efficacy, but with an increased risk of escape over time, response rates are lower with immunotherapy, but often with long response times. Rapid, targeted therapy is the treatment of choice for very symptomatic patients with tumor progression. For patients whose metastases do not pose a threat in the short term, the choice is less clear. The results of studies evaluating the outcome of patients treated with immunotherapy or vice versa following targeted therapy will assist the decision (NCT02224781). First-

line therapy for BRAF for patients whose melanoma has not been mutated is anti-PD-1 immunotherapy. Ipilimumab (anti-CTLA-4) can be used as a line 1 in combination with nivolumab or as a line 2 after escape with anti iPD-1. However, due to its toxicity, the combination of ipilimumab and nivolumab should be discussed only in patients who are in good general condition and have few comorbidities and have not been mutated for BRAF.

Conclusion

Melanoma is one of the deadliest cancers known, and 90% of cancer-related deaths occur in metastatic cancer cases. Therapeutic advances in recent years have revolutionized the management of metastatic melanoma, with dramatic improvements in survival rates. In current guidelines, immunological and targeted therapies have replaced chemotherapy. However, optimization strategies are still needed to increase the number of responders and reduce the toxicity of treatments. A large number of new biologic agents continue to be developed, and current treatment guidelines change as the clinical phase trial results of these agents are completed. The next step will be to use these treatments at earlier stages to prevent operated melanoma, which has a high risk of recurrence.

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CHAPTER 4

THE STRUCTURE OF THE GENOME

Serap KURT[1]

Fadime Çetin[2]

F. Sırrı CAM[3]

1 Arş. Gör., Manisa Celal Bayar University, Department of Medical Genetics, Manisa/TURKEY

2 Arş. Gör., Manisa Celal Bayar University, Department of Medical Genetics, Manisa/TURKEY

3 Prof. Dr., Manisa Celal Bayar University, Department of Medical Genetics, Manisa/TURKEY

Introduction

The genome is an important structural element of the nucleus. İn 1968, with the discovery of compositional genomics, genome organization was improved (Filipski vd., 1973).

The understanding of chromosome structure was first realized in 1968. At that time, while metaphase plant chromosomes were stained with quinacrine mustard; bands characteristic for each chromosome pair were seen by ultraviolet light fluorescence microscopy (Caspersson vd., 1968). These metaphase chromosome bands still remain a mystery. This is part of a larger mystery about chromosome architecture that remains to be explored (Ozer vd., 2015). With current studies, the organizational rules hidden behind the complexity of the chromosome structure have been revealed (Ozer vd., 2015; Pederson vd., 2015).

Chromatin structure is organized into higher level structures along the fiber and between genome regions on different chromosomes through electrostatic, hydrostatic and elastic interactions (Ozer vd., 2015). Chromatin interactions are crucial for cell identity, but how these interactions are established and regulated remains unclear (Dixon vd., 2016). However, the order in which chromatin is folded within the nucleus is still a matter of considerable debate (Schneider & Grosschedl, 2007). İmportant recent advance is that beyond the individual folding of chromosomes, chromatin is organized into different structural areas that can represent functional units of the genome (Kempfer & Pombo, 2020). The roles of chromatin fiber in forming and stabilizing chromatin organization are not fully understood, multiple polymer folding and interaction models have been proposed to explain higher order genome architecture (Ozer vd., 2015; Pederson vd., 2015). With recent observations, the chromatin fiber has been found to be 5-24 nm in diameter across the core, folding unevenly into higher features such as area and loop (Maeshima vd., 2019). The chromosome cohesion factor, cohesin, and chromatin architectural protein CTCF, which also plays a role in loop and field formation. Studies show that cohesin and CTCF cooperate to direct chromatin interactions (Rowley & Corces, 2018). Besides cohesin and CTCF, non-coding RNAs are also likely to contribute to higher order genome organization (Quinodoz vd., 2018). These features organize genomes at multiple levels and length scales (Misteli, 2020).

In eukaryotes, one-dimensional packaging of chromatin into nucleosomes is currently being studied in detail with genome-wide nucleosome positions and compositional maps (Ozer vd., 2015). Although we consider the mechanism of DNA folding into nucleosomes to be well defined, it still remains unclear how individual nucleosomes interact with each other (Ramani vd., 2016).

In the last two decades, studies emphasizing the importance of the position of genes for DNA to perform basic biological functions such as folding, forming chromatin, transcription, replication and repair have gained momentum (Schneider & Grosschedl, 2007).

The possible location of particular loci in the nuclei of cells within a population can be visualized using microscopy-based techniques, allowing for an understanding of how variation in the arrangement of individual chromatin fibers

at loci varies from cell to cell (Baldi vd., 2020). Although information on three-dimensional folding of the genome lags a little behind the one-dimensional picture, since a wide variety of technical approaches, such as chromosome divisions and topologically related domain (TAD) organization, have been readily observed in the last decade; has provided impressively cohesive views of in vivo chromosome architecture using various chromosome conformation capture (3C) methods, microscopy-based methods, and orthogonal methods such as genome architecture mapping (Cremer & Cremer, 2010). The DNA-FISH method has entered our lives as a revolutionary method that allows us to see the placement of chromosomes in the nucleus and how genes are organized. This method yielded very good information on a single-cell basis, but has limited efficiency, allowing only a small number of genomic loci to be analyzed at a time. Therefore, 3C-based approaches have been even more effective in identifying enhancer-promoter contacts by further segregating chromatin. High-throughput methods such as Hi-C map chromatin contacts on a genome-wide length scale from hundreds of kilobases to several megabases (Kempfer & Pombo, 2020). The organization of the genome must be such that the right gene expression programs occur at the right time and in the right tissue and cell type (Misteli, 2020). In summary, there is a correlation between chromosome architecture and genome organization.

Topology of the Genome

How the genome in the cell nucleus is organized in eukaryotes is a complex and dynamic process that is difficult to understand (Schneider & Grosschedl, 2007). The unique features of the nuclear architecture play an important role in the realization of basic processes such as DNA replication, DNA repair, transcription, and RNA processing. These features include the compartmentalization of molecular machinery and the three-dimensional arrangement of genomic sequences (Mahy vd., 2002). The basic organizational elements of the genome are composed of chromosomes, as well as chromatin fibers, loops, domains, and segments (Bickmore, 2013; Cavalli & Misteli, 2013).

A mammalian cell contains approximately 2m of linear DNA and it is usually compressed in the form of chromatin into a 10 mm diameter nucleus (Ozer vd., 2015). The human genome, containing about 25,000 genes and 3.2 billion base pairs of DNA, is compressed 400,000 times to fit in a nuclear volume of about 1000 μm^3. Packing DNA into chromatin is the most efficient way to store DNA in the nucleus (Mahy vd., 2002). The process of packaging DNA into chromatin begins with the wrapping of 147 base pairs (bp) around a histone octamer to form the nucleosome; this nucleoprotein complex acts as the basic repeating unit of chromatin. The histone octamer itself consists of eight subunits that combine as a histone H3 – H4 tetramer and two histone H2A – H2B dimers (Hauer & Gasser, 2017). Short-range interactions between nucleosomes shape the chromatin fiber into higher-order domains (Cui & Bustamante, 2000). Longer distance interactions along the fiber are facilitated by chromatin-associated proteins,

thereby promoting the formation of larger domains and causing chromosome formation (Rosa, 2013).

The quantitative strategy of DNA is the frequency of base compounds and short sequences. Classic CsCl ultracentrifugation does not have a satisfactory resolved power which breaks down DNA molecules according to GC levels. Conversly, Cs_2SO_4 / Ag^+ strategy can separate DNA fragments according to different densities of specific short sequences. Basically, since the short sequences determine the fine structure of the double helix and the interactions of DNA with proteins; compositional genomics is based on genome structure and function. Thus, the Cs_2SO_4/Ag^+ strategy led to the discovery of not only satellite DNAs, but also a small number of 10–20 Kb sized "baseband" DNA molecules in the genome that could not be resolved by CsCl ultracentrifugation (Corneo vd., 1968; Filipski vd., 1973). These 10-20 Kb DNA molecules were derived and "isochors" which are much longer stretches of DNA with homogeneous base composition were developed (Macaya vd., 1976). Segmentation of chromosome sequences by GC profiles provided full coverage of the human genome with ~3,159 isochors with an average of 0.9 Mb and a total size of 2,854 Mb (Costantini vd., 2006). Isochor families are divided into L1, L2, H1, H2, H3 and isochors interspersed from L1, L2, and H1 families are GC-poor; Interspersed isochores from the H2 and H3 families have two genome domains identified as GC-rich (Bernardi, 2005). All structural and functional features of the genome are related the GC grades of isochores. So, the relation of the genome with the isochor is an significant result. On the other hand, isochor maps of chromosomes are maps of structural and functional genome features (Pavlíček vd., 2002, s. 17). Structural proteins such as cohesin and CTCF form the protein scaffold (skeleton) of the chromosome (Bernardi, 2015). Chromosome architecture is conserved across mammalian species, and these observations parallel the conservation of isochor families in mammalian genomes (Rao vd., 2014). For all chromosomes, isochores provide detailed information on the sizes and GC levels of the prometaphase and metaphase bands (Costantini vd., 2006, 2007).

Chromatin is arranged 3D within the nuclear domain, allowing the genome to be packaged efficiently while also allowing proper expression and replication of genetic material (Baldi vd., 2020). Modifications of chromatin structure occur as a fundamental level of control of gene expression. Macromolecular complexes modify or preserve chromatin structures in specific regions of the genome, thereby enabling regulation of nuclear functions. While the initiation of DNA replication in the context of chromatin is still poorly understood, it is known that packaging DNA into chromatin can suppress DNA replication. Therefore, it seems likely that chromatin remodeling is involved in the spatial and temporal control of replication initiation. The architecture of interphase chromatin can be specified as a series of looped domains and domain boundaries. While the looped area corresponds to weak isochores in terms of GC; domain boundaries usually correspond to GC-rich isochores anchored by building proteins such as CTCF and cohesin. Looped domains are essentially composed of subdomains and most of them are anchored by CTCF and cohesin subunits (Bernardi, 2015). The 3-

dimensional linear chromatin structure is opened at a fiber size of 10 nm in the early stage of prophase. This open structure is folded into 30 nm fiber loops by structural proteins and single isochor bands are compressed into three early phases as R-G-R (Bernardi, 2015). Further compression of the nucleosomal DNA with 30 nm fibers and DNA loop domains provides an even more oppressive environment. (Fritz vd., 2019). Single isochor bands as R-G-R merge into multiple isochor bands (Bernardi, 2015).

Chromosome Regions and Interactions Between Chromosomes

In mammalian genomes, individual chromosomes preferentially occupy distinct nuclear sites called chromosome territories (CTs). Early studies suggested a different boundary pattern on the surface of the chromosome territories, with the region on one side and the interchromosomal spacing on the other side containing the transcription mechanism (Cremer & Cremer, 2010; Lazaris vd., 2020). At the cytological level, the eukaryotic genome is divided into euchromatin and heterochromatin (Ramani vd., 2016). Inactive genes associate near the nuclear border and with peripheral heterochromatin, but active genes are often found in the nuclear interior (Croft vd., 1999). Heterochromatin is evolutionarily conserved in most eukaryotic organisms (Bickmore, 2013). Recent studies using high-resolution in situ hybridization and chromosome conformation-capture methods have revealed that chromatin fibers from the periphery of chromosome regions are intermingled in interphase nuclei. Interestingly, inhibition of transcription also suggests that the regions of chromosomes can change the patterns of interlocking without changing their overall properties (Branco & Pombo, 2006).

Following the establishment of CT as the basis for genome organization in the cell nucleus, researchers focused on understanding how individual regions are arranged within the 3D architecture of the cell nucleus. For this purpose, firstly, the relative radial arrangement of chromosomes with respect to the nuclear periphery and the center was investigated. The question of whether the CT is arranged randomly or at a nonrandom level in their location in the cell nucleus has required much study. These studies clearly established a high level of order in the radial positioning of individual CTs in the cell nucleus. Many factors other than gene density or chromosome size were involved in the radial arrangement of CT. Interactions with other nuclear compartments have been proposed as a mechanism by which radial positioning is established. For example, interaction with the nucleolus is involved in the radial positioning of the nucleolar NOR-CT. The nuclear lamina has also been shown to be important for the radial organization of CT (Racko vd., 2019).

Topologically related domains (TADs) and Chromatin Loops

It has been known for more than 50 years that circular DNA molecules of a few mb in length and bacterial chromosomes are organized into topological domains where sequential, large DNA segments of 5-50 kb form superhelical loops (Racko vd., 2019).

Two types of distinctive patterns stand out in higher eukaryotes; the first is the checkerboard-like pattern that appears both within and between chromosomes in Hi-C maps. This pattern reflects the general phenomenon of active (euchromatin) and inactive (hetero) chromatin spatially separated in the nucleus, resulting in higher contact frequency between genomic regions of the same type and reduced contact frequency between regions of different types. The second type of pattern, evident in vertebrate maps, is cohesin-dependent and generates features associated with TADs. TADs are characterized as continuous regions where a higher contact frequency is observed between loci within each TAD than loci in neighboring TADs (Mirny vd., 2019). Although TAD formation appears to be mostly constant even after differentiation, intra-TAD interactions are variable between cell types, suggesting that regulatory interactions within TADs are highly dynamic (Rao vd., 2014). Chromosomes are tightly packed, folded in the interphase nucleus, but the molecular basis of this folding is not fully understood. Chromosome conformation capture methods such as Hi-C combine chemical cross-linking of chromatin with fragmentation, DNA ligation and high-throughput DNA sequencing to detect the neighboring locus genome. Hi-C technology revealed the division of chromatin into active and inactive compartments and folding of DNA into self-assembled domains and loops. The transcriptional silencing CTCF known as the CCCTC-Binding Factor consistently affects most domains and loops through extrusion of chromatin loops, reducing the amount of cohesin or cohesin-related proteins (Eagen, 2018).

There are 10,000 cycles in the human genome with some cycle boundaries, also called cycle anchors, shared between two or more cycles. FISH studies have shown that loops are apparently transient as loop anchors are greater than 250 nm apart in three quarters of a cell population (Rao vd., 2014). Although loop formation is a predictable mechanism that has been the subject of various studies since the 1990s, it was only discovered in 2010's (Alipour & Marko, 2012). In loop extrusion, a loop factor binds the chromatin fiber and compresses it from both sides, resulting in a growing loop. When the loop factor dissociates, the loop is left untidy. Early theoretical studies showed that this seemingly simple process, acting independently, could reproduce a large number of chromosomal phenomena. Knockdown experiments on cohesin and condensin, the proteins of the SMC family, clearly demonstrated that these proteins serve as loop factors and that during interphase this activity is entirely due to cohesin, not condensate (Chambeyron & Bickmore, 2004).

In humans, more than 85% of loop anchors are bound by cohesin or CTCF (Rao vd., 2014). Cohesin is thought to form a triple ring, suggesting that it can form or maintain chromatin rings by trapping two chromatin segments in the cis (Kim vd., 2007). CTCF is known to have the ability to bind isolators and prevent enhancers from communicating with promoters. CTCF has a characteristic non-palindromic binding motif (Eagen, 2018; Kim vd., 2007).

From the loops where both anchors can attach to a unique CTCF motif, about 90% of the motifs are oriented towards each other. This is called the convergent rule (Eagen, 2018). Convergent CTCF motif orientations at loop anchors are not

randomly dominant. If CTCF is included in the loop regardless of its motif's orientation, it is expected that one-quarter of the motif pairs will be convergently oriented, one-quarter divergently oriented, and half-tandemly oriented (Uhlmann, 2016).

Regulators of the Genome

One of the mechanisms by which the protein levels of the cell are controlled is transcriptional regulation. Certain regions on DNA, called cis-regulatory elements, are footprints of processing proteins involved in transcription, either by positioning or regulating essential transcriptional machinery. The basic transcription mechanism is DNA-dependent RNA polymerase (RNAP), which synthesizes various types of RNA, and core promoters on DNA are used to position RNAP. Other proximal regions regulate transcription: operators are involved in prokaryotic organisms; eukaryotic organisms have proximal promoter regions, enhancers, silencers, and insulators (Ogbourne & Antalis, 1998).

Enhancers

One of the hallmarks of eukaryotic gene expression is the presence of specific sets of DNA motifs that can segregate transcription factors (TFs), often from a great distance, to upregulate the rate of binding and formation of the preinitiation complex to the core promoter. These enhancer regions (enhancers) can be found in the 5' and 3' UTR regions of genes, upstream and downstream of the transcription start region within exons or introns. The precise mechanisms by which enhancers influence transcriptional activity are still debated, but their activation is often regulated by the binding of several transcription factors to cis-regulatory motifs themselves. When the enhancer is active, it can bind to the pre-initiation complex or binding elements in the proximal region of the promoter and influence the rate of transcription on its own (Gaszner & Felsenfeld, 2006; Ogbourne & Antalis, 1998).

As of today, it is unknown how many chromatin contacts are used for enhancer-mediated gene activation. However, a recent study showed that not only developers but also promoters can interact with each other. These interactions seem likely to have functional roles in the genome because they often occur in co-regulated genes.

Silencers

Although enhancers and silencers can act on more than one gene, sometimes these interactions can create problems for gene regulation. Specific cis-acting regulatory DNA sequence regions, called insulators, can inhibit such interactions. “Enhance blocking insulators” and “barrier insulators” are two different types of insulators that have been discovered (Gaszner & Felsenfeld, 2006). Enhancer blocking isolators protect from enhancer-activated gene activation and interfere with enhancer-promoter interaction only if the isolators are located between the enhancer and the promoter. Barrier insulators, on the other hand, are protected

against diffusion of heterochromatin and thus chromatin-mediated silencing and lie at the boundary of euchromatin and heterochromatin domains (Elkon & Agami, 2017).

Functional Role of Genomes

The organization of the genome within the nucleus has far-reaching effects on DNA-based processes ranging from transcription to DNA repair (Cremer & Cremer, 2010). One task of the genome, apart from its role in forming and maintaining the overall structure of the cell nucleus, is that heterochromatin can increase the structural robustness of the nucleus and strengthen the cell's ability to withstand physical challenges such as mechanical forces or during cell migration (Gerlitz & Bustin, 2011). Studies indicate that this function of the genome may be very important, especially in cells exposed to mechanical stress such as cardiomyocytes and migrating cells (Bustin & Misteli, 2016). Another non-genetic function of the genome is its ability to act as a scaffold. An important function of the genome, which it influences in non-genetic ways, is its role in forming and maintaining the nuclear structure (London & Biggins, 2014). The genome also contributes to the determination of nuclear size (Hergeth & Schneider, 2015).

Heterochromatin is evolutionarily conserved in most eukaryotic organisms (Bickmore, 2013). Compact heterochromatin is generally considered a tool to facilitate gene silencing (Pinheiro vd., 2012). It also provides mechanical support to the nucleus by reinforcing the nuclear membrane and nuclear lamina by non-genetic means. Downregulation of Prdm3 and Prdm16 methyltransferases in mouse embryoic fibroblasts leads not only to reduced chromatin density, but also to disruption of the nuclear lamina (Pinheiro vd., 2012). Inactive genes associate near the nuclear border and with peripheral heterochromatin, but active genes are often found in the nuclear interior (Croft vd., 1999).

An important function of the genome, which it influences in non-genetic ways, is its role in forming and maintaining the nuclear structure. In mitosis, the nuclear membrane separates and the membrane fragments are dispersed in the dividing cell. The nucleus regenerates when daughter cells are formed in telophase (Wandke & Kutay, 2013). The rigid nucleus limits the ability of cells to move easily in restricted spaces. The nucleus, which undergoes a shape change during cell migration, is exposed to significant mechanical stress (Harada vd., 2014). Nuclear lamina integrity plays an important role in the remodeling of the nuclear structure and its ability to withstand mechanical stress during migration. Changes in the nuclear lamina; reduces the migration rate. Decreased nuclear stiffness may reduce the viability of migrating cells (London & Biggins, 2014). The reduction in nuclear size due to chromatin condensation and increased stability of the nuclear lamina minimize potential damage during remodeling, thereby increasing the migration ability of cells (Gerlitz & Bustin, 2011).

The genome serves as the binding platform for a wide variety of cellular components. Binding of both transcription factors and chromatin shapers to the genome leads to changes in gene expression. In the separation of chromosomes towards the

poles, ıt is mediated by spindle microtubules that attach to align chromosomes at metaphase. The attachment of microtubules takes place in the centromere, which is a special chromosome region, in protein-structured kinethechores (London & Biggins, 2014). With the studies carried out; ıt was determined that loss of methylation of H3 Lys9 in the centromere caused both the attraction between sister chromatids and kinetochore defects. Thus, it causes chromosomal instability and leads to tumor progression (Bustin & Misteli, 2016). Binding of chromatin remodelers and transcription factors to the genome suggests that chromatin is actively involved in cellular signaling pathways through the binding-dissolution pathway, as well as in the regulation of gene expression(London & Biggins, 2014).

Another function of the genome is its contribution to the determination of nuclear size. The histone H1 family that contributes to chromatin condensation and destruction of condensins, which are responsible for the compression of chromosomes causes an enlargement of the nuclear dimension (Bustin & Misteli, 2016).

The link was found between heterochromatin organization and the night vision capacity of animals. In most cell types, heterochromatin regions are found in the nuclear periphery; In rod cells of night vision animals, heterochromatin regions accumulate in the nuclear interior (Solovei vd., 2009). Responsible for this mechanism are lamin A/C proteins and inner nuclear membrane protein lamin B receptor (LBR) (Solovei vd., 2013). Changes in optical properties of rod cells caused this change in heterochromatin organization in nocturnal animals (Solovei vd., 2009).

Despite all this, research is still ongoing regarding the non-coding parts of the genome that occupy a large part of it. In addition, although it has been examined in many aspects, how loops and compartmentalization affect biological function is still an unanswered question. As we mentioned above, the topology of the genome makes it accessible to a wide variety of cellular processes.

Conclusions

In addition to genetic material, many proteins are not randomly distributed in the nucleus, but concentrated in subnuclear fields. The important result obtained from the studies; the genome organization is not in a certain order and random. There are fundamental and longstanding biological questions linked to three-dimensional genome architecture. Whether the genome architecture itself defines cellular identity, and how chromatin status (such as histone modifications, DNA methylation) affects chromatin structure are some of them. The mechanisms that determine the chromosome or the position of a gene are largely unknown, but some protein and chromatin modifications related with the 3D position of some genes have been identified. The advent of high-throughput analysis of histone modifications has provided us with a genome-wide map for many chromatin modifications. Evidence has been provided for many areas, with the help of the map produced with Hi-C technology. With these studies mentioned; while clarifying the links between chromatin structure and gene expression/regulation; in order to solve the changes in the chromosome structure during the cell cycle, it will be useful to determine the relationship between chromatin structure and compositional genome features. Obtaining the knowledge necessary to answer

these questions requires a multifaceted approach using technologies such as microscopic, biochemical and computational tools. As generally reviewed here, these are requirements that the cytogenetic field is actively addressing and suggest that we will be in a good position to answer many, if not all, such questions in the coming years.

Although the genetic functions of the genome are well established, its non-genetic effects are not fully understood. It is quite possible that the non-genetic functions of the genome will be discovered with further studies. More research is needed to understand traits on various cellular functions of non-genetics effects.

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CHAPTER 5

BIOLOGICAL EFFECTS of RADIOFREQUENCY FIELDS: 5G (The Fifth Generation)

Mehmet Cihan YAVAS[1]

1 Assoc.of Prof., Kırşehir Ahi Evran University, Faculty of Medicine, Department of Biophysics.
https://orcid.org/0000-0002-2923-050X

Introduction

As a result of the continuous development of technology, radiofrequency bandwidths are constantly increasing to catch innovative technologies in the field of communication, as in every field. It seems that with the transition to 5G technologies, the increase in data download speed, low delay times, uninterrupted communication, and increase in bandwidth will open the door to important breakthroughs in education, industry, entertainment, service, and other fields. Therefore, the increasing usage rates of mobile applications, it will increase the rate of mobile data consumption. The 5th generation technology especially strengthens the connection situation with the phone. With the increase in mobile data traffic, businesses are investing in technological breakthroughs in wide and

different spectrum bands in many countries of the world. In addition, some authorities and professional organizations emphasize that there is no need for 5th generation technologies, they will be exposed to more radiation, and therefore there may be negative effects on human health. There is a need for scientific study reports on whether these new technologies have a possibly harmful effect on human/public health. When the study reports are examined, it is seen that there are research reports that exposures in the 5G frequency band can create some important biological effects, but more experimental studies are needed in the lower and upper band frequencies in the 5G frequency band.

a- Developments in communication technology

Telecommunication technologies based on radio frequency (RF) transmission, such as radio and television, have been widely used for decades. However, RF fields are ubiquitous in our environment. Increasing public exposure to RF fields makes their impact on human health a concern for scientists and the general public (WHO, 2010). Along with the latest technological developments, we continue to be exposed to man-made electromagnetic sources (EMF) and the latest 5G-generation communication in our daily and professional lives (Bortkiewicz, 2019).

When first-generation (1G) wireless networks were first introduced, they only offered the possibility for voice calls. Second-generation (2G) technologies started to support messages. It offered third-generation (3G) data transmission speed and multimedia support. The fourth-generation (4G) is gaining quite a lot of features from the previous generation. Fifth-generation (5G) mobile technology revolutionizes the mobile market and offers many technological features. 5G technology has given the following gains. It has been advantageous for the benefit of society with its high speed, high capacity, big data support in Gbps, the opportunity to watch multimedia newspapers, TV programs clearly, faster

data transmission compared to the previous generation, large phone memory, call speed, and clarity in voice/image. For this reason, 5G will provide us with many conveniences (Vora, 2015; Bhandari, et al. 2017; Javed and Siddiqui, 2017). 5G cellular mobile technology includes many radiofrequency (RF) bands. The frequency coverage can be roughly divided into two ranges: the sub-6-GHz bands and the 24-60 GHz frequencies that reach well in the mm-wave region. Frequency ranges are generally divided into low, medium, and high band gaps (Lin, 2021). The change process in communication technology from 1980 to the present is shown in Figure 1 (Alsharif et al. 2020). The 5G frequency bands are low (0.6 GHz - 3.7 GHz) are divided into medium (3.7 - 24 GHz) and high band frequencies (24 GHz and above) (Moskowitz, 2017).

In the last two or three decades, the growth in the mobile phone industry has also led to an increase in the number of base stations (Kostoff et al, 2020). The safety limits recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) for exposure to a radiofrequency electromagnetic field are shown in table 1 (ICNIRP, 2020).

In the compilation report investigating the public's exposure to electromagnetic energy from 5G wireless communication networks, it is stated that there is no supporting evidence of adverse health effects at exposures below specified limits, including 5G systems. (Bushberg et al. 2020).

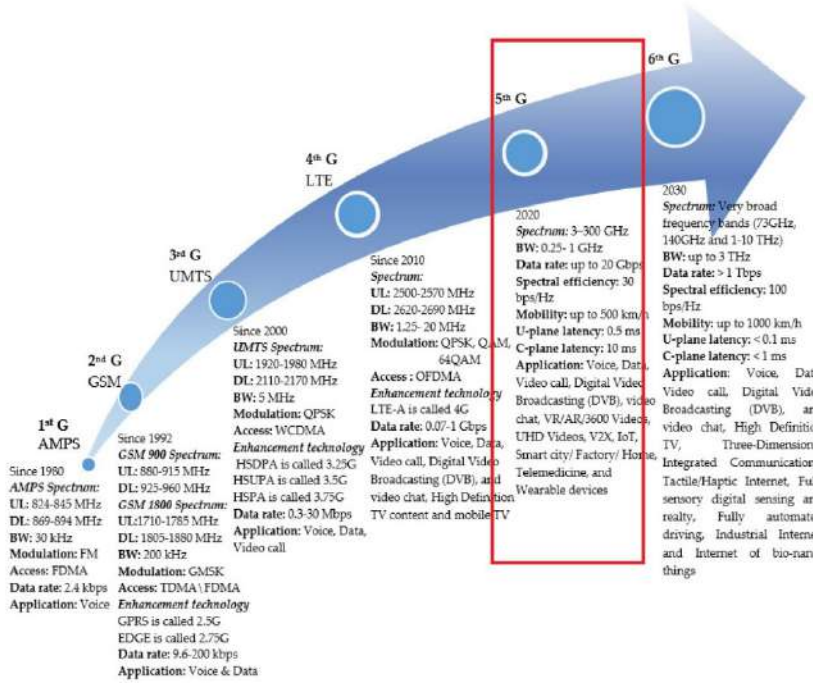

Figure 1. Changes and features in communication technology belong to different generations (Alsharif et al. 2020).

Table 1 Basic restrictions for electromagnetic field exposure from 100 kHz to 300 GHz, for averaging intervals ≥6 min.[a]

Exposure scenario	Frequency range	Whole-body average SAR ($W kg^{-1}$)	Local Head/Torso SAR ($W kg^{-1}$)	Local Limb SAR ($W kg^{-1}$)	Local S_{ab} ($W m^{-2}$)
Occupational	100 kHz to 6 GHz	0.4	10	20	NA
	>6 to 300 GHz	0.4	NA	NA	100
General public	100 kHz to 6 GHz	0.08	2	4	NA
	>6 to 300 GHz	0.08	NA	NA	20

[a]Note:
1. "NA" signifies "not applicable" and does not need to be taken into account when determining compliance.
2. Whole-body average SAR is to be averaged over 30 min.
3. Local SAR and S_{ab} exposures are to be averaged over 6 min.
4. Local SAR is to be averaged over a 10-g cubic mass.
5. Local S_{ab} is to be averaged over a square 4-cm^2 surface area of the body. Above 30 GHz, an additional constraint is imposed, such that exposure averaged over a square 1-cm^2 surface area of the body is restricted to two times that of the 4-cm^2 restriction.

b- Scientific reports maintained in the 5G radiofrequency range

5G technology will gradually increase the application areas of smart cities, smart transportation systems, and vehicle communication in agriculture, energy, and health fields in the coming years (Guevara and Auat Cheein, 2020). 5G technology has many unimaginable uses and benefits. It is stated that in case of widespread use, significant negative consequences on human health and ecosystems may occur. There is little study of 5G technologies, their human or environmental impact (Russell, 2018). Various studies are carried out to investigate the effects of radiofrequency fields. His research continues in epidemiology, human studies, animal studies, cell studies, interaction mechanisms, and dosimetry and exposure assessments (WHO, 2010). The results of the reports of independent scientists, health, and environmental advisory boards investigating the biological effects of radiofrequency radiation that we are exposed to will contribute to the risk assessment. Public health needs to keep these exposure standards up-to-date (Russell, 2018)

Millimeter waves (MMW) are characterized in the range of 30-300 GHz and constitute the extremely high-frequency band of RF-EMF (Di Ciaula, 2018). Some study reports with exposures of 5G and millimeter waves have been examined. There are reports that exposure to a frequency above 60.4 GHz-MMW may alter gene expression (Mahamoud et al, 2016), 60 GHz-MMW may have thermal effects on excitable cells (Shapiro et al. 2013), 60.4 GHz-MMW (20 mW/cm^2) may change the function of the endoplasmic reticulum (Le Quement et al. 2014). In another study, reports were presented that it can increase skin temperature in

the 20-100 GHz range (Zhadobov et al, 2015), stimulate cell proliferation (Li et al. 2014), and that 35GHz MMW rat exposure has effects related to inflammation and oxidative stress. (Sypniewska et al. 2010).

Scientific studies related to 5G have started to be made now. For this reason, studies are available at some frequencies. As the frequency increases, there is less penetration into body tissues and the absorption of energy becomes more limited on the surface of the body (skin and eye). No consequences for public health are expected, provided that overall exposure remains below international guidelines (WHO, 2020). In the study conducted by the European Parliament Research Service, it was reported that there are studies at low frequencies, but studies at high frequencies are needed in the compilation study on the effect of 5G on health (Belpoggi F. 2021). Although 5G technology brings new risks, it is reported that it is a really important factor in cell phone use and cancer. It is stated that 5G radio frequencies can increase the formation of free radicals with very high energy accumulation and low penetration per unit distance, and this may increase the risk of skin cancer (Yakymenko et al. 2016; Mehdizadeh and Mortazavi, 2019). The specific absorption rate (SAR) and temperature rise of 4G and 5G cellular frequencies of a brain tumor in a human head model have been studied. As a result of the study, it was determined that there was an increase in both SAR and temperature in the group of unhealthy individuals (Kaburcuk, 2019; Gultekin et al. 2020).

Researchers investigated the biophysical effect of 5 GHz electromagnetic radiation on the male reproductive system of *Mus musculus* mice. As a result of the study, physiological and histopathological harmful effects on the reproductive system were determined (Al-Dulamey et al. 2018). It was stated that the immune system parameters were affected after 61.22 GHz (30 min each day for 3 consecutive days) exposure applied on mice (Makar et al. 2016). The effects of 5 GHz Wifi on E. coli and human neuroblastoma cells were investigated. Long-term exposures have been reported to decrease growth rate, vitality, and spawning rate (Bircan et al. 2021). Study reports suggest that the potential health effects of 5G in real-life situations should be further investigated and tested. It is stated that 5G mobile network technology affects the skin and eyes, but also has adverse systemic effects (Kostoff et al, 2020). In 2011 IARC had evaluated RF radiation as one of the possible human carcinogens (IARC, 2013). Compared with the old techniques, it is emphasized that the radiation created by the high band spectrum in 5G can cause cancer and tumors. In addition, it is stated that low-intensity radio frequencies can cause oxidative effects in living cells by increasing reactive oxygen species. (Yakymenko et al. 2016). Children and pregnant women are

advised not to use cell phones and other wireless devices too much as they are more vulnerable (Bektas, 2021).

It is stated that at radiofrequency exposures above 6 GHz, limiting the thermal hazard to the skin is cutaneous pain for exposure times shorter than about 20 minutes, and thermal damage may occur in case of longer exposure (Foster et al. 2021). Scientists state that new thermal safety standards are needed to assess the health risks associated with 5th generation technology (Maisch, 2019).

c- Reports that require attention.

On health safety issues, it is unclear whether the biological responses to high-band 5G radiations will be similar to previous generations or to low-band 5G radiations, given the distinctive features of the mm-wave (Lin, 2021). As a result of the compilation of scientific study reports, a clear conclusion has not yet been reached due to the inconsistency of the findings obtained from in vivo and in vitro studies on the health effects of millimeter waves (MMW) in the 6-100 GHz frequency range (Simkó et al. 2019). In a study conducted in Switzerland, it is emphasized that the health risks arising from 5G technologies are ignored, biased reports can be prepared due to conflicts of interest and ties to the industry, and therefore, the absence of appropriate impartial risk assessment of 5G technology puts society at risk (Hardell et al. 2020). 5G continues to be a promising technology for people with its speed, reliability, and many innovations. In addition, there are study reports that RF-EMF fields have many negative effects (carcinogen, DNA damage, etc.). However, on the contrary, the need for more research in the field of 5G leads to a gap in this field. Since there is no consensus on the part of the international supervisory organizations, the discussion of this situation continues (Jiamjirarat and Rafflin, 2020).

d- As a result

When the study data is examined, it is seen that there are few sufficient and repeatable studies in all frequencies of 5G, and there is a need for studies by scientific authorities that may have a negative impact on health, and clarity has not yet been achieved.

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CHAPTER 6

SOURCES OF VIBRATION IN THE WORKING ENVIRONMENT AND PREVENTION OF VIBRATION EXPOSURE

Meleknaz KELES[1]

Dilek OZTAS[2]

Ergün ERASLAN[3]

1 PhD Candidate, Ankara Yildirim Beyazit University, Graduate School of Natural Sciences, Ankara TURKEY, e-mail: meleknazkeles@gmail.com, ORCID ID: 0000-0003-2988-0737

2 Associate Professor, Ankara Yildirim Beyazit University, Faculty of Medicine, Ankara TURKEY, e-mail: doztay@ybu.edu.tr, ORCID ID: 0000-0002-8687-7238

3 Professor, Ankara Yildirim Beyazit University, Faculty of Engineering and Natural Sciences, Ankara TURKEY, e-mail: eraslan@ybu.edu.tr, ORCID ID: 0000-0002-5667-0391

INTRODUCTION

In this article, information is given about the methods that can be applied to determine the exposure to vibration sources in the working environment. Detailed engineering and administrative measures that can be implemented to prevent or reduce exposure to whole body vibration and hand-arm vibration are included.

In particular, the properties, effectiveness and vibration permeability of vibration-reducing gloves were evaluated. Suggestions were made to the user about the situations in which these gloves should be used and what conditions they should meet.

1.BODY

1.1. Definition and types of vibration

Vibration; refers to mechanical oscillating movements around an equilibrium point. Here, vibration potential energy turns into kinetic energy and kinetic energy turns into potential energy. The wave pattern starts moving forward and backward, up and down, right and left. The keywords used to describe these movements are the frequency and intensity of the vibration.

The frequency of vibration refers to the number of vibrational movements occurring per unit time. Its unit is hertz (Hz). An object vibrating at a frequency of one hertz completes one cycle in one second.

The intensity of vibration is related to the intensity, power and size of the energy generated from the vibration source in unit area and time. Its unit is watt/cm2.

In addition to the wave patterns of vibration, there are also forms of physical movements of vibrating equipment. They have directions in the form of reciprocating motion (such as a drill bit or plunger), rotational motion (such as a drill or drive shaft), and oscillating motion (such as a pendulum or paint can shaker).

1.2.Vibration sources and exposure to vibration

Machines, devices, and equipment that are running and not well secured often generate vibrations.

Vibration can be acquired directly by direct contact with machinery, equipment, or vibrating surfaces, or indirectly by contact with another surface in contact with a vibrating machine or object. The indirect absorption of vibration can be exemplified by the transmission of a running engine from the floor to the wall, from the wall to the platform above it, and from the platform to the person working on it.

Vibration energy is taken from the palm and transmitted to the hand, arms, and shoulders. In this process, the power begins to fade, which is a positive indicator

for human health.

In the Regulation on the Protection of Employees from Risks Related to Vibration, it is stated that two types of vibration can be exposed. These are divided into two as exposure of a part of the body such as hand, arm, foot to vibration and exposure of a large part or all the body.

The exposure action value for vibration expresses the value that requires controlling the risks that may arise from exposure to vibration if this value is exceeded.

The exposure limit value for vibration, on the other hand, expresses the value that should not be exposed to a vibration above this value.

1.2.1. Whole body vibration

Whole body vibration refers to the mechanical vibration that is transmitted through the lower pelvis bones, feet and legs when the person stands or sits on a vibrating vehicle, machine or floor, poses a risk to health and safety, especially causing discomfort in the lumbar region and trauma to the spine.

In exposure to whole body vibration, the person is generally under the influence of vibrations with different characteristics (frequency, intensity, direction, etc.).

Bus, truck, forklift, mobile crane, grader, railway vehicles and transportation vehicles can be cited as examples of vibration sources that the whole body is under the influence of.

The daily exposure limit value is 1.15 m/s2 and the exposure action value is 0.5 m/s2 in the eight-hour working period in whole body vibration.

1.2.2.Hand-arm vibration

Hand-arm vibration refers to the mechanical vibration that occurs when a vibrating machine or tool is held or directed with the hands, which poses a risk to the health and safety of the person, and causes vascular, bone, joint, nerve and muscle disorders.

Exposure of the person to hand-arm vibration: It can be affected by many factors such as the temperature of the working area, whether to wear gloves, how the tool is held, the frequency and severity of the vibration, the duration of exposure, the age of the person, personal sensitivity, and health status.

Examples of hand-arm vibration sources are machine and hand tools such as chainsaws, impact drills, electric hammers, lawn mowers and sanders.

In hand-arm vibration, the daily exposure limit value is 5 m/s2 and the exposure action value is 2.5 m/s2 during the eight-hour working period.

1.3.Determination and assessment of vibration exposure

First, work operations, tasks, equipment, and tools that cause exposure to vibration should be determined by observing the platforms and surfaces on which workers stand.

In designated business activities, how often and for how long the equipment and machinery are worked should be determined by reviewing the records and by direct observation of the activities.

The exposure intensity is then estimated, considering the value ranges of the vibration levels. This estimated value is comparable to exposure limit and action values and if exposure to vibration is high, steps should be taken to reduce exposure.

1.4.Effects of vibration on human health

If the vibration is transmitted to the hands and arms through the palms and fingers, the nerves, blood vessels, muscles and joints in the hand, wrist and arm are affected. With continued exposure to vibration, symptoms such as tingling in the fingers, whitening of the fingers, pain when exposed to cold, reduced grip strength and finger dexterity may occur.

When the vibration is transmitted to the whole body from machinery, vehicles, or surfaces, while sitting or through the feet and legs, symptoms such as headache, chest pain, abdominal pain, loss of balance and fatigue. On short-term exposure, low back pain, damage to the spine, damage to the vertebrae with long-term exposure, damage or rupture of the discs can cause symptoms such as neck pain.

Chronic symptoms can often persist for months or even years after exposure has ended and are often persistent.

1.5. Prevention, reduction, and limitation of whole-body vibration exposure

When purchasing machinery and equipment, data on exposure to whole body vibration should be requested from the manufacturer and it should be determined at what level the maximum exposure should be. Vibration reduction systems in machinery and equipment should be identified and their maintenance requirements should be determined. The components that contribute most to the vibration generation of the equipment should be determined.

The maintenance of the suspension systems in the machines should be done periodically.

Suspension systems of vehicle seats should be maintained.

Vehicles should be equipped with air or suspension system seats, the seats should have adequate padding and suitable backrests, provide sufficient space to

allow the movement of employees, and have easy access for adjustments such as height.

To reduce the effect of vibration exposure of drivers, it should be ensured that the roads and driving surfaces are maintained, the vehicle type and tire suitable for the surface conditions are used, the appropriate headlight system is equipped to predict surface defects such as potholes and bumps, and warning signs should be placed for rough areas.

Driving techniques such as determining the driving speed that minimizes the vibration of the vehicle, avoiding pits and bumps, and avoiding driving for a long time should be applied.

It must be ensured that the tires are inflated correctly, that no worn tires are used, and that proper wheel alignment is maintained.

Vehicle cabins should be isolated in a separate section from the chassis and engine, and vibration-isolating material should be placed on the floor inside the cabin.

Engine maintenance should be done regularly so that defective, worn parts can be repaired and vibration exposure can be reduced.

If engineering measures are insufficient, the duration and frequency of exposure to vibration should be reduced. Rotation should be provided among the employees, and the operators should take rest breaks at certain intervals.

1.6. Prevention, reduction, and limitation of hand-arm vibration exposure

New tools and equipment tend to generate less vibration than older models, so old tools and equipment should be replaced with new ones.

Tools with damping and insulating properties should be used with materials that absorb vibration energy.

Rotary power tools apply a rotational force to the hand, while the user applies counterforce to prevent it, increasing the level of vibration exposure. This effect can be reduced by using tools with the center of gravity close to or just below the hand.

Appropriate power and size tools and tools should be preferred, otherwise the work will take longer, and the user will be exposed to vibration for a longer time.

Appropriate and adequate maintenance programs should be applied to tools and machines. Inadequate maintenance can cause parts to loosen, misalign, wear, and become unstable, which can cause increased vibration.

Correct handling of vibrating tools must be ensured. The muscles are stretched due to the excessive force applied to grip the arm or handle, so the vibration is easily transmitted from bone to bone and from bone to muscle.

Exposure to vibration can be reduced by placing the tool more closely on the material being worked and holding it with minimal contact.

Workstations and tasks should be ergonomically designed so that workers do not strain and burden their hands, wrists, and arms.

Changes should be made to tools and equipment, such as increasing the mass of the vibrating field, installing vibration isolators, placing foam, pads or wraps on the handles.

Where engineering measures are insufficient, the duration and frequency of exposure to vibration should be reduced. Work programs should be planned to consider the radiated vibration level and rotation of employees should be ensured so that vibrating tools are not used continuously.

Employees using vibrating handpieces should be trained on vibration-related hazards, ways of protection from vibration, and the effects of vibration on human health.

1.7. Using anti-vibration gloves

Leather is generally used for the outer coating of the anti-vibration gloves, and viscoelastic material is used for the inner linings. Some types of gloves have multiple viscoelastic layers, and compressed air pockets are placed between the layers.

Anti-vibration gloves can provide the benefit of basically reducing vibration when exposed to high-frequency vibration. In most of these gloves, the reduction in vibration transmission at frequencies below 25 Hz on the palms and below 160 Hz on the fingers is very low.

Gloves can often become more effective by reducing the force exerted by the hand.

According to the test described in the ISO 10819:2013 standard applied to anti-vibration gloves; how much vibration is transferred from the glove to the palm of the user holding the vibrating handle was measured. In the standard, the glove's performance is analyzed only by vibration in the z-axis, but the vibration total value is the combination of vibration measured in the three axes. Since the effective mass in the palm is usually highest in the z-axis, reducing the glove's vibration is most effective in this direction, while the overall effectiveness of the glove can be estimated since tests are performed only in this direction.

Glove permeability values were also calculated in the test. If the transmittance value is 1.0, it means that all vibration is transmitted from the glove to the user, if it is lower than 1.0, the glove reduces the amount of transmitted vibration, if it is more than 1.0, the glove increases the vibration.

If the permeability criteria and material thickness requirements are met, the CE marking can be given to the gloves and the gloves can be put on the market.

The vibration permeability of the finger parts of the vibration protection gloves is much higher than the palm, which shows that the effect of reducing the vibration transmitted to the fingers is low.

If protective gloves against vibration are to be used, the gloves should not reduce the grip strength, should not alleviate the tactile sensation of the employee, provide protection against cold, provide freedom of movement so that hand tools can be easily grasped, and have a high level of protection against other hazards such as cuts, punctures, and tears. Thick gloves can increase the need for grip strength to hold and control the tool, thus causing rapid operator fatigue.

CONCLUSION

Anti-vibration gloves can reduce the vibration power and effects, especially when low force is applied and with high frequencies (≥500 Hz), but the protection of the gloves is negligibly low in low-frequency vibrations and during the protection of the fingers.

Therefore, priority should be given to engineering measures such as renewing and maintaining tools and equipment and using vibration dampers to reduce exposure to vibration. Administrative measures such as organizing work schedules and job rotation should be included in cases where engineering measures cannot be implemented or are insufficient. Vibration protective gloves should be used in conjunction with other measures in the risk prevention hierarchy mentioned above or should be used as a last resort when all these measures cannot be taken.

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CHAPTER 7

INCISIONAL HERNIAS

Metin LEBLEBICI[1]

1 MD, Associate Professor of Surgery, Department of General Surgery, Faculty of Medicine Istanbul Medeniyet University

A hernia is a protrusion, bulge, or projection of an organ or part of an organ through the body wall that normally contains it. Abdominal wall hernias are typically classified by location or etiology. Most abdominal wall hernias should be evaluated by a surgeon when identified. Abdominal hernia is the displacement of intra-abdominal organs or tissues under the skin through a defect in the abdominal wall. Abdominal wall hernias, which are caused by the disruption of the continuity of some or all of the abdominal wall in areas where surgical incisions have been made before, are called incisional hernias. Incisional hernias are secondary hernias and they are iatrogenic. Incisional hernias are a common and potentially serious complication of open abdominal surgery (1, 2, 3, 4). Incisional hernias are hernias that occur as a result of insufficient closure and inadequate healing of the fascia, or due to long-term high intra-abdominal pressure (5, 6, 7). 2-20% of all abdominal incisions result in the development of an incisional hernia (8, 9).

Two-thirds of incisional hernias develop within the first five years after the operation, and the remainder develop between the fifth and tenth years (10).

The first complaint described by patients diagnosed with incisional hernia is swelling originating from the abdominal wall and involving the scar tissue on the skin. With coughing and stretching, the complaints increase and the hernia contents come out through the defect in the wall. The development of ischemia or pressure necrosis in some large incisional hernias is called Frank ulceration. A history of recurrent colic abdominal pain and persistent nausea may be associated with incomplete bowel obstruction. It is easy to determine the boundaries of the hernia on physical examination. There may be more than one hernia along the incision scar line. In obese patients with suspected incisional hernia, computed tomography is the best method for visualizing the contents of the hernia sac (11).

Approximately 17% of incisional hernias present with incarceration, and the mortality associated with the repair of cases with such complications was found to be 3 times higher than in cases with elective repair (12, 13).

Materials used in the repair of hernia defects cause intra-abdominal adhesions and intestinal fistula formation as a result of contact with the visceral peritoneum. The structure and design of the prosthesis used in the repair plays an important role in adhesion formation (14, 15).

Obesity, wound infection, hematoma and seroma accumulation at the wound site, advanced age, male gender, postoperative pulmonary complications, emergency surgery, malignancy, postoperative chemotherapy and radiotherapy, steroid use, acid, peritoneal dialysis, old incision in incisional hernia development Many factors are effective in the etiology of scar reuse, malnutrition, diabetes

mellitus, smoking, inadequate incision closure technique and use of inappropriate suture material (16).

Local factors are more important than systemic factors in the formation of incisional hernia. The most important risk factor is wound infection. When considered alone, wound infection in the incision increases the risk of hernia development by 4 times (17, 18).

The chosen incision is also important in the formation of incisional hernia. As Maingot says, the basic principles of an incision are; accessibility, extensibility and reliability. Transverse incisions cause less incisional hernias than vertical incisions, although the operation time is longer and there is a greater bleeding tendency (11, 16). Midline incisions have the highest incidences of incisional hernias (3 to 20 percent). In a systematic review, the risk of incisional hernia was higher for midline incisions than transverse incisions (relative risk [RR] 1.77, 95% CI 1.09-2.87) and paramedian incisions (RR 3.41, 95% CI 1.02-11.45), respectively (16, 17).

Treatment options in incisional hernias

The treatment of incisional hernias is surgery. Incisional hernias can be managed expectantly or operatively, depending on the acuity of presentation and severity of symptoms. Incisional hernia repairs can be performed open or laparoscopically with techniques selected based on the location of the hernia. Anterior (mostly midline) incisional hernias are managed as other ventral hernias (ie, primary ventral hernias). Flank incisional hernias are managed as other flank or lumbar hernias (17). In surgical repair, there are basically two different methods of primary repair and the use of prosthetic materials (mesh). The method in which prosthetic materials are used is divided into two as open repair and laparoscopic repair. Nowadays, prosthetic materials are frequently used in hernia repairs. Undoubtedly, the preference for repair with mesh is that the recurrence of incisional hernias repaired with mesh is 25% less than primary repair (19).

The hernia is reached by an elliptical skin incision made just above the defect in the fascia to remove the scar tissue from the old incision. Adhesions between the hernia sac and the subcutaneous tissue and fascia edges are separated by both sharp and blunt dissections, and the intra-abdominal organs in the hernia are reduced into the abdomen. If this is not possible, the sac is usually opened and the excess is excised, resulting in reduction. The hernia sac is tried to be excised as little as possible. Because in cases where mesh will be used, direct contact of the abdominal organs with the mesh is prevented. The upper and lower surfaces of the intact fascia are exposed a few centimeters laterally, and the fascia is closed with primary or

mesh, without creating tension, with the preferred method. A drain can be placed to prevent seroma formation in the dead space on the fascia (7).

1) Primary repair methods:

In the primary repair of incisional hernias, it is a method in which the defect is completely closed by making the edges of the intact fascia face each other, with late-absorbable or preferably non-absorbable suture materials, with continuous suture technique or by knotting one by one. When it is preferred except for very small defects, the recurrence rate is reported to vary between 49-58%. The main reason for the recurrence rate is the weakened fascia and the inability of the fascia to hold the sutures with sufficient strength due to mechanical stress and tension (9, 20).

In order to reduce the fascial tension in the primary repair method, relaxing incisions can be made by incising the anterior rectus fascia bilaterally, as in the Keel procedure (21).

Mayo repair is described by closing the fascia in two overlapping layers. With this method, recurrence rates are 29-54% (22).

In the Shoelace method, which is another primary repair method, incisional hernia repair is performed anatomically by bringing the anterior sheath of both rectus muscles to the appropriate position with frequent and continuous sutures and creating a strong midline (23).

2) Mesh repair methods:

Because the recurrence rate in incisional hernia repair is generally between 0-15%, mesh repair is frequently used today (5, 24). Although the preperitoneal (sublay) area is frequently used as the place where the mesh will be placed, there is still debate about the location of the mesh (25). At the same time, there are discussions about the fixation method of the mesh to the fascia. Inlay placed meshes can migrate into the bladder or intestine (26).

Onlay, sublay, inlay and sandwich and cuffed mesh repair have been described as the place where the mesh is placed.

In onlay repair, the intact fascia is freed approximately 4 cm from the edge and the mesh is fixed on the fascia with individual sutures at least 2 cm away from the defect edge. The defect in the fascia is primarily repaired in a way that does not create tension, and a barrier is created between the mesh and the intra-abdominal organs. With this method, the recurrence rate is 8%, and wound site infection is 8%, chronic sinus is 12%, and seroma is 4% as other complications. The reason for recurrence is usually the separation of the mesh fixed to the fascia edge (27).

In the sublay method, the mesh is placed between the peritoneum and the posterior rectus fascia preperitoneally, and in the inlay method intraperitoneally (28). In the study of Trupka et al., sublay repair was performed in 33 cases with incisional hernia and no recurrence was observed in their 9-month follow-up (29). If the mesh is placed intraperitoneally, if there is no barrier or tissue between the mesh and the intra-abdominal organs, postoperative adhesions and intestinal erosion and subsequently enterocutaneous fistula may develop (26).

In the repair method with sandwich and cuffed mesh, both onlay and inlay or sublay techniques are used together. Condon described the repair performed by applying an onlay-style polypropylene mesh on PTFE (polytetrafluoroethylene) placed in an inlay manner and passing matress sutures from both layers (30). After fixing two pieces of mesh in the form of cuffs on the anterior and posterior surfaces of the fascia, Rubio joined the two pieces in the midline (31). In addition to the technical difficulties of sandwich and cuff mesh application, there are disadvantages such as fluid accumulation between two layers and preparing the ground for infection. The disadvantages of using two-piece mesh have been tried to be reduced by developing the modified cuff method. In the modified cuff technique, one piece of mesh is folded 2 centimeters above and below the edge of a fascia, and the mesh is sutured to the defect edge (31).

In recent years, surgeons have preferred laparoscopic mesh repair more frequently in mesh repair because of less recurrence and lower wound infection rates. With the laparoscopic, that is, minimally invasive surgical approach, less dissection is performed compared to the open mesh repair. In the laparoscopic approach, the hernia sac is left in place and attached to the abdominal wall with a mesh hernia stapler, usually with a transabdominal approach. Although the recurrence rate is 4,2%, it is an effective treatment method in the treatment of incisional hernia today (32, 33). The most important complication of the laparoscopic method is the adhesion of the intraperitoneally placed mesh to the intra-abdominal organs and this mesh leads to intestinal erosion and fistula (34-36).

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CHAPTER 8

BILIARY CANDIDOMA MIMICKING CHOLANGIOCELLULAR CARCINOMA COEXISTING WITH LUNG CANCER

Murat SEVMIS[1]

Elbrus ZARBALIYEV[2]

1 Department of General Surgery, Gaziosmanpaşa Hospital, Yeni Yüzyil University, İstanbul, Turkey
orcid.org/0000-0003-0512-6027

2 Department of General Surgery, Gaziosmanpaşa Hospital, Yeni Yüzyil University, İstanbul, Turkey
orcid.org/0000-0002-4595-8314

Introduction

Acute cholangitis is a clinical syndrome characterized by fever, jaundice and abdominal pain and develops as a result of stasis of the biliary tract accompanied by infection. Fungal cholangitis, especially Candida cholangitis can occasionally be seen in malignant diseases and immunosuppression (1). Candida species can reach the biliary tract through ascending, hematogenous routes (fungemia, sepsis) as a result of invasive procedures (ERCP /percutaneous transhepatic cholangiography (PTC)) (1, 2). In this case report, we present a 60-year-old male patient, applied to our outpatient clinic with symptoms of cholangitis. He had a radiological suspicion of a cholangiocellular carcinoma (Klastkin tumor) and was diagnosed with an incidental lung adenocarcinoma. After receiving chemotherapy histopathological interpretation of the right hepatectomy specimen of the patient revealed fungal cholangitis.

Case Report

A 60 year old male patient applied to our general surgery outpatient clinic with fever, jaundice, nausea and vomiting for 20 days. Serum total bilirubin level was 4.5 mg / dl, direct bilirubin: 3.6 mg / dl, ALT: 123 U / L, AST: 102 U / L of ALP: 160 U / L and GGT: 1067 IU / L. Abdominal USG and abdominal MRI revealed a soft-density mass compatible with a cholangiocellular carcinoma (type IV Klatskin tumor), which initiated from the cystic duct in the proximal common bile duct and extended to both hepatic ducts (Figure 1a). The patient's tumor markers were normal. In addition, several masses with irregular borders with diameters of 6.5 mm, the largest of which was located peripherally in the middle lobe of the right lung, were observed. Those masses were thought to be metastatic in the foreground (Figure 1b). The result of the brush cytology of the patient with internal choledochal stent was not diagnostic. PET-CT SUV-Max value of the mass localized in the lobe of the right lung was detected to be 3.3 and SUV-Max value of the mass localized in the proximal common bile duct and perihepatic lymph nodes was 7.7. (Figure1c). The mass in the right lung was treated by a thoracoscopic wedge resection. Histopathological interpretation revealed a T1N0 primary lung adenocarcinoma. Considering a possible cholangiocellular carcinoma, together with the lung carcinoma, the patient received 12 cycles of Gemcitabine + Splatin treatment. No significant activity uptake was observed in the right lung and proximal common bile duct in the PET-CT taken after the treatment. Control abdominal MRI showed persistence of the mass localized in the proximal common bile duct (Figure 1d). Approximately 1 year after the his admission, with the decision of the oncology

council the patient who could not get a tissue diagnosis underwent a right hepatic lobectomy together with a choledochus resection following selective right portal vein embolization and pericaval lymph node dissection with the pre-diagnosis of a cholangiocellular carcinoma. Fungal cholangitis (candidoma) was detected in the patient whose pathological examination did not reveal any malignancy. (Figure 2). A wig drainage catheter was inserted into the patient who developed a right subdiaphragmatic abscess after surgery. There was no Candida growth in the preoperative and postoperative blood cultures. Candida growth was observed in the abscess drainage culture of the patient. Intravenous Fluconazole 1x400 mg treatment was initiated. The patient who was positive for Candida for up to 3 weeks in percutaneous drainage catheter culture continued Fluconasole treatment for 3 months, and then the catheter was removed. No problem was observed in the patient's first year controls.

Discussion:

Although bacteria are the most common agents in sclerosing cholangitis cases, fungi are being more prevalent and are associated with a poor prognosis. It has been reported that the frequency of fungal pathogens in bile fluid varies between 15% and 50% depending on the underlying disease (3-5). In a study conducted by Lenz et al., the authors stated that advanced age at the time of diagnosis and therapeutic endoscopic interventions were independent risk factors for biliary candidiasis. (6). Diagnostic ERCP does not increase the risk. (1). In a multivariate cohort study, biliary candidiasis was independently associated with decreased survival in the presence of dominant biliary stenosis (<1.5 mm for common hepatic duct; <1 mm for right and left hepatic duct) (6). Unlike transient biliary candidiasis, persistent biliary candidiasis has been shown to increase the incidence of cholangiocellular carcinoma with significantly higher GGT levels. A similar GGT elevation was observed in our case. Especially in the presence of high grade biliary stenosis or tumor stenosis, the probability of reproduction of Candida species increases. In the present case, stenosis due to the mass image caused by candidiasis (Candidoma) in the proximal common bile duct raised the suspicion of a cholangiocellular carcinoma. Development of a candidioma as a result of candidiasis, has previously been reported in the esophagus in immunosuppressive patients (7). Our case is the first example in the literature of the development of biliary candidoma. It is recommended to take a bile culture in cases with recurrent candidiasis together with blood culture sampling, due to the possibility of candidemia (2). In our case, although Candida growth was observed in the drainage culture, there was no candidemia. Antifungal

agents such as fluconazole, caspofungin, voriconazole and amphotericin B are used in the treatment of fungal cholangitis (2, 5). It has been demonstrated that antifungal therapy was effective on candidoma as well as on candidemia (5, 7). In the present case, we applied 1 x 400 mg Fluconazole for 3 months. No Candida growth was detected in any blood or drainage culture starting as of the 3rd week postoperatively. In another study, no difference was observed in the survival rates of transient biliary candidiasis patients; Survival has been found to be significantly reduced in patients with persistent biliary candidiasis (8). Our patient also had transient biliary candidiasis, and no morbidity or mortality was experienced in surgical follow-ups after PTC and ERCP.

Conclusion:

We recommend biliary aspiration and microbial analysis including fungal species, during PTC and ERCP procedures performed for biliary stenosis especially in immunosuppressive patients. The necessity of persistent biopsy for biliary pathologies was obvious in the present case as the candidoma developing after fungal cholangitis demonstrated findings mimicking cholangiocellular carcinoma in CT, MRI, and PET-CT.

Author Statement

All authors meet the ICMJE authorship criteria.

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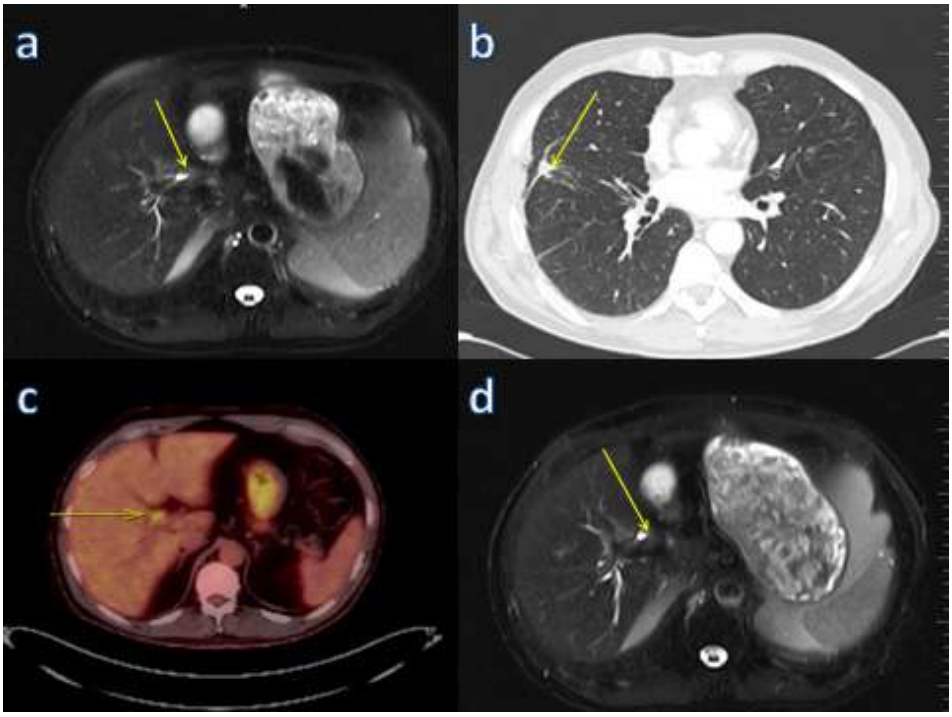

Figure 1. **a)** Mass image in proximal common bile duct (Abdomen MRG), **b)** peripherally localized irregularly circumscribed mass in the right lobe of right lung (CT scan), **c)** PET-CT image of the mass localized in the proximal common bile duct, **d)** Persistent mass in the proximal common bile duct after chemotherapy (Abdomen MRG)

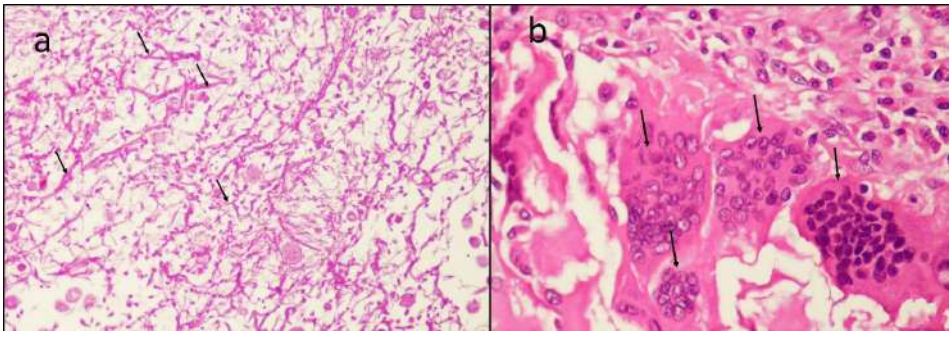

Figure 2: **a)** fungal hyphae and spores in the lumen (arrows) (PAS stain, 20X), **b)** chronic inflammation rich in multinucleated histiyocytic giant cells (arrows) (H&E, 40X).

CHAPTER 9

THE EFFECTS OF NOISE ON BIOLOGICAL SYSTEMS AND FETAL DEVELOPMENT

Mustafa YILDIZ[1]

[1]Canakkale Onsekiz Mart University, Can School of Applied Sciences, Department of Occupational Health and Safety, Can/Canakkale, Turkey.
e-mail: mustafayildiz@comu.edu.tr; mustafayildiz17@yahoo.com

1. Noise

Noise is defined as unwanted sounds that have negative effects on living beings. With the development of technology, increasing noise pollution has become an environmental issue, leading to physical and psychological problems in human life. Major sources of noise can be expressed as office work, recreation events, motor vehicles, construction machinery, aircraft, and appliances (1, 2).

The unit of measurement that indicates sound intensity and is used to measure noise is the decibel (dB). The ear perceives sounds between 0-140 dB (2). In the Noise Regulation, published in the official newspaper dated 23.12.2003 and numbered 25325, it has been expressed that the weekly noise exposure level determined by adequate measurement will not exceed the exposure limit value of 87 dB (A) (3). Sounds exceeding 90 dB are harmful and may cause hearing loss (1). Also, the duration of the noise exposure is important. With noise at 140 dB level, effects such as pain and rupture of the eardrum may occur. This leads to permanent damage in the ear (2).

It is a matter of curiosity what impact the noise-exposed in many activities in daily life can have on human health. In this review, the effects of noise on the systems of the body and fetal development were focused and its possible negative effects were comprehensively evaluated.

2. Effects of Noise on Nervous System

In the studies related to the nervous system, it has been found that noise exposure increased levels of norepinephrine, epinephrine, dopamine, serotonin, heat shock protein 70, and acetylcholinesterase in the brain (4-7), while reducing the level of acetylcholine (6, 8). Also, it was revealed that hippocampal serotonin and dopamine levels decreased in rats exposed to noise compared to the control (9). However, it was determined that while the levels of superoxide dismutase (SOD) and lipid peroxide (LPO) increased (10-14); the levels of catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx) decreased in the brain of noise-exposed rats (10, 11, 13). Thus, it has been stated that noise triggered oxidative stress in the brain.

In the examinations conducted as histomorphological and histopathological has been found that noise exposure reduced brain and hippocampus volumes, medial prefrontal cortex area, cortical thickness, amygdala area, and neural density in the medial prefrontal cortex and dentate gyrus (15, 16). It has also been observed that noise increased the number of pyknotic cells (17), reduced neuron cell sizes, and disrupted histological structure in the brain (10). In addition, it has been reported that Nissl bodies decreased and cytoplasmic vacuolization was observed in cells of the hippocampus and medial prefrontal cortex with the effect of noise (12).

When studying the effects of noise on brain function, it has been determined that it caused memory dysfunction (17), altered cognition functions (18), as well as adversely affected balance and motor coordination (15). It has also been stated that noise exposure caused anxiety and depression-like behaviors (19) and impaired spatial learning ability (20).

3. Effects of Noise on Cardiovascular System

In the studies conducted on the cardiovascular system, it has been reported that noise exposure increased blood pressure and heart rate (21-24), as well as triggered ischemic heart disease and acute myocardial infarction (25, 26). In addition, it was indicated that noise impaired endothelial functions and may induce stroke and coronary artery disease (27, 28). Also, it has been found that noise stress increased the levels of plasma corticosterone, adrenaline, noradrenaline, endothelin-1 and malondialdehyde (MDA) while reducing the level of SOD. Based on these findings, it has been stated that noise stress may have many negative effects on the cardiovascular system by increasing the plasma levels of stress hormones, oxidative stress, and endothelial dysfunction (29).

In the examinations conducted as histomorphological and histopathological, it has been determined that noise exposure increased heart volume (30), led to degeneration in myocardial muscle fibers, reduced the diameter of muscle fibers, and caused collagen increase between muscle fibers (31, 32). Nevertheless, it was observed that noise increased the amounts of mitochondria and myofibril in heart tissue, caused chromatin condensation and nuclear envelope changes (30). It was also reported that noise stress increased heart weight, left ventricular wall thickness, number of microvessels, and ischemic myocardial lesions in spontaneous hypertensive rats. In light of these results, it has been stated that hypertensive heart disease in spontaneous hypertensive rats may be aggravated by noise stress (33).

4. Effects of Noise on Digestive System

In the studies related to the digestive system, it has been found that noise exposure increased the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in serum (34, 35) while reducing the level of GSH in the liver (36). It also increased glycogen, triglyceride, and MDA levels in liver tissue (37). However, it has been reported that noise reduced liver weight and liver/body weight ratio (38).

In the examinations conducted as histopathological, it has been observed that noise stress increased the amount of collagen in the liver (39), caused the storage of lipid droplets in hepatocytes, as well as induced the formations of apoptosis

and superoxide in hepatocytes (34). Also, it has been noticed that noise caused irregularity, vacuolization, cellular swelling, and karyolysis in remark chords of the liver (35). Besides, it has been determined that noise exposure increased Bax expression and the number of pyknotic cells in the liver, as well as triggered degeneration, sinusoidal dilation, lymphocyte infiltration, and necrosis formation in hepatocytes (35, 40). On the other hand, it was found that noise increased the level of MDA in the stomach while reducing the level of SOD. It has also been observed that noise stress raised the damage score by leading to gland irregularity, inflammatory cell infiltration, and interstitial edema in the stomach (41). In the small intestine, an increased number of degranulated mast cells and eosinophils, dilation of lamina propria and lymph vessels, and spillage of epithelial cells have been observed with the effect of noise (42).

5. Effects of Noise on Urinary System

In the studies conducted on the urinary system, it has been found that exposure to noise increased levels of serum creatinine (43), urea (44), albumin, and uric acid (45). Also, it was reported that noise decreased the level of GSH in kidney tissue while increasing the level of MDA (44). In examinations conducted as histopathological, it has been observed that noise stress led to tubular degeneration, tubular and glomerular vacuolization (46), tubular brush border loss, glomerular disorganization, and glomerular congestion in kidney tissue (45). Besides, thickening in artery walls (43), dilation in tubules, swelling in tubular cells, and increased collagen amount in the kidney have been determined due to noise (44).

6. Effects of Noise on Respiratory System

In the studies related to the respiratory system, it has been found that exposure to noise increased the level of MDA and reduced the level of GSH in the lung. It has also been noticed that noise caused inflammatory cell infiltration, thickening in alveolar cell walls, and hypertrophy in type II epithelial cells in the lung (47). In addition, it was reported that alveolar obliteration, thickening in pulmonary vessel walls, pyknosis in interstitial cells, collagen accumulation, and the existence of apoptotic cells in the interstitial area of the lung were observed with the effect of noise stress (31).

7. Effects of Noise on Male Reproductive System

In the studies conducted on the male reproductive system, it has been determined that noise stress elevated levels of serum adrenocorticotropic hormone (ACTH), cortisol (M24), follicle-stimulating hormone (FSH), and luteinizing hormone

(LH), but decreased testosterone level in rats (48-50). Also, it was reported that noise exposure stimulated the release of the hormone melatonin from the epiphysis gland, and melatonin causes reproductive dysfunction by leading to gonadotropin suppression, too (51). In contrast, some studies have demonstrated reduced levels of serum FSH and LH (52-55) and increased levels of testosterone in rats exposed to noise stress (56).

It has been determined that noise stress decreased testis, seminal vesicle, and ventral prostate weights in rats (57-59). It was also found that noise reduced sperm count, sperm vitality, and sperm motility (52, 53, 59, 60) while increasing abnormal sperm count (58). In addition, it was reported that noise exposure decreased the diameter of the seminiferous tubule (60) and the germinal epithelial thickness, while the ratio of the interstitial tissue area to the total testicular tissue increased in the testis (50).

The degradation of inter-tubular areas, increase in connective tissue, atrophy in tubules, (50, 60), germ cell loss (53), and vacuolization (50) have been observed in the testes of the noise-exposed group in researches conducted as histopathological. Also, maturation arrest in germinal layers and breakage in the basal membrane in some tubules of testis have been determined (61). Besides, hyperchromatic nuclei and vacuoles in the cytoplasm of germ cells were noticed (62). Nevertheless, irregularity, deterioration, and separation between the Sertoli and germinal cells were observed in the noise group (57). It was also reported that the testicular capsule was thick and irregular, as well as the blood vessels in the capsule were blocked and dilated in the noise group. In addition, dissipated Leydig cells and wide gaps between seminiferous tubules have been seen in the noise group (63). Besides, it was found that noise stress increased the apoptotic process in the testis (57).

In studies conducted ultrastructurally, it has been determined that there were large amounts of lipofuscin in Leydig cells of testis in the noise group (64). It was also noticed that Leydig cells in the noise group had a thick nuclear membrane and their cytoplasms were inadequate and dense. Besides, irregular granular endoplasmic reticulum and degenerated mitochondria were present in their cytoplasms (51). Furthermore, it has been observed that there were gaps between the seminiferous tubule cells and basal lamina of seminiferous tubules, disfigurement in germ cells, and fragmentation in some nuclear membranes in the testis of noise group (63). In the prostate gland of the noise group, it has been determined that the boundaries of epithelial cells were indistinguishable, the epithelial cell nucleus had an irregular nuclear membrane, as well as degeneration was observed in the granular endoplasmic reticulum (51).

8. Effects of Noise on Female Reproductive System

In the studies related to the female reproductive system, it has been found that ovary weight, follicle count, and granulosa cell layer decreased, in contrast the theca cell layer increased in animals of the noise group (65). In addition, it was noticed that the rats in the noise group had low gonadal somatic index values. Furthermore, the spills and separations in the germinal epithelium (66), degeneration in primordial and primary follicles (65), as well as edema and congestion in the medulla were observed in the ovary of animals in the noise group histopathologically. On the other hand, the reduction in CAT, SOD, and GSH levels and an increase in LPO level have been determined in rat ovarian tissue of the noise group. Thus, it has been concluded that noise may cause oxidative stress and tissue damage in the ovary (66).

9. Effects of Noise on Fetal Development

In studies on women's health and fetal development, it has been reported that the incidences of the irregular menstrual cycle, dysmenorrhea, lumbago, pregnancy hypertension, the threat of miscarriage, preterm birth, dystocia (67), and low birth weight increased in women exposed to noise (67, 68). Also, it has been found that noise shortened gestational duration (69), raised mother and baby's cortisol levels, and led to restriction in fetal growth. On the contrary, it has been stated that noise cannot be associated with premature birth (70).

It has been found that the fetus weight was lower (48, 49, 71, 72) but the numbers of malformed, dead, and absorbed fetuses were higher in the noise group (54, 71, 73-75). On the other hand, it has been reported that noise might induce hearing loss by causing changes in fetal auditory brainstem response (76). In addition, it has been observed that the brain weight (77), the number of neurons, and the size of neuronal nuclear area decreased but the number of glia cells increased in the group exposed to noise during the prenatal period (78). Thus, it has been stated that these findings may be an indicator of developmental retardation following noise exposure (77). Also, it has been found that damage was seen in the inner and outer cilium cells of the cochlea in noise-exposed fetuses (79).

In a conclusion, the negative effects of noise on body systems and fetal development have been revealed through many studies. Therefore, pregnant women should not be employed in noisy environments so that development and body functions are not adversely affected. Also, the level of noise exposure in the working environment should be determined. Exposure duration should be reduced, and personal protective equipment should be used in noisy environments.

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CHAPTER 10

THE EFFECTS OF KETOGENIC DIET ON GUT MICROBIOTA

Mehmet BERKOZ[1]

Oruc YUNUSOGLU[2]

1 Assist. Prof. Dr. Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Biochemistry, Van, Turkey.
e-mail: mehmet_berkoz@yahoo.com, ORCID: 0000-0003-4219-8054

2 Assist. Prof. Dr. Bolu Abant İzzet Baysal University, Faculty of Medicine, Department of Medical Pharmacology, Bolu, Turkey.
e-mail: orucfarm@yahoo.com, ORCID: 0000-0003-1075-9574

Introduction to Ketogenic Diet

The ketogenic diet is a nutritional approach characterized by high-fat, adequate protein and very low levels of carbohydrates, which can mimic the metabolism found in the fasting state (1). As a matter of fact, the record of fasting used to treat epilepsy can be dated back to the Hippocratic era, and the use of fasting in seizures treatment was also documented in Biblical times (2). Over the past three decades, the ketogenic diets have experienced a reemergence and an explosion in clinical utility as well as research interest. A large number of studies have used ketogenic diets as therapeutic tools in the treatment of various common and rare diseases, such as neurological diseases, astrocytomas, hyperlipidemia, polycystic ovary syndrome, Type 2 Diabetes, and even certain kinds of cancer (1, 3, 4). Moreover, ketogenic diets have recently become quite popular in western countries because of their effective weight-loss effects (5).

The initial version of ketogenic diet was composed of 1 g per kilogram of body weight of protein, 10-15g/day of carbohydrates, and the rest of energy intake in fat (6). In order to make ketogenic diet more palatable, several modified ketogenic diet versions have been established, such as the Atkins Diet, the Modified Atkins Diet, low-calorie-ketogenic diets, or diets lowering the fat to non-fat ratio. However, the most commonly used ketogenic diet has a 4:1 or 3:1 of fat to protein ratio in combination with reduced carbohydrates (usually below 50 g/day), thus approximately 70% of daily energy intake is derived from fat (1, 7). Ketogenic diets with lower fat to protein ratios were also successfully used in Asian countries, where rice is the staple food (8).

After a few days of ketogenic diets, in which carbohydrate consumption is drastically decreased, the body is compelled to find substitute energy source. Thus, a shift in metabolism that resembling to fasting state is seen, that is, the metabolic pathway of obtaining energy is changed from glycolysis to lipolysis (7). This alteration leads to the overproduction of ketone bodies in the liver, which become the major energy source when short in carbohydrate supplyment (9). Higher-than-normal levels of ketone bodies are released to circulating blood and result in ketonemia and ketonuria. This process resulted from ketogenic diet is called ketogenesis (1).

Gut Microbiota and Host Physiology

A huge number of microorganisms comprising of bacteria, archaea, fungi, viruses, and unicellular eukaryotes are inhabited in the human body (10). The collection of microorganisms that lives in a particular environment, such as the gastrointestinal tract, is termed a microbiome. The human intestine, especially the colon, is a preferred

site for microbial colonization due to its rich nutrient environment. It is estimated that there are more than 100 trillion microbes live in the intestine, approximately 10 times the total number of human cells, and the unique genes encoded by the gut microbiota are 150-fold larger than our own genome (11). At present, a large fraction of bacterial species cannot be cultured, but owing to the addition of modern molecular approaches, such as 16S ribosomal RNA (16S rRNA) gene sequencing, metagenomics sequencing and gene chips, the microbial population can be identified and classified. Although gut microbial species vary widely between individuals at lower taxonomic level, they can be predominantly classified into 9 phyla and one archaea, namely *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Spirochaeates*, *VadinBE97* and *Meitianobrevibacter smithii*, among which *Bacteroidetes* and *Firmicutes* account for 90% of the total gut microbial population (11-13).

Recent findings indicate that the gut microbiota has complex interactions with the host and acts as a virtual organ with many essential metabolic, nutritional, immunologic and endocrine-like effects that affect human health (14, 15). The effect of gut microbiota on food digestion and nutrition uptake is largely dependent on its ability to ferment and degrade indigestible food components through a variety of hydrolytic enzymes that cannot be synthesized by the host (16, 17). The products of microbial fermentation activity, short-chain fatty acids (SCFAs), bile acids and choline, are reported to have complex metabolic interactions with the host (18, 19). For example, SCFAs can bind to G protein-coupled receptors (GPRs) and activate subsequent signaling pathways that affect insulin sensitivity, appetite and gut motility, thereby regulating host energy harvesting and fat storage (20, 21). In addition to supporting the food digestion and energy metabolism, gut microbiota is also essential for maintaining normal immune function. It can shape and strengthen the intestinal mucosal barrier to prevent epithelial cell surface from directly contacting the pathogenic bacteria, assist in immune cell differentiation and immune tissue maturation, and activate intestinal adaptive immune responses (22). Collectively, gut microbiota has a fundamental impact on host nutrition uptake, energy storage, metabolism, and systemic immunity, and maintaining host-microbiome homeostasis is critical to the overall health of the host.

Role of Gut Microbiota in Obesity and Metabolic Diseases

In 2004, Bäckhed et al. (23) pioneered the study of gut microbiota as an environmental factor regulating fat storage and obesity. They observed that although conventionally raised mice consumed 29% less food than germ-free mice, they had a 42% higher body fat content. After transplanting the microbiota harvested from

the cecum of conventional mice into germ-free mice, the total body fat of germ- free mice increased by 66% in two weeks despite reduced chow consumption. And such increment was accompanied by increases in serum glucose and leptin, adipocyte hypertrophy, and decreases in insulin sensitivity (23). These findings indicate that the gut microbiota has the ability to increase dietary energy harvest, resulting in rapid weight gain and body fat accumulation. Gut microbial transplantation experiments further showed that this trait can be transmitted through colonization (24, 25). When the fecal microbiota obtained from human twin pairs discordant for obesity was transferred into germ-free mice, mice received microbiota from the obese twin showed metabolic changes and more weight gain compared to the mice received microbiota from the lean twin (25). In the follow-up experiment, the researchers cohoused the obese and lean mice. Surprisingly, the increment of body mass and total fat mass in obese mice was prevented, and the metabolic profile of the microbiota was also corrected (25). These breakthrough studies support a causative role of gut microbiota in host energy storage, metabolic homeostasis, and the development of obesity and associated metabolic disorders. More importantly, the gut microbial community is transmissible.

The efficiency of gut microbiota in regulating host metabolism is due in part to its ability to extract additional energy from indigestible dietary polysaccharides (24, 25). The degradation products, monosaccharide and SCFAs, are absorbed by the small intestine and promote glycogenesis and lipogenesis in the liver, which further accelerate fat accumulation in the host. Moreover, mechanistic studies have revealed that the reason for germ-free mice being more resistant to diet-induced obesity is involved with elevated muscle and liver levels of fasting-induced adipose factor (Fiaf) and phosphorylated AMP-activated protein kinase (AMPK), the expression of which are inhibited by gut microbiota in the conventionally housed mice (23, 26). Fiaf is a circulating inhibitor of lipoprotein lipase (a cell lineage limits the rate of triglyceride- derived fatty acids uptake). By inhibiting the expression of Fiaf, gut microbiota can increase the activity of lipoprotein lipase in adipocytes, and thus enhancing fatty acids uptake and triglyceride storage in host adipocytes (26). In addition, AMPK can function as a *fuel gauge* to monitor the cellular energy status. Activation of AMPK can facilitate the ATP-generating catabolic pathways and increase fatty acid metabolism. The gut microbiota has also been shown to inhibit fatty acid oxidation in skeletal muscle via phosphorylated AMPK metabolic pathways (26). Besides serving as an important energy source for the host, SCFAs also act as signaling molecules that regulate energy harvest and metabolism. SCFAs are ligands for GPR41 and GPR43 which can activate subsequent signaling pathways of free fatty acid receptor 2 (FFAR2) and free

fatty acid receptor 3 (FFAR3) (20, 21). It has been shown that FFAR2 activation (mainly activated by acetate) can modulate energy balance by reducing insulin sensitivity and fat storage in adipose tissue, and increasing insulin sensitivity in liver and muscle (27). In contrast, FFAR3 activation can stimulate the secretion of satiety hormones, such as leptin and peptide YY, resulting in decreased appetite and reduced diet energy harvest (21, 28). As summarized in review articles (15, 29), the gut microbiota can functionally affect the host's physiology by releasing numerous metabolites that can interact directly or indirectly with other endocrine hormones, or by stimulating the parasympathetic nervous system, thereby activating the corresponding metabolic processes.

A state of gut bacterial dysbiosis is related to a series of physiological disorders, including excessive fat accumulation, low-grade inflammation, impaired insulin action, and metabolic disorders, further aggravating the development of cardiometabolic diseases (15, 30). Compared to people with high gut bacterial richness, individuals with low gut bacterial richness are characterized by more marked overall adiposity, dyslipidemia, insulin resistance and a more pronounced inflammatory phenotype (31). It has been revealed that the genetically obese mice have a higher relative abundance of *Firmicutes* and a lower relative abundance of *Bacteroidetes* than their lean siblings (24, 32). This characteristic of gut bacterial division is also found in obese and lean human volunteers (24). Comparing the composition of gut microbiota between individuals with and without Type 2 Diabetes indicates that the diabetic individuals have a moderate degree of gut bacterial dysbiosis, a reduced abundance of butyrate-producing bacteria species, but opportunistic bacterial species relating to sulphate reduction and oxidative stress resistance are enriched in Type 2 Diabetes patients (33). Specifically, the proportion of *Firmicutes* phylum and *Clostridia* class is significantly reduced, whereas the *Betaproteobacteria* class is highly increased in the Type 2 Diabetes patients compared to the non-diabetic subjects (34). As a matter of fact, metabolic diseases, including obesity, Type 2 Diabetes, and cardiovascular disease are accompanied by a low-grade chronic inflammation state caused by metabolic cells responding to excess nutrients and energy (35, 36). A high-fat diet can increase the lipopolysaccharide-producing bacteria in the gut, thus increasing the plasma concentration of lipopolysaccharide. High levels of plasma lipopolysaccharide are triggers of low-grade inflammation that contribute to the progression of obesity and Type 2 Diabetes (37). Exogenous infusion of lipopolysaccharide has been shown to increase inflammation markers, and triggers insulin resistance and weight gain in mice (37). It has been shown that altering gut microbiota through antibiotic treatment can significantly reduce metabolic endotoxemia in high-fat fed and ob/

ob mice, and this effect is associated with improved glucose tolerance, reduced body fat and fat mass, and lowered inflammation and oxidative stress (38). These findings suggest that both compositional and functional changes in gut microbiota are responsible for the onset of metabolic diseases.

Due to differences in demographic characteristic of the studied population and discrepancies in DNA extraction and gene sequencing techniques, individual studies have reported inconsistent findings regarding the specific disease-promoting bacterial species that have been changed. Even so, the associations between gut microbiota and the presence and progression of obesity and metabolic diseases are undeniably strong. However, the role of specific microbiomes and their derived metabolites that are directly related to the pathology of specific diseases has not been fully revealed, and how external factors affect the composition and function of gut microbiota remains largely unexplored. In order to develop strategies to prevent or treat metabolic diseases by manipulating the gut microbiota, future studies are needed to identify and characterize gut microbial characteristics and biomarkers associated with specific metabolic symptoms, and to discover the cellular and molecular mechanisms involving in the microbiota-host interaction.

The Effect of Ketogenic Diet on Gut Microbiota

Alteration in diet is the most rapid and direct way to change the gut microbial composition and gut microbiome activity (39). It has been reported that approximately 60% of the structural variation in gut microbial community is attributed to diet perturbations, whereas genetic mutation only accounts for no more than 12% (40, 41). The diversity of diet is closely associated with gut microbial diversity, individuals with similar diet pattern are tended to have a similar functional composition of gut microbiome, constituting the core microbiome of the population. According to the predominant resident bacteria species, the human gut microbial communities can be clustered into three main enterotypes, namely the *Bacteroides* enterotype, the *Prevotella* enterotype, and the *Ruminococcus* enterotype (42-44).

Increasely more studies demonstrate that altering diet can shape the gut microbial ecosystem. In animal studies, changing from a low-fat, high-fiber diet to a high-fat, high-sugar diet altered the gut microbial structure, microbial gene expression and representative metabolic pathways in mice within a single day (45). Similarly, switching from a standard chow diet to a high-fat diet induced significant weight gain in wild-type mice and accompanied with decreased *Bacteroidetes*, and increased *Firmicutes* and *Proteobacteria* (46). In order to examine whether shifts in gut microbiota are reversible in accordance with dietary alteration, Zhang and

colleagues induced the adult mice into insulin-resistant obese mice using high-fat diets, and then switching back to normal chow feeding, they found that the gut microbial structure can rapidly move back to its original state upon switching back to normal diet, and the corresponding metabolic disorders induced by high-fat diet were also significantly alleviated, suggesting that the gut microbiota is resilience to diet alterations (47). Another study pointed out that the diet-induced alteration in gut microbiota could be largely reversed within a single generation, nevertheless, the diet-induced reduction in gut microbial diversity and extinct bacterial species across generations could not be restored even if reintroduced to the original diet. Administration of the disappeared taxa and reintroduction of dietary were required to recover the microbiome to its initial condition (48). Apart from alteration in dietary macronutrients, caloric load is another major factor affecting the structure of gut microbiota. It has been reported that diets varying in nutrient load (2400 vs. 3400 kcal/d) caused rapid changes in human gut microbiome within three days, and such changes were associated with the energy harvest of lean subjects; every ~150 kcal increase in nutrient absorption was associated with a 20% increase in *Firmicutes* and a 20% decrease in *Bacteroidetes* (49). Comparing the fecal microbiota of European children with a modern western diet to that of children from a rural African village with a fiber-rich diet has revealed distinct gut microbiota variation between the two populations (50). Besides a significant enrichment in *Bacteroidetes* and reduction in *Firmicutes*, the African children possessed a unique gut microbial genus of *Prevotella* and *Xylanibacter* which contained gene sets for encoding cellulose and xylan hydrolytic enzymes, however, totally lacking in the European children. In addition, there were more SCFAs and lower abundance of *Enterobacteriaceae* (*Shigella* and *Escherichia*) in African children than in European children, thus enabling them to maximize energy intake from a fiber-rich diet and protecting them from inflammations and noninfectious colonic diseases (50). In a dietaty intervention study, the researchers altered subjects' macronitrient intake by providing them with diets composed completely of plant or animal products. Within five days, the subjects showed a diet- specific shift in microbial community structure and microbial metabolic activity (39). The abundance of 22 bacterial clusters were changed in subjects consumed animal- based diet, but only 3 clusters were changed in subjects consumed plant-based diet. Specifically, enrichment of bile-tolerant microbiome (*Alistipes*, *Bilophila* and *Bacteroides*) and decrement of *Firmicutes* processing dietary fiber (*Roseburia*, *Eubacterium rectale* and *Ruminococcus bromii*) were observed in response to animal- based diet (39). These findings demonstrate that both composition and function of gut microbiome can be rapidly altered in adapting to a specific diet. Therefore, dietary intervention

maybe a promising and easy way to modulate individuals' gut microbiota and thus to improve health.

Modified versions of ketogenic diet are becoming increasingly popular in recent years for their beneficial effects on weight control and metabolic health, though the precise underlying mechanisms are not fully understood. Given the effectiveness of ketogenic diet in obesity and associated co-morbidities, and the interrelationship between gut microbiota and the host metabolic health, it is reasonable to assume that the gut microbiota may be a potential mediator of the ketogenic diet-induced modification in obesity and correlated morbidities (1, 5).

A recent study to examine whether different dietary fat contents change gut microbiota and cometabolites conducted a randomised feeding-control trial, 217 healthy adults were provided with three isocaloric diets differing in fat content for 6 months. The proportions of fat, carbohydrate and protein in the three diet groups were 20: 66: 14%, 30: 56: 14% and 40: 46: 14%, respectively. The researchers found that with the increment of dietary fat content, the abundance of *Bacteroides* and *Alistipes* which associated with Type 2 Diabetes were enriched, health-promoting metabolities (butyrate acid and SCFAs) were reduced, whilst the adverse cometabolites including arachidonic acid, lipopolysaccharide and plasma pro-inflammatory biomarkers were elevated. In contrast, in lower-fat diet group, the α-diversity and beneficial bacteria species were increased, and co-metabolites associated with metabolic disorder were decreased (51). Although the proportion of fat content in this study is lower than that of a ketogenic diet, these findings indicate unfavourable impacts of high dietary fat on gut microbiota. At present, only few studies have examined the impact of ketogenic diet on gut microbiota and intestinal health. It has been reported that ketogenic diet intervention counteracted the diseased microbial phenotype, and reversed increased abundance of *Akkermansia muciniphila* in mice with autism spectrum disorder (52, 53). In children with refractory epilepsy, ketogenic diet treatment significantly alleviated the onset of seizures and corrected an imbalanced gut microbiota (54-56). Further animal data revealed that the ketogenic diet-mediated seizure protection effects were functioned through modulating specific gut bacterial species that increase hippocampal gamma- glutamylated amino acids/glutamate levels (57). Moreover, ketogenic diet intervention enhanced neurovascular function and reduced risk of Alzheimer's disease in healthy mice was also reported (58). Simultaneously, ketogenic diet increased the relative abundance of *Akkermansia muciniphila* and *Lactobacillus* which are presumptively beneficial microbial genera, and decreased *Desulfovibrio* and *Turicibacter* which are presumptively pro-inflammatory bacterial genera (58). On the contrary, some studies proposed that ketogenic diet might

have adverse effects on gut microbiota, because diminished total bacterial levels, lower α-diversity, enriched pro-inflammatory bacterial species and decreased anti-inflammatory microbial species were observed following ketogenic diet (52, 55, 58-60). However, another study pointed out that the reductions in bacterial richness and diversity in response to ketogenic diet were temporary, the total bacterial concentrations and diversity were transiently reduced on the first two weeks of ketogenic diet therapy and started to recover at week 12 and reached values of healthy condition at week 23/24 (61). Multiple observation points used in this study provide us a more complete understanding of the dynamic fluctuation of gut microbiota in response to ketogenic diet.

The nature of ketogenic diet is extremely high in fat and low in carbohydrates, which has been suggested may induce negative effects on gut microbial ecology. Although the initial findings are mainly derived from animals or subjects with specific diseases, concerns are raising about the safety for utilizing such a diet in healthy humans. Given the growing popularity of ketogenic diet in weight loss and regulating metabolic disorder in healthy populations, it is necessary to ensure whether ketogenic diet would induce deleterious effects on gut microbiota and thus confer negative health consequences, and to examine whether ketogenic diet-mediated modifications in cardiometabolic profiles are modulated through changes in gut microbiota.

Conclusion

Besides having broader therapeutic actions on polycystic ovary syndrome, Type 2 Diabetes, cancer, neurological and cardiovascular diseases, literatures from western countries indicate that ketogenic diets are useful tools for fighting obesity and many studies even point out that they are more effective in weight-loss than the low-fat diets. Increasingly more studies suggest that the gut microbiota plays a vital or even causative role in host nutrition uptake, energy metabolism, fat storage, and the further development of obesity and associated metabolic diseases. Numerous gut microbial taxonomies and functional components are recognized as biomarkers of human health and disease. Diet is the most rapid and direct way to shape gut microbial ecosystem and change the composition and function of gut microbiota, which is associated with the regulation of cardiometabolic health in the host. At present, evidence regarding the safety and effectiveness of ketogenic diets in the Turkish population is rare. Therefore, it is meaningful for further studies to investigate more in detail about the safety and effectiveness of ketogenic diet on cardiometabolic health, and its therapeutic use and potential mechanisms in gut microbiota.

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CHAPTER 11

EFFECTS OF NOISE ON HUMAN HEALTH

Samet TOSUN[1]

Dilek OZTAS[2]

Ergün ERASLAN[3]

1 Lecturer, Tokat Gaziosmanpasa University, Zile Vocational School, Department of Architecture and Urban Planning, Tokat TURKEY, e-mail: samet.tosun@gop.edu.tr, ORCID ID: 0000-0002-5454-3057

2 Associate Professor, Ankara Yildirim Beyazit University, Faculty of Medicine, Ankara TURKEY, e-mail: doztay@ybu.edu.tr, ORCID ID: 0000-0002-8687-7238

3 Professor, Ankara Yildirim Beyazit University, Faculty of Engineering and Natural Sciences, Ankara TURKEY, e-mail: eraslan@ybu.edu.tr, ORCID ID: 0000-0002-5667-0391

INTRODUCTION

Noise caused by machines or devices in working life and in almost every area around us constitutes a serious health problem for employees. There are various control methods used to reduce the exposure in the workplaces that cause noise, to control it and to prevent it from being harmful to the health of the employees. Among the preventive methods, the most effective ones are engineering methods that control the noise at the source and in the propagation environment. The need for technical personnel and technical equipment in the implementation of these methods and the relatively additional costs cause employers to stay away from these practices. As a result, employees are constantly faced with the harmful effects of noise. For these reasons, in this study, the harmful effects of noise on human health and the measures that can be taken to avoid these effects are mentioned.

1.DEFINITIONS

1.1 Sound

The sensation created by small pressure fluctuations or changes that the hearing sense can perceive in an elastic environment is called sound. In order for these pressure fluctuations occurring around the equilibrium pressure of the environment to be perceived as sound, they must have certain characteristics (in terms of magnitude and fluctuation speed). Sound requires a sound source and an elastic medium with mass in which pressure fluctuations will propagate. For example, it is impossible for sound to propagate in a vacuum or vacuum. It is clear that the necessity of the existence of a sensor that will detect the pressure fluctuations described in such elastic environments as sound cannot be denied (Dedeler, 2008).

1.2 Noise

Sounds that are unwanted or that do not make sense to the affected person are considered noise. A sound that one person perceives as music may be described as noise by another person. For example, a person who likes classical music may consider other types of music as noise. For this reason, it is possible to say that noise has a subjective side. However, noise types such as industrial noise are considered as noise in all circumstances, regardless of personal taste (ILO, 1977).

Noise consists of physically irregular sounds. Physiologically, any unwanted and unpleasant sound is noise. A sound evaluation unit that emphasizes the middle and intense frequency, which the human ear is very sensitive to, is dBA (Tufaner, 2010). The sounds that make up the noise can be defined in three types. Infrared sounds are sounds with a frequency lower than 20 Hz, audible sounds are sounds with an approximate frequency of between 20 Hz and 20 kHz, and ultra sounds are

sounds with a frequency higher than 20 kHz (Özmen, 2014). Noise is one of the foremost industrial and environmental issues of our time. The noise produced by industrial machinery and equipment can cause significant harm, especially to those working in the sector, if appropriate and effective measures are not taken (Yağımlı and Tozan, 2017).

1.2.1 Noise Measuring Devices

There are two types of devices for measuring sound level; sound level meters and dosimeters, also known as personal noise exposure meters. Some devices can be used in two ways, both as a sound level meter and as a dosimeter. Sound level meters give the user the opportunity to read the sound level directly. Dosimeters are sound level meters designed for use by employees and are devices that measure noise exposure during the whole working day or during a certain part of the day (Özmen, 2014). Whether the noise is harmful or disturbing is depends on the level, frequency and duration of the noise. These three factors are combined into equivalent noise levels. Noise measurements are taken for certain periods and then the time weighted average (LEX,8h) is calculated (Yağımlı & Tozan, 2017).

2. EFFECTS OF NOISE ON HUMAN HEALTH

The concept of noise is a risk to human health, defined by the World Health Organization (WHO) as "a state of complete mental, physical and social well-being". Defined as "obscene sound".

The fact that today's social life is too complex and the physical and mental wear of the employees reduces the tolerance and tolerance to noise. However, the noise problem does not seem to be given due attention. Noise problems have different dimensions like other environmental problems. In our developing country, this problem is growing rapidly and discomforts are emerging, but the measures taken are insufficient. Environmental problems caused by noise end abruptly when the cause of the noise disappears and generally does not leave any residue. There is no contamination such as material contamination, poisoning, burning or destruction of living things due to noise. However, noise causes sleep disturbance, its effect on voice communication, hearing impairment, physiological and cardiovascular effects, psychological effects, performance and general behaviors in residential areas (Toprak and Aktürk, 2004).

2.1. Noise-induced Hearing İmpairment

Around the world, noise-induced hearing loss is an irreversible occupational hazard. In developing countries, not only environmental noise but also occupational

noise is an increasing risk factor for hearing impairment. According to a study conducted by the World Health Organization (WHO) in 1995, it is assumed that there are 130 million hearing-impaired people impairment worldwide. Studies have been able to show that men and women are equally at risk of hearing loss.

A safe noise level depends on the sound level. Most countries generally accept 85 dBA per day as a standard, which indicates the precautions to be taken to protect people from negative impact (Erdoğan, 2016).

2.2 Effect of Noise on Voice Communication

Conversation can be defined as the presence of two or more individuals in oral conversation, consultation and negotiation. Most of the acoustic energies in speech are between 100-6000 Hz and the most important energy occurs in the 300-3000 Hz range. The speech act is a masking process that simultaneously interferes with the noise. The higher the speech levels, the more energy there is in the frequencies, resulting in more inappropriate sounds for the listeners (Kürklü, Görhan & Burgan 2013).

Noise can have an impact on speech understanding as well as result in multiple behavioral changes. Problems related to concentration fatigue, disorder, misunderstandings, indecision, anger, self-confidence, decreased work performance, stress reactions and some problems in human relations can be listed. Particularly vulnerable to these effects are the elderly, the deaf, people who do not speak the spoken language and children in the process of learning to read.

2.3 Noise-Induced Sleep Disorder

It is assumed that uninterrupted sleep is a prerequisite for good mental and physiological functions of healthy people, and environmental noise is thought to have a significant effect on sleep disturbance. It is predicted that most of the sleep disorders detected in noisy environments are caused by reasons that are not caused by external noise. Experimental research in controlled environments is used to understand the effect of noise exposure on sleep. In normal life, the number of field studies with people is not enough. The latest research on sleep disturbance examines airplanes, road traffic, and railroad noise.

The primary sleep disturbance effects are: frequent awakenings, inability to fall asleep, and variations in sleep depth or stages, particularly decreased REM 2 sleep rate. Other physiological effects are vasoconstriction, increased finger pulse tension, respiratory changes, increased blood pressure, increased body movements, and increased noise-induced heart rate during sleep, including heart palpitations. Both response threshold and noise threshold values may not be the same for all of

these physiological effects. Different sounds may also have different information content, which may affect physiological threshold and noise response relationships.

Noise exposed in the evening also causes secondary effects (side effects/ secondary effects) called delayed effects. The effects seen are those measured after hearing noise at night while the person is not sleeping the next day. Delayed (secondary) effects may include increased fatigue, decreased sleep quality, decreased happiness and performance, or depression.

2.4 Cardiovascular and Physiological Effects

Epidemiological and laboratory studies involving workers exposed to occupational noises; hypothesizes that noise has permanent and temporary effects on physiological functions in the general population living around industries, airports and noisy streets. Noise is recognized as an environmental stressor. Increased blood pressure activates the hormonal and autonomic systems, causing discontinuous changes such as acute exposure to noise, constriction of blood vessels and accelerated heart rate. Susceptible individuals in the general population may experience persistent effects, such as ischemic and hypertension heart disease, associated with continued exposure, exposure to non-low sound pressure levels. The magnitude and duration of the impact are determined in part by lifestyle behaviors, personal characteristics, and environmental conditions. Sounds also amplify reflex responses when there are sudden and unusual starts (Plomp, 1986).

2.5 Psychological Effects of Noise

Mental health is the individual's being in harmony and balance with himself and his environment. Environmental noises are not assumed to be a direct cause of mental illness, but are assumed to intensify and accelerate the development of mental disorders. Studies on mental illnesses of environmental noises include different symptoms such as anxiety. They are general psychiatric disorders such as emotional stress, nausea, headache, indecisiveness, aggression, sexual impotence, mood changes, increase in social conflicts, psychosis, neurosi and hysteria. Large population studies show the link between mental health assessment and noise exposure. Examples of these are standard psychological symptom profiles, intake of psychotherapeutic drugs, and consumption of tranquilizers and sleeping pills.

Exposure to excessive occupational noise is associated with the development of irritability and neurosis. However, the findings showing the link between mental health and environmental noise are not clear. The only research in the field has shown a link between minor psychiatric disorders and road traffic noise level (Berglund & Lindvall, Community Noise., 1995).

2.6 Effects of Noise on Performance

It is observed that noise has a negative effect on cognitive task performance in employees exposed to occupational noise (Reyner & Home, 1995). Environmental noise on children disrupts many motivational and cognitive parameters. However, no studies have been found that show how much environmental noise in the home affects cognitive performance in adults. Few field studies of the effects of noise on safety and performance have shown us that noise can increase the number of errors in the job and create a dysfunction, but the effects depend on the task and type of noise.

Continuous noise exposure has adverse effects on performance. For example, it has been determined that students studying at schools around the Los Angeles airport are inadequate in literacy and have persistence through challenging experiments. In addition to the intensity of the noise, the difficulty of controlling it was determined to be the most challenging variable (Berglund & Lindvall, Community Noise., 1995).

2.7 Controlling Noise

There are three steps in the prevention of noise pollution. First and foremost is to reduce noise at the source. For this, the following measures should be taken..

- Replacing used machines with low-noise machines,
- Replacing a high-noise operation with a less-noise operation,
- Taking the noise source into a separate compartment.

Precautions to be taken in the noise environment;

- Taking adequate precautions against noise and vibration on the ground where the machines are placed,
- Putting a noise-canceling barrier between the noise source and the person exposed to the noise,
- Increasing the distance between the noise source and the person exposed to the noise,
- Covering places such as walls, ceilings, floors, where sound can pass and reflect, with sound-absorbing material.

Precautions to be taken for people exposed to noise;

- Ensuring that the person exposed to the noise is placed in a well-insulated compartment,
- Reducing working time in noisy environment,
- Using effective personal protection against noise.

The process of removing the noise from the source is an engineering process. It is important to prevent noise in the design phase of machines and vehicles. The second measure of noise suppression is to control the noise at the receiver. It is taking protective measures on the person who is exposed to the noise in case the sound cannot be limited at its source and on the path it follows or if the precautions cannot be taken.

The third measure is environmental control. In this case, the most important step is to create noise awareness in people.

Measures such as the implementation of appropriate maintenance programs for the workplace, working systems and work equipment, a work organization that will reduce noise, limiting the exposure time and noise level, arranging working times by giving sufficient rest breaks should also be taken.

In case the noise exceeds the highest exposure effective value, the exposure should be reduced by making an appropriate organization, a risk map should be prepared by marking the workplaces, the entrances to the marked places should be controlled, the resting places should be suitable for the purpose of the noise level, the necessary precautions for the protection of sensitive risk groups such as women, children, the elderly and the disabled. must be taken.

3. CONCLUSION

The noise created by the machinery and equipment used in industries can cause irreversible damage to the worker. Noise is among the factors that cause environmental pollution for developed and developing countries. Noise, which is one of the important problems of our age, increases its place in environmental problems. For this reason, preventing noise is considered an important step in preventing environmental pollution. Hearing impairment remains the greatest danger for workers working in noisy environments, and the hearing loss caused by this damage is irreversible.

Although there are regulations regarding occupational health and safety in our country, employers avoid taking measures to avoid costs because the necessary safety and health culture is not formed. In addition, employees who do not care about occupational health or are uneducated do not use PPE (Personal Protective Equipment). This causes various work accidents and occupational diseases. It has been explained that when all the provisions in the regulations regarding the protection of employees from risks related to noise and vibration in the legislation are fulfilled, employees will be protected from the bad effects of noise and vibration.

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CHAPTER 12

THE RELATIONSHIP BETWEEN VITAMIN D AND COVID-19

Sevtap HAN[1]

1 Dr. Öğr. Üyesi, Gazi Üniversitesi Eczacılık Fakültesi Farmakoloji Ab.D., 06330, Ankara.
E-posta: sevtap.han@gazi.edu.tr

Vitamin D is a fat-soluble vitamin essential for maintaining health, growth and the health of bones. It can be produced in the skin by exposure to sunlight. Vitamin D deficiency is associated with cardiovascular disease, metabolic diseases, cancer, depression, and decreased cognitive function (Amrein et al., 2020; DeLuca, 2004; Galesanu & Mocanu, 2015). While the definition of vitamin D deficiency is still controversial, the use of vitamin D supplements has increased over the years. There are different definitions of vitamin D deficiency based on different serum thresholds of 25-hydroxyvitamin D (25(OH)D). There is widespread acceptance that vitamin D deficiency is prevalent, even if the most moderate serum 25(OH)D threshold <25/30 nmol/L is used (Cashman, 2020). Vitamin D deficiency is found in less than 20% of the population in Northern Europe, in 30-60% in other European regions, and up to 80% in Middle Eastern countries (Lips et al., 2019).

Many studies, from experimental studies to clinical applications, have reported a strong association between chronic diseases, as well as acute conditions and low vitamin D levels. The effects vitamin D on the lungs have been demonstrated due to its immunomodulatory, anti-inflammatory, and anti-infective effects that has been reported in patients with community-acquired infections, acute respiratory failure and lung transplantation recipients (Amrein et al., 2020). A strong association has been shown between pre-hospitalization vitamin D supplementation and improved outcomes among critically ill, mechanically ventilated adults (Leclair et al., 2019). It has been suggested that vitamin D deficiency is common in the pediatric population with mild to moderate persistent asthma and is associated with a higher likelihood of severe exacerbations (Brehm et al., 2010). In addition, a positive correlation has been found between vitamin D level and lung function in adults with asthma (J. Liu et al., 2019). Vitamin D supplementation has been shown to be safe and protects against acute respiratory infections (Martineau et al., 2019).

COVID-19, which has a high death rate, still affects almost all countries of the world. Severe acute respiratory syndrome, which develops due to an exacerbated inflammatory reaction, is one of the causes of death in COVID-19 disease. The fact that there is still no definitive treatment continues to cause concern (Yisak et al., 2021). Studies in different parts of the world have shown that the prevalence of COVID-19 patients is higher in vitamin D-deficient regions, and that severe COVID-19 cases and mortality rates are higher in vitamin D-deficient patients (Farid, Rola, Koch, & Nakhoul, 2021; Kalia, Studzinski, & Sarkar, 2021; Yisak et al., 2021). In this section, clinical studies and meta-analysis on the relationship between COVID-19 and Vitamin D have been compiled.

Clinical Trials

The aim of the COVIT-TRIAL study, an open-label, multicenter, and randomized controlled trial, has been to compare the effect of a single oral high-dose cholecalciferol administered and a single oral standard dose on all-cause mortality in adults 65 years of age and older who are at higher risk of severe COVID-19 disease. It has been reported that COVIT-TRIAL is the first randomized controlled trial to test the effect of vitamin D supplementation on COVID-19 prognosis in high-risk elderly patients. Patients have been diagnosed with COVID-19 by RT-PCR and/or chest CT scan. Individuals who had no contraindications for vitamin D supplementation and who had not received >800 IU/day vitamin D supplementation in the previous month have been included in the trial. Two 200,000 IU drinking vial of high-dose cholecalciferol have given to 130 subjects at the same time. The other part has given a standard dose of cholecalciferol in a 50,000 IU drinking vial. Two hundred and sixty volunteers have been included and followed for 4 weeks. Researchers have suggested that high-dose vitamin D supplementation could be an effective, well-tolerated, and easy treatment for COVID-19 (Annweiler et al., 2020).

In another study, it has been aimed to compare the clinical outcomes of COVID-19 positive elderly patients who were given a combination of vitamin D, magnesium and vitamin B12 (DMB) with those who did not. Patients aged 50 and over who did not require oxygen therapy have been included in the study and given 1000 IU/day oral vitamin D_3, 150 mg/day oral magnesium and 500 mcg/day oral vitamin B12 at the time of admission. The primary outcome was disease state requiring any oxygen therapy, intensive care unit, or both. 17 of the 43 identified patients have been given DMB prior to the initiation of primary outcome and 26 were not. The basic demographic features between the two groups have been differed significantly by age. The rate of initiation of oxygen therapy in treated patients has been found to be lower than in the control. DMB exposure in elderly patients has been associated with a significant decrease in the proportion of patients with the condition requiring oxygen therapy, intensive care support, or both. The authors have noted that the study showed promising results regarding the benefit of the DMB combination in preventing clinical deterioration in high-risk aged patients, but the findings need further confirmation in well-designed randomized controlled trials (Tan et al., 2020).

A parallel pilot randomized open label, double-masked clinical trial conducted in Spain has evaluated the effect of calcifediol (a form of vitamin D) treatment on ICU Admission and Mortality among hospitalized patients for COVID-19. 76 COVID-19 positive patients have been included in the study. A combination of

hydroxychloroquine and azithromycin has been administered to all hospitalized patients. Two-thirds of proper patients have been randomly determined to receive oral calcifediol (0.532 mg) on the initial day, while one-third did not. Patients in the calcifediol treatment group have been given 0.266 mg of oral calcifediol on days 3 and 7. Afterwards, weekly drug administrations have continued. Admission to the intensive care unit and mortality rates of the patients who did or could not be applied have been evaluated. One of 50 patients (2%) given calcifediol required admission to the intensive care unit, while 13 (50%) of 26 untreated patients required hospitalization. No death has been observed in patients who received calcifediol treatment and all were discharged without complications. Of the 13 patients who did not receive calcifediol, 2 died, while the remaining 11 were discharged. The results have showed that administration of calcifediol in hospitalized patients with a diagnosis of COVID-19 reduces the severity of the disease and the rate of need for intensive care unit admission (Castillo et al., 2020).

In the study conducted by Ling et al. in England, it has been aimed to determine whether mortality rates due to COVID-19 are affected by serum 25(OH)D levels, vitamin D status or cholecalciferol treatment. The data of 986 patients hospitalized with the diagnosis of COVID-19 have been collected retrospectively. In the primary cohort of 444 patients, cholecalciferol treatment has been associated with a reduced risk of COVID-19 mortality. This result has been replicated in a validation cohort of 541 patients. Regardless of baseline serum 25(OH)D levels, treatment with high-dose cholecalciferol seems to be associated with a decreased risk of mortality in hospitalized patients with a diagnosis of COVID-19 (Ling et al., 2020).

The relationship between 25OH-Vitamin D serum levels and pulmonary involvement in elderly patients hospitalized for COVID-19 infection has been investigated in a clinical trial. Data from 65 consecutive elderly COVID-19 patients and 65 gender and age matched individuals as the control group have been analyzed. Various clinical and laboratory parameters have been collected. Vitamin D serum levels of COVID-19 patients have been found to be lower than the control group. A significantly positive correlation has been shown between vitamin D serum concentrations and PaO_2, SO_2, PaO_2/FiO_2. A negative correlation has been found between serum levels of vitamin D and the severity of pulmonary involvement. Among hospitalized COVID-19 patients, those who died had lower vitamin D levels than those who survived. In conclusion, the authors have confirmed that aged COVID-19 patients with low serum vitamin D levels have more severe pulmonary involvement, longer duration of illness, and risk of death (Sulli et al., 2021).

The clinical and demographic data of 464 patients have been analyzed retrospectively in the study, in which the relationship between vitamin D levels,

disease severity and mortality in COVID-19 patients from the United Arab Emirates population has been evaluated. It has been reported that vitamin D concentrations lower than 12 ng/mL are associated with the severity of the disease and the risk of death (AlSafar et al., 2021).

In another clinical trial, inflammatory markers have been evaluated in COVID-19 patients with vitamin D deficiency. Patients have been randomized to either standard therapy or standard therapy plus additional Pulse D therapy. Pulse D therapy is a targeted daily supplement of 60,000 IU of vitamin D for 8 or 10 days, depending on their BMI. It has been started with one hundred and thirty subjects aged 20–83 years, and eighty-seven completed the study. There has been no difference between the groups in terms of vital parameters. After pulse D treatment, patients' vitamin D concentrations have been increased from 16 ± 6 ng/ml to 89 ± 32 ng/ml. In addition, a statistically significant decrease has been recorded in all evaluated inflammatory markers. There has been no difference before and after treatment in all inflammatory parameters except CRP in the standard treatment group. Improvement in serum vitamin D up to 80-100 ng/ml in COVID-19 patients have significantly decreased inflammatory markers without any complications. The authors have noted that additional Pulse D therapy could be added to existing COVID-19 treatment protocols for better outcomes (Lakkireddy et al., 2021).

The dose of vitamin D to be administered has also been a matter of debate, following studies that demonstrated the beneficial effects of vitamin D therapy in COVID-19 patients. A multicenter randomized clinical trial has aimed to compare the effects of 5000 IU versus 1000 IU daily oral vitamin D_3 supplementation on symptom relief among vitamin D-deficient COVID-19 patients. 36 patients with mild to moderate COVID-19 received 5000 IU of oral vitamin D_3 once daily for 2 weeks. In 33 patients, 1000 IU oral vitamin D_3 has been given once a day for 2 weeks. Before and after the application, blood samples have been taken and fasting blood glucose, 25(OH)D level, lipids and inflammation markers have been evaluated. It has been found that serum 25(OH)D concentrations increased and BMI and IL-6 values decreased in the group receiving 5000 IU vitamin D supplement daily. 5000 IU vitamin D supplementation has also shortened the recovery time in COVID-19 patients with insufficient vitamin D levels (Sabico et al., 2021).

Another question that needed to be answered was the effects of a single high dose vitamin D administration. A randomized clinical trial have investigated the effect of a single high dose of vitamin D on the length of hospital stay in hospitalized COVID-19 patients. Moderate to severe COVID-19 patients older than 18 years of age have been included in the study. Vitamin D_3 has been given

orally at a dose of 200 000 IU. The primary outcome was the length of hospital stay of the patients, the secondary outcomes were mortality during the hospital stay, the number of transitions to the intensive care unit, the number of patients in need of mechanical ventilation, serum levels of 25(OH)D, total calcium, CRP and creatinine. The length of hospital stay and mortality have not differed between the group receiving vitamin D_3 and the placebo group. There has been no difference between the groups in the need for intensive care unit and mechanical ventilation. While serum 25(OH)D levels have increased in the vitamin D_3 group, no change has been observed in other measured parameters. The results have indicated that the use of a single high dose of vitamin D_3 has no beneficial effect in moderate to severe COVID-19 patients (Murai et al., 2021).

Table 1. Effects of Vitamin D treatment in COVID-19 patients

References	Dose of Vitamin D	Outcomes	Patient profile
Tan et al., 2020	1000 IU/day oral vitamin D3, 150 mg/day oral magnesium and 500 mcg/day oral vitamin B12	• Lower initiation of oxygen therapy • Decreased need for intensive care	Age ≥50
Castillo et al., 2020	0.266 mg of oral calcifediol	• Decreased required admission to the intensive care unit • No deaths were observed in patients who received calcifediol treatment.	45 men and 31 women Mean age: 53 ± 10
Ling et al., 2020	Cholecalciferol (approximately ≥ 280,000 IU in a time period of up to 7 weeks)	• Reduced risk of COVID-19 mortality	Mean age: 74 199 female and 245 male
Lakkireddy et al., 2021	Daily supplement of 60,000 IU of vitamin D for 8 or 10 days	• Decreased inflammatory markers	Aged 20-83
Sabico et al., 2021	5000 IU of oral vitamin D_3 once daily for 2 weeks	• Decreased IL-6 values • Reduced time to recovery for cough • Shortened the recovery time in COVID-19 patients with insufficient vitamin D levels	15 females and 21 males
Murai et al., 2021	Oral Vitamin D_3 at a single dose of 200 000 IU.	• No difference between the groups in the need for intensive care unit and mechanical ventilation	Age ≥18 Mena age: 56.2 104 female and 133 male

Meta-Analysis Studies

After the increase in studies investigating the relationship between Vitamin D and COVID-19, meta-analysis and systemic review studies have also started.

First, studies on whether vitamin D deficiency is a risk factor in patients with COVID-19 were analyzed. Six selected studies have been evaluated in a study aiming to systematically investigate the relationship between serum concentrations of vitamin D, an immune modulator, and the severity of COVID-19. In the aforementioned study, a systematic review of studies reporting the severity of the disease, admission to the intensive care unit or mortality rates in COVID-19 patients has been conducted. A standardized mean difference or odds ratio and 95% confidence interval have been applied to estimate the pooled results of the selected studies. It has been found that patients with poor prognosis have significantly lower vitamin D levels than patients with good prognosis. The authors have suggested that serum vitamin D values may play a role in the prognosis of COVID-19 and that the diagnosis of vitamin D deficiency may assist in assessing patients' risk of developing severe COVID-19 (Munshi et al., 2021).

In the meta-analysis study conducted by Liu et al., the relationship between low vitamin D levels and COVID-19 has been investigated. Studies on COVID-19 and Vitamin D have been systematically searched using PubMed, Embase, and Cochrane Library databases. Considering all the results, a relationship has been observed between vitamin D deficiency and an increased risk of COVID-19 infection. It has also been found that people with COVID-19 disease have lower vitamin D values than those who are not infected. Therefore, it has been recommended to be careful in individuals with vitamin D deficiency (N. Y. Liu et al., 2021).

In the meta-analysis conducted by scanning PubMed, preprint servers and google scholar databases of studies between December 2019 and December 2020, it has been aimed to reveal the effect of oral vitamin D supplementation on the need for intensive care unit and mortality in hospitalized COVID-19 patients. The cumulative effect of vitamin D therapy on intensive care unit transition in COVID-19 patients has been evaluated using a meta-analysis. It has been observed that the rates of need for intensive care unit in patients who received vitamin D treatment were significantly different from those who did not ($p<0.0001$). The effect of vitamin D therapy on mortality in hospitalized COVID-19 patients has also been evaluated using a meta-analysis. It has been found that mortality rates in patients who received vitamin D treatment were not significantly different from those who did not. According to the authors,

this study is the first meta-analysis to evaluate the cumulative evidence of the effect of vitamin D administration on intensive care needs and mortality rates in hospitalized COVID-19 patients. As a result, it has been observed that vitamin D supplementation reduced the severity of COVID-19 disease, but it did not reach a statistically significant improvement in mortality rates (Shah, Saxena, & Mavalankar, 2021).

In another systematic review and meta-analysis, the relationship between serum 25(OH)D levels and mortality and clinical outcomes related to COVID-19 has been evaluated, as well as the effects of vitamin D therapy on the course of COVID-19 disease. Medline (OVID), Embase.com, CINAHL (EBSCO), and Cochrane databases have been searched until 18 December 2020 and clinical trial records until 20 January 2021. Outcomes evaluated were mortality rate, admission to intensive care unit, invasive and non-invasive ventilation, hospitalization, length of hospital stay, COVID-19 disease severity and positivity. In addition, the risk of complications has also been evaluated in the study. A correlation has been found between low 25(OH)D values and mortality rates. In patients with serum 25(OH)D concentrations less than 20 ng/ml, an increase in the rate of admission to the intensive care unit has been observed. Patients with serum 25(OH)D concentrations less than 20 ng/ml have been showed an increased trend in invasive ventilation requirement, whereas patients with serum 25(OH)D concentrations below 12 ng/ml have been showed an increased trend in non-invasive invasive ventilation requirement. In patients with serum 25(OH)D levels less than 30 ng/ml, an increasing trend in COVID-19 disease severity has been reported. In patients with serum 25(OH) D levels less than 30 ng/ml, the rate of COVID-19 positivity has been found 1.5 times higher than in patients with adequate Vitamin D levels. In conclusion, the authors have stated that more clinical trials need to be completed in order to reach a clear conclusion about the relationship between vitamin D and COVID-19 (Bassatne et al., 2021).

Conclusion

Vitamin D deficiency is fairly common worldwide, but the literature supporting vitamin D treatment is inconclusive. Vitamin D can be an important, inexpensive, and safe complementary treatment for many diseases and life stages, including more sensitive periods. Therefore, studies to prevent vitamin D deficiency should be further encouraged (Amrein et al., 2020).

Clinical and meta-analysis studies suggest that vitamin D treatment in patients with COVID-19 is associated with a decrease in the severity of the

disease, a decrease in the need for intensive care admission, and a decrease in mortality rates. On the other hand, some issues such as at which stage of the disease vitamin D administration is more effective and the effect of the patient's initial vitamin D levels on treatment need to be further clarified. Ideally, all health-related decisions are made based on evidence, but a crisis situation may require slightly different rules. It is suggested by different study groups that vitamin D therapy may be beneficial in patients with COVID-19. Therefore, vitamin D therapy may be safely added to existing COVID-19 treatment protocols for better outcomes.

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CHAPTER 13

MIDPALATAL SUTURE MATURATION

Ufuk OK[1]

Tugce Unal Kaya[2]

1 Assist. Prof. İstanbul Gelisim University

2 Ph.D. Student İstanbul Aydin University

1. INTRODUCTION

Skeletal anomalies that occur in the craniofacial bones due to hereditary, environmental or functional factors may cause malocclusions on the anteroposterior, coronal and transverse planes by affecting maxillary growth negatively[1].

Transverse growth deficiency or deficiency of the maxilla is accepted as one of the most common skeletal complications. Posterior crossbite that is the most important clinical evidence of maxillary deficiency may appear as unilateral or bilateral as well as involving one or more teeth[2,3].

In this case, apical bone base is restricted, posterior teeth reveal palatinal inclination and crossbite. The orthopaedic forces applied to the midpalatal suture (MPS) by the rapid maxillary expansion (RME) method allows expansion of the apical bone base and correction of crossbite[4].

The idea of RME has been mentioned in an article published by H. Angell in 1860 and RME started to be used by Haas in 1960s[5,6]. The morphology and maturation of midpalatal suture has been analyzed in the histological studies, an autopsy study, occlusal radiographic studies and animal studies conducted using computed tomography (CT)[7]. Maturation occurs with advancing age in the facial skeleton and skeletal resistance increases during maxillary expansion[8,9]. Haas has stated that this resistance is produced by zygomatic buttress[10]. It has been reported that SARME should be performed to reduce this resistance in the adult patients[11,12].

The MPS fusion varies depending on age and sex, and individual assessment of MPS maturation for the young adult patients is crucial in determining RME as an alternative method which is a less invasive alternative implementation compared with SARME.

MPS has been defined as an end-to-end type of suture with its morphologically characteristic changes during growth[13-17]. Melsen[18] has reported that MPS is morphologically wide and Y-shaped in its frontal sections during the infantile period[16,19]. The ossification of MPS starts with bone spicules with “islands” (masses composed of acellular tissues and inconsistently calcified tissues) in the middle of suture[7,16,17,20]. The spicules increasing with maturation emerge in many locations throughout the suture and form scalloped fields as separated by connective tissue in some juxtaposition regions[15,17,20,21].

The fusion of MPS progresses from posterior to anterior with cortical bone resorption and spongious bone formation[15,17], and fusion occurs previously in the posterior region[16,19].

Persson and Thilander have reported that the MPS fusion occurs between

the ages of 15-19 years[17]. However, it has been noted in some studies that MPS revealed no sign of fusion at the ages of 27, 32, 54 and even 71 years[14,15]. It has been clarified that MPS maturations is not directly associated with chronological age in the young adults[7,14].

In the present caphter, it was aimed to evaluate the maturation of MPS rather than chronological age in determination of the indication for RME or SARME in late adolescent or young adult patients who need maxillary expansion.

2. GENERAL INFORMATION

A. The Growth and Development of the Maxilla

The maxilla develops by intramembranous ossification. The "premaxillary ossification point" and "postmaxillary ossification point" grow and join in the maxilla[22]. Premaxillary ossification point forms incisive bone, spina nasalis anterior, the external alveolar lamina of the incisor teeth region and a portion of the maxillary frontal protrusion. On the other side, postmaxillary ossification point forms the external alveolar bone of the canine and molar teeth regions, processus zygomaticus and a portion of the orbital basement. Maxillary prominences form from the posterolateral parts of the first branchial arch in the late third week of prenatal period. The primary palate is formed by fusion of medial nasal prominences and maxillary prominence in the 7th week of the embryonic life. Toward the 9th week, the vertical palatinal segments are reoriented to horizontal position by the effect of the tongue and form the secondary palate by the disappearance of the epithelial tissue[12] (Figure 1). The palatal clefts are known as the most commonly seen deformity that develop during the formation of the palate[23,24].

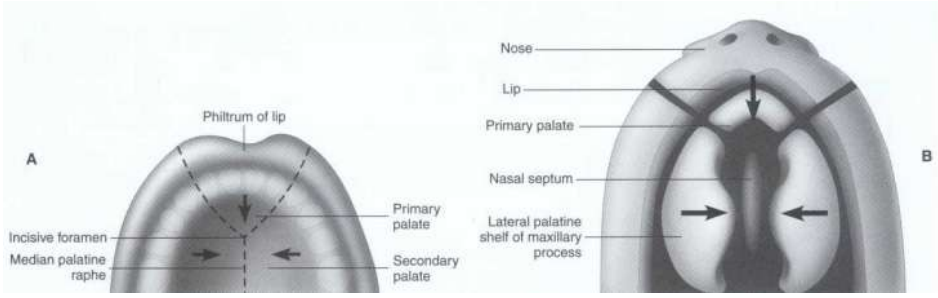

Figure 1: A, The appearance of primary and secondary palates (occlusal appearance) B, Palatal development (occlusal appearance) [25]

The surrounding and intermaxillary sutures are the bone growth sites in the postnatal development of the maxilla[1]. The growth occurs by bone apposition in the frontomaxillary, zygomaticomaxillary, zygomaticotemporal and pterygopalatine sutures (Figure 1). Spheno-occipital, sphenoethmoidal and frontoethmoidal synchondroses also play role in the postnatal development of the maxilla[26].

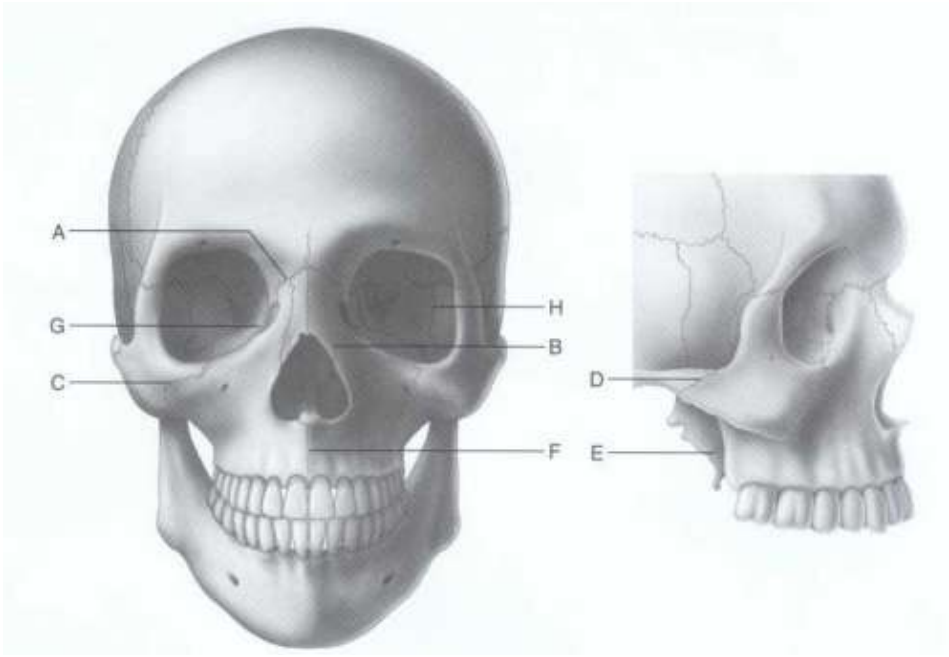

Figure 2: Circummaxillary complex A, Frontomaxillary suture B, Nasomaxillary suture C, Zygomaticomaxillary suture D, Zygomaticotemporal suture E, Pterygopalatine suture F, Intermaxillary suture G, Ethmomaxillary suture H, Lacrimomaxillary suture [25]

Midpalatal and transpalatal sutures are considered to be significantly associated with transverse and anteroposterior growth of the maxilla. Keith[27] has reported that midpalatal suture plays an active role in the transverse growth of the maxilla.

B. The Definition and Development of the Suture

1. The Definition of the Suture

The sutures are the fibrous joints in the vertebrate skull (Figure 3). It is composed of two bone ends that differentiate from the embryonic mesenchyme and intervening fibrous tissue. Sutures are not only the joints between the bones, but also, they are the primary sites of osteogenesis by involving osteoprogenitor cells

that proliferate, differentiate and function along the bone margins or osteogenic lacunae. The bones forming the sutures have generally intramembranous origin.

The skull bones can differentiate to the structures supporting the nasal passages, oral cavity and pharynx, viscerocranium forming the face and neurocranium surrounding the brain. Neurocranium can differentiate to skull base and calvaria (the dome of the skull). Skull base bones are formed by endochondral ossification, and cartilaginous articular structures between the bones are called as synchondrosis. The bones of the calvaria and face are formed by intramembranous ossification.

Fontanelles are located in the calvaria where three or more bones join. Fontanelles are bigger than the sutures at birth, however, postnatal growth rate of the calvarial bones decreases by time. The suture and fontanelles are the intact but flexible structures that allow a temporary compression[28].

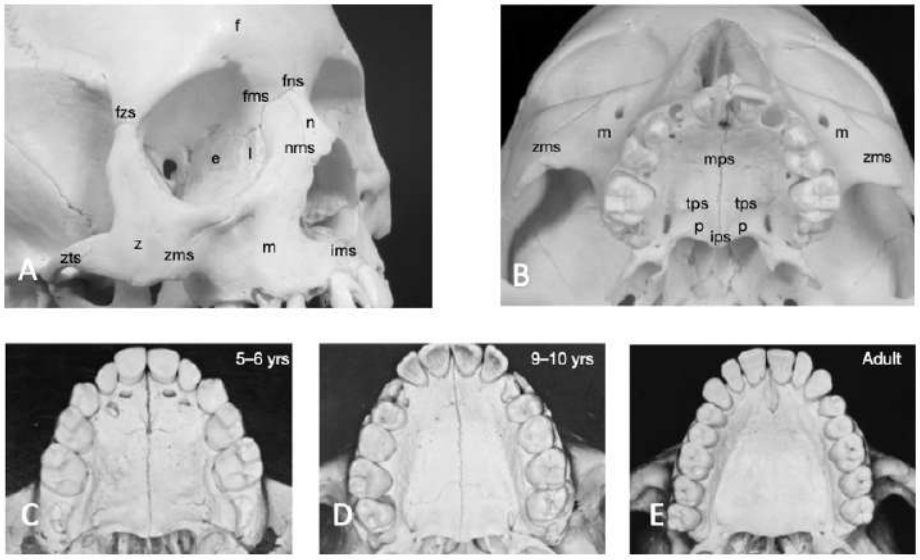

Figure 3: Sutures: A-B; a 7-year-old human. C-E; The closure of midpalatal suture in human. The growth of MPS approximately continues for 17 years. The suture fuses between the ages of 30-35 years. e: Ethmoid bone, f: Frontal bone, fms: frontomaxillary suture, fns: Frontonasal suture, fzs: Frontozygomatic suture, ims: Intermaxillary suture, l: Lacrimal bone, m: Maxilla, mps: midpalatine suture, n: Nasal bone, nms: Nasomaxillary suture, p: Palatine bone, ips: Interpalatine suture, tps: Transverse palatine suture, z: Zygomatic bone, zms: Zygomaticomaxillary suture, zts: Zygomaticotemporal suture.[28]

2.The Origin of the Craniofacial Skeleton

The skeletal structures of the skull are derived from embryonic mesoderm and cranial neural crest (CNC). CNC cells originate from the neural epithelium in the neural folds. These cells are subjected to epithelial-mesenchymal transition and they migrate to their final localization in the neck and craniofacial regions[29]. The studies carried out in both birds and mammals have shown that facial skeleton and anterior cranial base completely originate from CNC and that posterior cranial basal skeleton is derived from the paraxial and somitic mesoderm[30,31,32-34].

3. The Ossification of the Craniofacial Skeleton and the Formation of the Suture

The ossification of the craniofacial skeleton starts with condensation of neural crest or mesodermally derived cells to firmly packed masses. The cells are divided to chondroblasts or osteoblasts that form respectively cartilage and bones in these centers. Endochondral ossification involves the formation of a cartilage template or scaffold before transition to the bone that will be formed by the osteoblasts subsequently. During intramembranous ossification, osteoblasts secrete osteoid and then become calcified without cartilage. In intramembranous ossification, bones develop in the mesenchymal tissue layer or membrane which usually contacts with dermal layer of the skin. Thus, it is termed as dermal bone. This mesenchymal tissue also contacts with underlying dura mater that covers the brain. The signals coming from both skin and dura mater regulate intramembranous bone development and suture closure[35,36]. The craniofacial skeletal structures that demonstrate intramembranous ossification essentially grow with ossification in the sutures besides modeling and remodeling in the other surfaces. For instance, an increase in maxillary width is obtained by bone apposition on the external surfaces and by resorption on the internal surfaces to increase the aeration of the maxillary sinuses and to allow their development as well as growth in the median palatinal suture. In addition, modeling and remodeling occurs around the teeth in the alveolar part of the maxilla at the occurrence time of eruption after the completion of the development of maxillary teeth. An essential coordinated balance exists in the calvaria between the apposition continued by the osteoblasts formed on the ectocranial surface and the resorption continued by the osteoclasts formed on the endocranial surface[37,38]. These synchronized processes are important in the control of the bone thickness and individual bone configuarion[39].

C. Maxillary Malocclusions

Malocclusion which was first described by Edward Angle is misalignment of the teeth or abnormal relationship between the jaws[40].

Malocclusions may be in the sagittal, vertical, or transverse directions. The anterior (maxillary prognathism) or posterior (maxillary retrognathism) positioning of the maxilla relative to the cranial base is sagittal skeletal malocclusion[41]. The teeth are divided into groups according to Angle's Dental Malocclusion Classification based on the malocclusion in the sagittal direction. Incorrect overbite or anterior open-bite can be given as the examples for vertical skeletal malocclusions of the teeth and jaws[42]. Transverse developmental deficiency of the maxilla due to environmental and genetic factors or combination of those causes skeletal crossbite. Maxillary deficiency is observed together with many craniofacial anomalies. The most common craniofacial anomalies are labial-palatal clefts[43]. Long-term sucking habits also lead to maxillary deficiency by causing increased intraoral pressure. Thumb sucking, mouth breathing, and hypertrophic tonsils are the other causes of maxillary deficiency[44-46].

The teeth and jaws can be classified with respect to transverse malocclusions based on the fact whether posterior crossbite and its severity. Posterior crossbites may occur due to one tooth, a tooth group, skeletal factors and combination of those. It may be unilateral or bilateral. On the other side, functional crossbites emerge resulting from the shift of the mandible to any direction in order to find the maximum occlusion due to the early contact on the dental arch[42].

The clinical appearance of the maxillary deficiency involves unilateral or bilateral crossbite, palatal inclination in the teeth, dental crowding, high palatal vault, narrow and dental arch form with anterior prominence, complications of nasal breathing or their combinations. Extraoral findings are narrow alar base, deep nasolabial fold and paranasal collapse[47].

1. Rapid Maxillary Expansion (RME)

Rapid Maxillary Expansion is a successfully applied treatment method since many years to obtain a midpalatal suture separation of approximately 0.2-0.5 mm per day in a short time interval such as 1-3 weeks by applying heavy orthopaedic forces of 1.5-4.5 kg. The maxillary bones are separated from each other by this expansion and buccal inclinations are produced in the posterior teeth. In this method, it is expected to create higher skeletal effect and minimum tooth movement by applying heavy forces[5,48-51]. During RME applied after MPS fusion; several complications such as pain during activation, failure in the activation of the apparatus, necrosis in the tissues, tilting posterior teeth, posterior rotation

of the mandible and the detection of anterior open-bite, gingival recession, the detection of root resorption and increased probability of relapse[52].

a. Indications of Rapid Maxillary Expansion

It is indicated in the cases of unilateral or bilateral posterior crossbite due to maxillary dental or skeletal deficiencies[10,51,53,54].

Maxillary expansion can be applied to obtain space in the patients with a maxillary crowding of 3-6 mm[53,55,56].

Dark sites can be observed in the mouth sides of the individuals with maxillary deficiency. RME provides reduction or removal of these areas[57].

It has been reported that maxillary deficiency occurs due to the pressure of the buccal muscles in the patients with labial-palatal cleft, and maxillary expansion has been recommended in these patients[10,49].

Maxillary expansion is recommended for also correction of the axial inclination of the posterior teeth and alignment of Wilson plane[57].

It has been reported that application of maxillary expansion facilitates operating the functional apparatuses more effectively prior to jaw orthopaedic treatment by correcting the arch deficiency and arch size deficiency in most of the severe Class II cases who will undergo functional jaw orthopaedic therapy[57].

In the orthopaedic treatment of Class III malocclusions with maxillary retrusion; some researchers have recommended to perform maxillary expansion prior to orthopaedic correction of Class III malocclusion by considering that maxillary expansion can contribute to the recovery of Class III malocclusion by providing the mobilization of the sutural structures connected with the maxilla[5,10,49,57,58].

b. Contraindications of Rapid Maxillary Expansion

RME is contraindicated in the poorly cooperative patients, periodontally unhealthy patients and the patients with only a single tooth posterior crossbite, severe anterior open-bite that requires orthognathic surgery, and verticle mandibular plane inclination and convex profile patients[51,59]

In addition, rapid maxillary expansion can be performed in the adult patients only with surgical assistance since midpalatal suture is completely closed[1,5,17,60]

c. The Impact of Age in Rapid Maxillary Expansion

Melsen[18] has examined the age-related morphological changes in the midpalatal sutures in the histological studies conducted on the cadavers. The suture has a wide and sinuous structure and is Y-shaped in the infantile period while the T-shaped suture has a squamous structure with palatine part covering

the maxillary part in the juvenile period whereas its sinuous appearance increased in the adolescent period (Figure 4). The suture is composed of a very narrow connective tissue and inactive osteoblasts at the ages of 15 and 17 years in girls and boys, respectively.

The age of MPS closure shows individual variation and different studies in the literature have assumed that it closes between the ages of 15-18 years and 20-25 years[17,18]. The actual studies have exhibited the presence of a limited level of obliteration in the MPS in the adults[14,15]. The growth of the bones involved in the suture continues while bone apposition occurs and bone production process terminates at the end of growth[12].

Latham has noted that transverse growth of the maxilla is completed at the age of 3 years[61]. However, Björk and Skieller[62], Krebs[63], Korn and Frantz[64] and Snodell, and Nanda and Currier [65] have then reported in their studies that transverse growth of the maxilla in the midpalatal suture continues until the completion of the growth of the other facial sutures.

It is considered that mean annual increase in the transverse MPS is approximately 0.18-0.43 mm while the overall increase from the age of 4 years to the adulthood age is supposed to be 6.5 mm[62]. Of this increase, 5 mm of this increase occurs after the age of 7 years. After the age of 10 years, 25% of the increase in the dental arch results from the transverse growth of the midpalatal suture in the region of first molars[66].

Björk and Skieller have observed in their implant study that the amount of maxillary growth on the transverse plane was 6.5 m between the ages of 4-20 years. They have noted that sutural growth of the maxilla on the sagittal and transverse planes is completed at the age of 17 years[67].

Lines[45] has reported that RME can be effectively performed in the children and adolescents, however, the resistance that will develop against the expansion in the adults may originate from the maturation in MPS or circummaxillary rigidity.

Melsen[18] has stated that the growth in the midpalatal suture continued until the ages of 16 and 18 years in girls and boys, respectively, and that the increase in the size of the hard palate until the ages of 13-15 years results from the growth in the transverse suture and apposition in the posterior edge of the palate, and that the growth in the suture is completed after this period whereas apposition continues some years more.

Işeri and Solow[68] have denoted in their implant study on girls that horizontal growth of the maxilla peaked at the age of 11 years and terminated at the age of 18 years.

Bishara and Staley[51] have reported that optimal age for RME is 13-15 years

and that the potential results of expansion cannot be predicted and relapse may occur in the long term even performed in the adults.

Persson and Thilander[17] have stated in their study on human autopsy material that MPS can be closed in the juvenile or in a latter period. They have detected in their study that intermaxillary suture was closed in a 15-year-old girl whereas the suture was not closed in a 27-year-old woman. They have suggested that individual variations and sex may cause changes in the optimal application age of RME.

Glassman et al.[69] have noted that MPS and osteotomy of anterior and posterior walls are needed after the closure of maxillary sutures and synchondroses between the ages of 15-18 years.

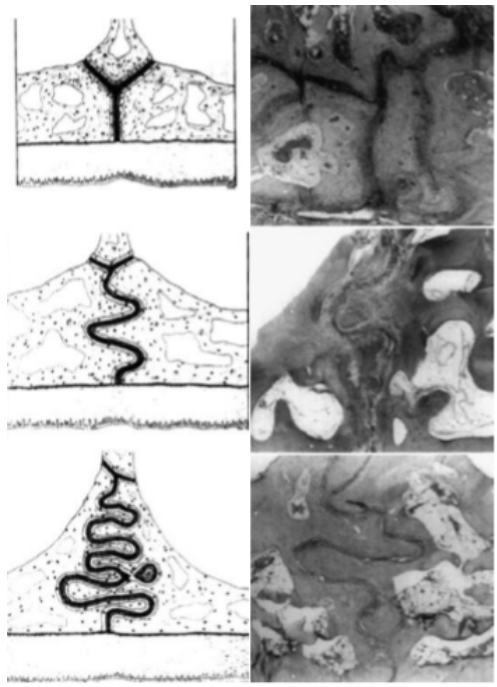

Figure 4: The histological sections and schematic images of midpalatal suture in the infantile, juvenile and adolescent ages[18]

2. Surgically Assisted Rapid Maxillary Expansion

RME is routinely performed for the separation of MPS to correct maxillary deficiency in the adolescent patients. This technique is not appropriate for the skeletally mature subjects.[70] Isaacson et al.[71] have demonstrated that the resistance of the facial skeleton increases against expansion as it ages and matures. The extrusion of the teeth and periodontal complications may emerge by the activation

of RME appliance in the adults in whom MPS fusion was encountered[72]. Therefore, surgically assisted rapid maxillary expansion (SARME) or segmental Lefort I osteotomy is performed in the adult patients with maxillary deficiency since the fusion of the midpalatal suture occurred.

a. Indications

SARME is indicated in the subjects with the indication of RME[11].

The age of the patient should be evaluated before the treatment of RME. There are different conclusions for the appropriate patient age in the literature. Bishara and Stanley[51] and Proffit[73] have evaluated the efficacy of RME to be suspicious after growth-development. Whereas Haas[10,50] has reported that RME can be performed until the age of 18 years. Epker and Wolford have recommended SARME after the age of 16 years whereas Timms and Vero[59] have accepted the age of 25 years as the upper limit. SARME should be applied in the patients aged over 14 years according to Mommaerts[74]. On the other hand, Suri and Taneja[11] have reported that RME can be successful in the chronologically old but skeletally immature patients whereas success cannot be expected in an exactly opposite situation.

b. Risks and Complications

The morbidity rate of SARME is very low compared with other orthognathic surgical procedures[75]. Pain, gingival recession, periodontal destruction, root resorption, devitalization in the teeth, infection, damage in the branches of the maxillary nerve, sinus infection, unilateral expansion, widening of the alar base and relapse may be observed as the complications due to SARME[74,76-82].

The postoperative residual resistance sites may cause asymmetrical fractures in the maxillary sutures. These asymmetrical fractures may cause gingival recession, periodontal defect in the upper incisor region and increased tooth mobility[77,78].

3. The Techniques Used in Decision Making for Surgically Assisted Rapid Maxillary Expansion

The accurate diagnosis, appropriate treatment planning and timing, and the success of RME may depend on the evaluation of craniofacial development and maturation parameters to predict the growth pattern of the patient. The craniofacial parameters are evaluated radiographically. The most commonly used skeletal parameters in the timing of orthopaedic treatments such as RME are hand-wrist radiograph[83] and the assessment of cervical vertebral maturation (CVM). It is easier to predict the treatment approaches in the early stages of the

craniofacial development and more consistent, uncertainty continues as skeletal maturation progresses[84-86].

The pubertal stage of a patient can be determined using hand-wrist radiograph or CVM method; however, it has been concluded that the stage of MPS maturation is more associated with treatment of maxillary expansion rregarding the prediction of the response to maxillary expansion. The stage of MPS maturation has been previously evaluated in the histological and radiological studies, and histological studies revealed the presence of various maturation stages in the same age group[87,88]. In addition, Angelieri et al.[86] have noted that the children with same chronological age may have different MPS maturation stages.

MPS fusion is completed at the ages of 14-15 and 15-16 years in females and males, respectively[50]. It is considered that RME has a mainly skeletal effect in the subjects younger than these ages whereas the expansion exhibits dental and dentoalveolar characteristic rather than skeletal effect in the patients older than these ages[89]. Age is generally used as a parameter in maturity evaluation of the patients; however, skeletal maturation stages are more reliable than chronological age[14,86].

In the previous years, information about morphology, development, maturation and fusion of MPS have been provided by the histological[20,90], radiographic[18,21] and tomographic[85,86,91,92] studies in the literature. Although MPS contributes to the prognosis of surgical approaches, no relationship has been established between the chronological age and maturation stages yet. In a similar way, no threshold age for RME has been defined based on the analysis of MPS[20,93-95].

Revelo and Fishman have evaluated MPS by occlusal radiographs before RME[94]. Nevertheless, several years later, Wehrbein and Yıldızhan have demonstrated that occlusal radiographs are also not histologically reliable with respect to diagnosis of midpalatal suture fusion because of the superposition of vomer and external nasal structures onto midpalatal region[21].

CT has allowed the evaluation of skeletal maturation by providing detailed imaging of bone morphology. The three-dimensional images may be necessary to making clinical decisions related with craniofacial development. CT-dependent techniques have been developed to evaluate the stages of MPS maturation[86,96,97]. The use of CT was limited since CBCT provides advantages such as three-dimensional imaging of oral and maxillofacial structures with relatively lower costs, easy-availability and low radiation exposure[98].

Due to the lack of clinical parameters to predict the success of RME in the late adolescents and young adults, Angelieri et al. have carried out an individual evaluation about MPS maturation using the CBCT images[86].

Angelieri et al. have developed[88] a classification system to classify the stages of MPS maturation by utilizing the previous histological studies[7,16,19] (Figure 5, 6). It is estimated that lower resistance and higher skeletal effect will be encountered in A and B stages compared with Stage C[86] in the maxillary expansion procedure performed with RME method. Even a diastema is observed at anterior MPS, opening of the suture by RME will be prevented by the fusion in the posterior region. In a such case, extrusion and periodontal destruction may be observed in the molar or premolar teeth in the posterior maxilla while a transverse increase is monitored in the anterior maxilla[86].

The "bone islets" in MPS has been defined as ossification areas by Melsen[20]. It has been stated that timing of RME is critical since fusion begins in Stage C in the palatinal part of MPS. Since MPS fusion partially or completely occurs in Stage D and Stage E, the patients can be treated by surgically assisted RME[86].

Angelieri et al. have noted that Stage CS1, CS2 and CS3 of CVM are equal to A, B and C stages of MPS maturation, respectively. Accordingly, Stage CS5 was accepted to correspond to Stage D and E[85].

Angelieri et al.[86] have evaluated a sampling composed of 140 subjects aged between 5.6 to 58.4 years and confirmed the fusion of MPS in the girls aged over 11 years old (Stage D and E) and boys aged over 14 years old (Stage D) on the CBCT images. Clinically, this sexual dimorphism on MPS fusion has been documented, women generally mature earlier than men.

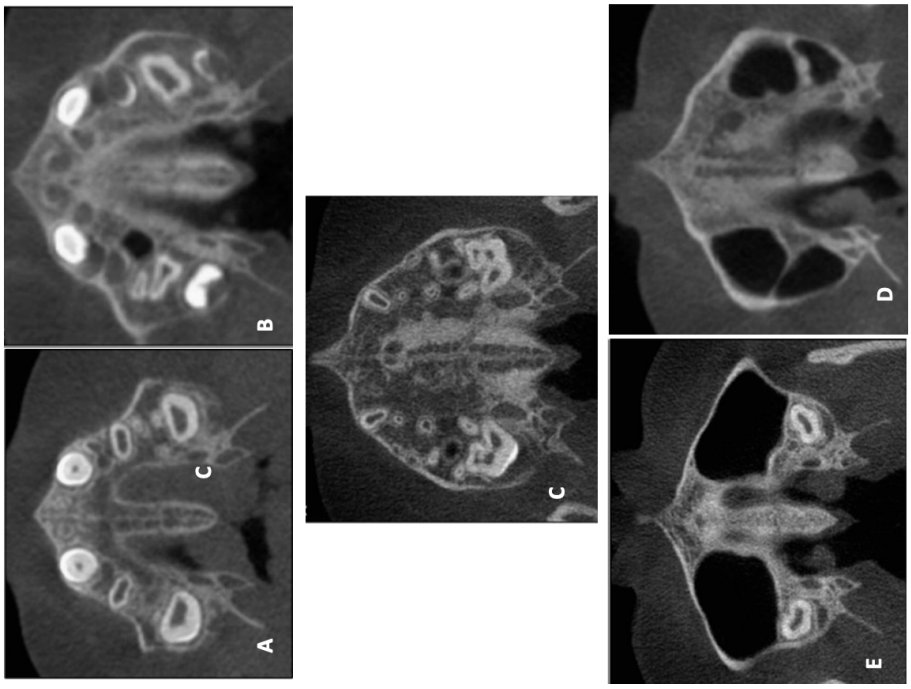

Figure 5: MPS maturation classification by Angelieri et al. A: MPS appears as a straight line of high-density without interdigitation. B: MPS appears as an irregular and scalloped-shaped line of high-density. C: MPS appears as two parallel, closely located and scalloped-shaped line of high-density. D: fusion occurred in the portion of the MPS in the palatinal bone while no fusion occurred in its maxillary part. E: The fusion of midpalatal suture was also completed in the maxillary part, and no suture was encountered while bone density increased in whole palate[86].

Tonello et al.[99] have evaluated MPS maturation in their study according to the classification by Angelieri et al.[86] and observed that age groups of 14 and 15 years reveal mostly Stage D and E maturation and identified similarities between the sexes in the Stage D and E.

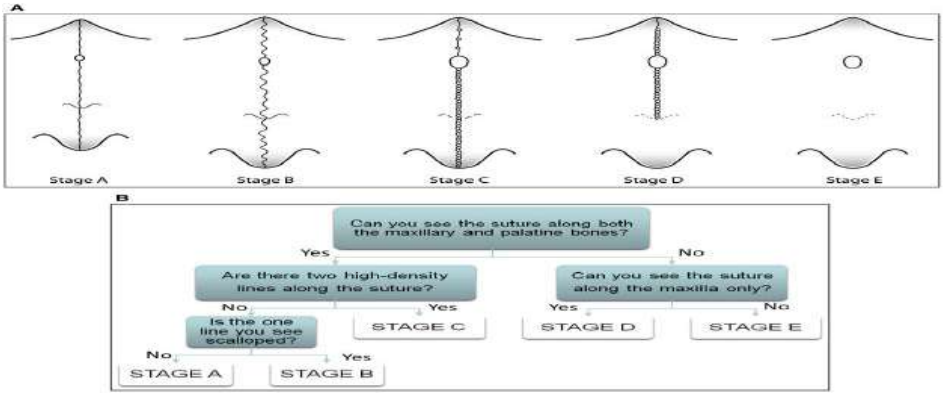

Figure 6: The schematic diagram of the maturation stages encountered in MPS[86]

D. Imaging Techniques

1. Two-Dimensional Imaging Techniques

a. Panoramic Radiography

Panoramic radiography, also known as pantomography, is an imaging technique that allows to create the image of maxilla until the 1/3 upper region of the orbit, maxillary sinuses, temporomandibular joint, mandible and whole dental arch in a single film[100].

Panoramic radiographs are very commonly used in dentistry because of the advantages such as low cost, easy applicability, time-saving application, providing comprehensive image, non-invasiveness, applicability in the trismus patients and use of minimum radiation dose. The fields of usage in dentistry are jaw fractures, localization determination of third molar teeth, osseous diseases, lesions, tooth development and tooth eruption (particularly in the mixed dentition period), impacted teeth and roots, temporomandibular joint diseases and developmental anomalies[100]. However, panoramic radiographs may be inadequate because of the disadvantages such as inability to provide data related with buccolingual width of alveolar bone, distortion, magnification, artefacts, head positioning errors and superposition of hard and soft tissues[101,102].

A magnification of 20-25% was reported for panoramic radiographs in the documents issued by American Academy of Oral and Maxillofacial Radiology (AAOMR) while another study has reported a magnification degree higher than 25%[103].

b. Lateral Cephalometric Radiographs

Cephalometric radiography provides data about the relationships of maxilla and mandible on the midsagittal plane. Its magnification rate is known between

7% and 12%. The soft tissue profiles of the patients can be displayed and the changes in the profile after the orthodontic treatment can be evaluated by lateral cephalometric radiography[104].

The growth pattern can be determined by performing angular and distance measurements from the reference points on the lateral cephalometric radiography images frequently used in the field of orthodontics, however, it has disadvantages such as iniability to provide three dimensional details, magnifications in the view and superposed image[105,106]. This superposed image may be due to facial symmetry or non-coincidence of right or left edge[107].

c. Postero-Anterior Cephalometric Radiographs

Postero-anterior cephalometric radiographs allow the assessment of the mandibular and maxillary widths and positions on the transverse plane, determination of nasal cavity width and analysis of dental and skeletal asymmetries in the maxilla and mandible, and transverse and vertical facial asymmetries. The assessment of difference in the intermolar distance is important for diagnosis of both asymmetry and crossbite[108].

2. Three-Dimensional Imaging Techniques

a. Computed Tomography

Diagnostic imaging is important for clinical evaluation in dentistry. Intraoral and extraoral radiography techniques are based on tissue attenuation and transmission of x-rays by recording on a single planar medium. The most accurate image formation is obtained by establishment of optimal geometric regulation of the x-ray device, patient and sensor. The image obtained from three-dimensional object is presented as two-dimensional. The presence of exposure or geometric errors may cause the image to be suboptimal regarding the diagnostic procedure[109].

Many imaging techniques such as stereoscopy, tomography and tomosynthesis have been tried to obtain volumetric image. The computed tomography (CT) has been introduced in 1972 as a revolutionary medical imaging technique as the independent inventions of Hounsfield and Cormack. In this device, the image is created by x-ray tube with a rigidly connected detector located to place the object in the middle. The tube and detector panoramically scan the surrounding of the subject taking a thin slice via narrow X-ray beams. The reconstruction of the transmitted X-ray attenuation data produces slices of the volume created by a specific software algorithms on the axial plane[110]. CT has been regulated subsequently by addition of a fan-shaped beam, helical or spiral synchronous motion and multislice detector acquisition (MSCT) enabling to obtain faster scan

times and higher quality images[109].

CT is commonly used in dentistry for traumatic cases, the evaluation of the maxillary and mandibular structures before dental implant implementation, planning before orthognathic surgery, diagnosis of temporomandibular joint disorders and detection of calcifications, tooth or root fractures, foreign substances and localizations of the cysts[111].

CT is used in orthodontics for detection of the localizations of supernumerary and impacted teeth, the determination of the inclinations and angulations of teeth and analyses of the lateral cephalometric radiographs performed before the placement of the miniscrews used to obtain skeletal anchorage[112].

The use of CT was limited in dentistry because of device costs and use of high radiation doses[113].

b. Conic-Beam Computed Tomography (CBCT)

CBCT is an alternative imaging technique with faster reconstruction and cheaper radiation detector compared with conventional CT. The conventional CT uses a limited number of x-ray beams and measures attenuation. CBCT uses conical or pyramidal-shaped source of ionizing radiation and contains an area detector that converts multiple transmission to the direct volumetric information. In CBCT; X-ray are used more productively, the device occupies less space, it decreases the equipment cost and requires lower electrical energy[114].

3. CONCLUSION

The implementation of RME for the treatment of maxillary deficiency is supported within the adolescence period in the literature[95].

The obliteration of MPS has higher dental impact and lower skeletal impact parallelly with its rate. Side effects such as buccal inclination in teeth, mucosal ulceration, pain, and gingival recession may accompany with this condition[51,93,115]. Therefore, surgically assisted maxillary expansion is preferred in the adult patients despite its higher cost and some potential morbidities since there is no reliable parameter to assess the probability of sutural opening[93].

Since the maturation stage of MPS may show individual variation, it would be beneficial to improve the diagnosis by the accurate images and treatment protocols that enable the orthodontist to create an individualized prognosis for RME patients[17].

There are studies conducted on MPS maturation in the literature, however, further radiological, and histological studies on predominantly three-dimensional imaging techniques are needed to carry out a detailed and comprehensive analysis.

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CHAPTER 14

GENOSENSORS AND THEIR APPLICATION AREAS

Umut KOKBAS[1]

Umit YASAR[2]

Zehra Gul YASAR[3]

1 Nevşehir Hacı Bektaş Veli Üniversitesi, Diş Hekimliği Fakültesi, Tıbbi Biyokimya AD

2 Ardahan Üniversitesi Nihat Delibalta Göle MYO, Laborant ve Veteriner Sağlık Bölümü

3 Ardahan Üniversitesi Nihat Delibalta Göle MYO, Eczane Hizmetleri Bölümü

Biosensors

Biosensors are complex devices that can perform qualitative and quantitative analysis, consisting of the words “bio” (vital) and “sensor” (sensor). Biosensors were first used in the late 1950s when Lenand C. Clark developed the surgery to monitor the amount of blood oxygen with the aid of an electrode. In those years, the term biosensor, which was referred to as bioelectrode, enzyme electrode or biocatalytic membrane electrode in articles, was first used by Clark and Lyons in 1962. It consists of the combination of two interlocking systems, one biochemical and the other transducer. The biochemical part interacts with the substance to be analyzed and recognizes it. The biochemical product formed as a result of this interaction is converted into a readable numerical value by the converter part[1, 2].

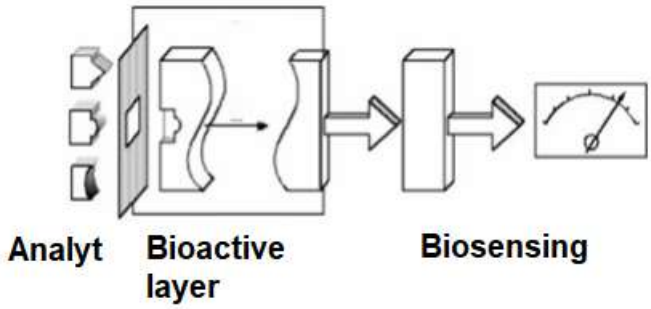

Figure 1. Principle of the biosensor.

Biosensors are analytical systems formed by combining physicochemical analysis systems and biological materials. In biosensors, the high specificity of the biological system and the detection sensitivity of the physical analysis system are combined. Many biosensors have been developed to use in the analysis of many bioorganic molecules and some inorganic molecules. Today, biosensors, especially in health; It is used in environmental analysis, military, food, drug production and chemical industries[3, 4].

Biosensors basically; It is based on the principle of formation of a signal proportional to the amount of analyte on the transmitter surface as a result of the interaction of the substance to be analyzed with the biocomponent on the biosensor surface and transmitting this signal to the measuring device[2, 5].

All biosensors perform two basic tasks: sensing at the molecular level and signal processing[2].

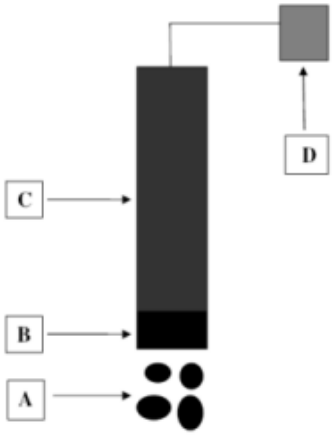

Figure 2. A) Analyt B) Bioactive layer C) Electrode D) Transducer[2]

The first biosensor studies were conducted by L.C. It is a glucose biosensor prepared by Clark by monitoring the oxygen level in the blood with an oxygen-sensitive amperometric electrode during surgery and then immobilizing the glucose oxidase enzyme on the oxygen-sensitive amperometric electrode surface in 1962. After this development, many biosensors have been developed. Biosensors are defined by the International Union of Fundamental and Applied Chemistry (IUPAC) as 'shrunken devices that selectively and reversibly respond to chemical compounds or ions and generate concentration-dependent electrical signals'. Biosensor technology is developing so fast that; Although this biosensor definition, which was prepared and published by the Commission for Classification and Naming of Biosensors formed by IUPAC in 1996, has already lost its validity with the development of biomicrochips, we can generally classify biosensors as given below[2, 6, 7].

- Electrochemical Biosensors
- Amperometric/Voltametric Biosensors
- Conductivity/Capacitance/Impedance Biosensors
- Optical Biosensors
- Absorption and Reflection Biosensors
- Surface Plasmon Resonance Biosensors

Determination of nucleic acid from biological materials in traditional methods; While a three-step process sequence is required, including sample preparation, amplification and determination of the nucleic acid sequence to be examined, determination can be performed without the need for these processes in new generation studies[8].

Genosensors (Genetic Biosensors)

Genosensors; It recognizes a genetic defect or bacteria and viruses bioelectrochemically using a probe DNA sequence. The high sensitivity of the converter and the high selectivity of the hybridization that converts this event into a measurable physical signal; genosensors in medicine, agriculture, food, pharmacy, environment etc. its use in analysis. Today, with the development of technology, the design of genomic DNA chips, which are designed for the diagnosis of hereditary and infectious diseases and are ready for clinical applications, is also based on the genosensor principle[9].

Detection of the DNA sequence with the electrochemical genosensor is based on changes in the oxidation signal of one of the DNA bases (indicatorless method (direct measurement) or in the oxidation or reduction signal of a hybridization indicator interacting with at least one of these bases (indicator method (indirect measurement)). In recent years, hybridization determination studies have been continuing rapidly in electrochemical DNA biosensors by using piezoelectric based vibration signals without using any indicator[10].

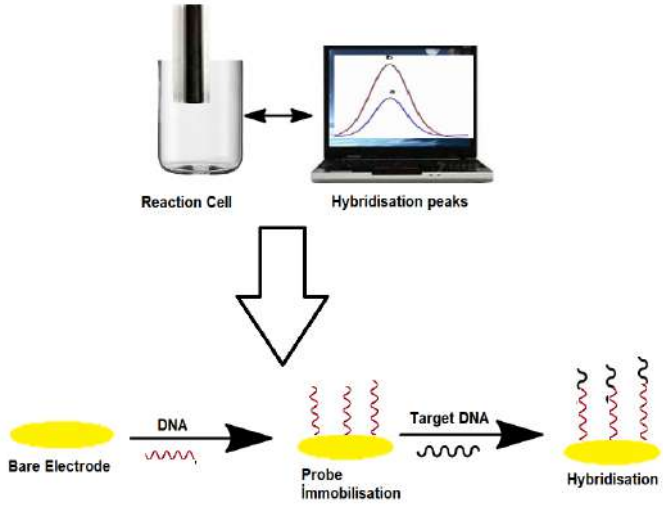

Figure 3. Working prencible of the genosensor

Prenatal (prenatal) diagnosis is one of the optional preventive medicine service tools because today's technologies cannot perform the curative treatment of genetic diseases routinely. One of the factors that contributed significantly to the routine applicability of the prenatal diagnosis tool was the developments in bio and nanotechnological fields. Developments in these areas; It has enabled the reliability of diagnosis to increase, diagnosis of more diseases, earlier and faster diagnosis, as well as the widespread use of screening tests with the development. In prenatal diagnosis applications, it is very important to be able to make an early diagnosis and make the necessary decision according to the result. The purpose of the methods used for prenatal diagnosis, besides not being a tool for termination of pregnancy; It is to provide accurate information about the condition of the fetus and to enable the family to make their own decisions within the framework of personal, social and ethical principles[11].

Prenatal diagnosis of inherited genetic disorders has been routinely applied in many countries since the practice in the 1970s, with nearly 50 years of experience. The fact that hemoglobin synthesis disorders are among the first single gene disorders identified at the molecular level has created a prototype for the development of many polymerase chain reaction (PCR)-based techniques in the literature used in mutation detection and ultimately in the diagnosis of globin gene mutations[12].

DNA Biosensors by Translator Systems

According to the transducer systems, DNA biosensors are classified as electrochemical, optical, piezoelectric and thermal biosensors. DNA optical biosensors are based on the conversion of the emission signal of the fluorescent label by the fiber optic. Fiber optics are devices that carry light from one place to another. In the operation of fiber optic DNA biosensors, the fluorescence change obtained by placing a single-stranded DNA probe at the end of the fiber and forming a double-stranded DNA hybrid labeled with a fluorescent compound (indicator) is examined. The first DNA optical biosensor was developed by Piunno et al and is based on the use of ethidium bromide as an indicator. Walt's group has developed a fiber-optic DNA biosensor that allows simultaneous analysis of different DNA sequences. A different optical transducer system can detect DNA hybridization in real time, unmarked. These biosensors are based on monitoring the change in surface optical properties as a result of surface bonding reactions[13].

In situ, unsigned optical detection can be achieved by changes in other optical properties. For example, a new nanoparticle-based colorimetric assay holds great promise for direct detection of DNA. In this case, the change in distance

resulting from the hybridization event results in changes in the optical properties of the total functional gold nanoparticles. Another innovative approach for direct fluorescence detection of DNA hybridization is based on the use of molecular beacons as probes[14].

Molecular lanterns (MB) consist of a lantern-shaped head and body. There is a fluorescent fluorophore at one of the two ends of the body and a quencher at the other end. This structure becomes fluorescent as a result of hybridization. Besides direct tracking features, molecular beacon probes offer high sensitivity and specificity[12].

Mass sensitive devices are based on the use of another indicatorless determination method, a quartz crystal microbalance (QCM) converter. The QCM is a highly sensitive mass measuring instrument and allows dynamic observation of the hybridization event. QCM hybridization biosensors consist of quartz crystal immobilized to the surface of the DNA probe. The increasing mass with the hybridization reaction causes a change in quartz frequency. A high-sensitivity microgravimetric device has been developed for the determination of Tay-Sachs genetic defect with QCM[12, 15].

Determination of Hybridization Event in Electrochemical DNA Biosensors

Electrochemical genosensors rely on base pair recognition to convert it to the appropriate electrical signal. These can be guanine signal-based (unsigned) or electrocatalytically mechanized (signal-based); They can use the electrocatalytic mechanism that uses the redox indicator (cationic metal complexes, daunomycin, electrochemical dye, methylene blue) by inserting it between the double chain or the enzyme labeling method that can be attached after the hybridization step. The enzyme-based method is based on obtaining the signal after the enzymatic reaction[3].

The Importance of Electrochemical DNA Biosensors

Compared with other methods, electrochemical methods have significant advantages. These; being easily downsized, easy, fast and cheap. Thus, in recent years, the importance of electrochemical DNA genosensors has increased in the diagnosis of genetic diseases and in other biological analyzes due to their fast and cheap cost. Modified DNA electrodes are very important in the development of electrochemical biosensors and in studying the interactions of DNA with other molecules. However, the hybridization efficiency is low in the immobilization of DNA on the electrode surfaces by conventional methods[9].

The interaction between the specific DNA probe and the target molecule has a broadly important role in the performance of the DNA biosensor. Most

typical single-stranded DNA probes designed for sensors show poor stability or specificity. Therefore, there is still a need to develop sensitive and selective DNA probes for hybridization analysis between DNA probes and their cDNA fragments. Recently, hybridization and synthesis of a new nucleotide called locked nucleic acid (LNA) has been described in some papers. LNAs have many advantages, including significant antisense activity, no toxicity in vivo, and nuclease resistance[4].

Features that an Ideal Electrochemical Biosensor Should Have

Selectivity

One of the most important parameters in an ideal biosensor is selectivity. If there is not enough selectivity, long additional processes must be added to compensate for this deficiency[2].

Lifetime

The most important factor limiting the lifetime of the biosensor is the decrease in the activity of the biological sensing surface. This situation also affects other parameters such as calibration frequency, stability and repeatability of the biosensor[2].

Calibration Requirement

An ideal biosensor needs no or minimal calibration. However, this feature is not as planned in theory, it could not be realized in practice. During their lifetime, biosensors must be calibrated frequently[2].

Repeatability

It is desirable to obtain almost the same results in successive measurements of the electrode containing the sensing surface under the same conditions. Considering this situation, which is not possible in practice, the repeatability parameter should definitely be examined in the studies carried out. It can be said that the better the repeatability, the better the applications of the biosensor[2].

Stability

High electrode stability is required for ideal biosensors. Stability depends on the physical durability of the biological material used. Also; It is also affected by parameters such as pH, temperature, humidity and oxygen concentration of the environment[2].

High Sensitivity

One of the characteristics of ideal biosensors is that the biological material immobilized to the biosensor is sensitive only to certain substances[2].

Limit of Designation

The detection limit of a designed biosensor should be below a certain concentration value. This specified limit is affected by factors such as the size of the electrode surface, the affinity of the biological material to the substance to be determined, and the amount of immobilized substance[2].

Wide Measuring Range

The region called measurement range in biosensor applications is the concentration range in which the current-concentration curves obtained from biosensors are linear[2].

Fast Response Time

The response time of a biosensor electrode can be understood from the current-time curves obtained. For example, in the curve obtained, if the shape of the steps is flat and wide, the response time is long (slow), and if the opposite is the case, the response time is short (fast)[2].

Turnaround Time

The turnaround time determines how long after the first sample the second sample can be measured, for example, in amperometric studies. In other words, if the constant current values can be observed in a short time after the addition of the first sample, it can be added after the same time in the second sample[2].

Simplicity and Cost Effectivity

Biosensors that are simple in design and inexpensive and easy to use are ideal biosensors. Therefore, the complex and expensive structures in the first biosensors were later simplified and made as cheap as possible[2].

Reducibility and Sterile

Sterile electrodes and minimizing their size are important in biosensor design[2].

Technical Problems and Market Potential

The viability of biosensors in the market depends on their versatility and

inexpensiveness in a wide range of applications. Regardless of the biosensor platform, many technical issues pose problems. First, a commercially available biosensor should have a shelf life of at least one month. Most biosensors, with the exception of the glucose meter, cannot meet this stringent requirement and this is due to the delicacy of the bio-recognition element. Second, only a few biosensors can accurately assay a biological sample in less than a few minutes, while many devices have assay times ranging from 15 minutes to several hours. Problems associated with matrix interference, sensor degradation due to the adsorption of many endogenous components in the sample to be analyzed, signal bias, microbial contamination are common to all biosensors. A successful biosensor must be variable enough to support different biorecognition elements, miniaturized to allow automation, and affordably priced[8, 13].

The medical biosensor market is dominated by the USA and Europe in 2020 with a 73% market share. Glucose meters account for more than half of biosensors worldwide, and the United States has the largest market share. Public safety and concern, new laws, food contamination in some countries have spurred the environmental and food/agricultural industries into major research initiatives[16, 17].

About 5000 people die each year in the United States from poisoning due to Salmonella or E.coli. The worldwide food production industry is $600 billion in size and needs biosensors to identify pathogens and contaminants in foodstuffs that are expected to increase in the near future. In addition, a significant amount of research has focused on the identification of biological warfare agents. Advances in the areas of toxicity, bio-availability, multi-pollutant screening will potentially enhance the biosensor market and allow it to compete with traditional laboratory-based methods[18-20].

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CHAPTER 15

IMMUNO-BASED BIOSENSORS

Umit YASAR[1]

Umut KOKBAS[2]

Zehra Gul YASAR[3]

1 Ardahan Üniversitesi Nihat Delibalta Göle MYO, Laborant ve Veteriner Sağlık Bölümü

2 Nevşehir Hacı Bektaş Veli Üniversitesi, Diş Hekimliği Fakültesi, Tıbbi Biyokimya AD

3 Ardahan Üniversitesi Nihat Delibalta Göle MYO, Eczane Hizmetleri Bölümü

1. Introduction

Worldwide leading health institutions, competent institutions on infectious diseases, have mentioned the danger of a general resistance in the use of drugs with antibiotic class. It has been determined that this situation has the potential to cause a serious public health threat in all societies. As long as the development of resistance that develops with the evolution of pathogens is not prevented, resistance to antibiotics and antivirals in diseases becomes widespread. Some studies have reported that only about one hundred thousand deaths from pathogens that have developed resistance to antibiotics per year.

As a matter of fact, while the discoveries of new antibiotics have slowed down considerably in recent years, the frequency of encountering microorganisms with multi-antibiotic resistance has increased significantly, and a future where antibiotics have lost their effect has begun to get closer [1].

In some studies in the UK, it is estimated that the problem of antimicrobial resistance will put the lives of millions of people at risk every year by 2050, and it has been estimated that a total of 100 trillion dollars will be spent in this case [2].

For this reason, early diagnosis of all pathogenic microorganisms in living organisms and early treatment of diagnosed diseases gains great importance.

Nowadays, techniques used in microorganism diagnosis include microscope studies, microorganism culture studies, Enzyme-Linked ImmunoSorbent Assay (Elisa) , Pcr (Polymerase Chain Reaction) (or real time pcr), multi-tube fermentation method, Raman spectroscopy and similar techniques.

Microscopy studies are a very effective technique for determining the morphology of unicellular organisms. However, this technique has a disadvantage that it has low efficiency than microorganism culture techniques. In addition, microorganism culture techniques are costly, time consuming and have relatively low sensitivity [3].

Elisa, It is possible to search for antibodies against antigen or antigens against antibodies. It is a diagnostic method used in virus and parasite infections. In Enzyme-Linked ImmunoSorbent Assay and similar methods, antigenic markers on the bacterial surface can be detected specifically. However, ELISA-based diagnostics are time-consuming, costly, complex, and have a narrow detection range, and often have the potential to exhibit undesirable activity in studies [4].

Polymerase Chain Reaction is defined as a common name given to the reactions applied to enzymatically amplify a unique region between two segments of known sequence in DNA. This method, which is suitable for small samples, also often produces false positive results. This situation reduces the reliability of the PCR method [5].

Multiple tube fermentation technique; It is done in 3 stages as estimation test, verification test and completion test. The aim is to put the sample volume determined according to the estimated degree of pollution in the waters into the fermentation tubes and to examine the gas formation in these tubes. Depending on bacterial growth, this test may take over 90 hours [6].

Laser-equipped Raman spectroscopy is achieved by irradiating a sample with a strong visible monochromatic beam source. This technique is very complex and very costly. [7]. Of course, the difficulties of such techniques and methods have brought along new detection techniques in the determination of biological materials. Analytes can be identified in these new techniques that are being developed, and they can work in a short time, with low cost, precision and high efficiency. Biosensors are designed by integrating biological and physicochemical parts. Biosensors working with this design can find the biochemical differences in the environment in a very short time.

Basically, it is a system that includes the transducer, output and biological molecule main headings. The differences of biological molecules (nucleic acid, antibody, enzyme, cell and tissue) used in this type of sensor systems ensure that the device is specific to the biochemical analyte. This is important in the selectivity of the desired analyte. The biochemical signals produced by the analyte to be recognized in the biochemical process in the reaction medium are detected by the transducer and converted into measurable signals. The converted signals can be displayed as electrical signals.

These devices, which can use different transducer elements (thermal, ion selective, electrochemical, optical, piezoelectric), vary in shape and size. Nowadays, sensors with optical and electrochemical transducers are used in particular. The reason for this is actually the characteristic advantages of this type of sensors [8]. Electrochemical biosensors can detect electrons and ions produced between biomolecules in a biochemical reaction. Different methods can be used in sensors with this type of electrochemical structure (potentiometric, amperometric or impedimetric) [9,10].

Basically, the current resulting from the oxidation-reduction of electroactive materials in the environment is measured in amperometric biosensors whose interelectrode potential is adjusted. The measured current is then correlated with the concentration of the analyte to be identified [11].

The correlation of the signal produced with the difference of ion concentration with the analyte concentration to be measured is evaluated in potentiometric sensors. The electrodes used are ion selective [12].

Frequency and response are also important in electrochemical impedance

spectroscopy (EIS). It can be used to obtain information about the changes (chemical, physical) caused by a molecule immobilized to the electrode with the analyte to be measured. The affinity between the analyte and the immobilized biomolecule is monitored in real time [10].

Optical biosensors identify the changes in the optical properties of the analyte to be measured and convert it into a signal. These sensors have advantages such as small size, ease of use and simplicity of measurement. Thanks to these advantages, they are preferred in many fields (medicine, environment, food, etc.) [13].

Thermal sensors measure the concentration of the target analyte using the change in reaction enthalpy. This type of biosensors is highly sensitive to even small changes in temperature. In thermal-based biosensors, the biomolecule is fixed in a container and the reaction takes place there. The enthalpy change is followed by monitoring the change in the temperature of the container [11].

Surface Plasmon Resonance (SPR) method can also be used in target analyte measurements. In this method, the interaction of biological molecules on the surface of the transducer can be detected. The method with components such as detector, light source, gold film, prism is a very sensitive method. The purpose of analyte measurement is to follow the resonance shifts formed by the conformationally changing biomolecules and to correlate this with the substrate concentration [7].

Recently, biosensors have been used in many fields and they continue to be developed by researchers. The use of some commercialized biosensors in fields such as medicine, food and environmental monitoring is increasing day by day. In addition, the ease of use, fast results and ease of use of these sensors provide advantages over other methods.

2. Immunosensors

They emerged from the classical immunotest approach. These are the types of sensors in which immunoactive substances are used as a specific element. They are sensors produced by immobilization of antigen or antibodies to the sensor surface to form a stable complex. The antibody-antigen reaction is highly selective. They are generally classified according to the measuring principle. Electrochemical, optical, acoustic, piezoelectric and thermometric sensing elements are used as sensor platforms for immunosensors [14]. In particular, his contributions in the field of medicine, immunology and clinic provide high success rates in diagnosis. Therefore, it is among the current development technologies by researchers [15].

When the structures of the developed and tried to be developed immunosensors are examined, the immobilization of analytes or biological molecules is encountered. In the literature, it is seen that sulfhydryl, covalent bond, carboxyl

and amino bonds are mostly preferred among these surface bonding techniques. The stability of these bonds ensures the stability of the attached molecule. The result is the usability and selective specificity of the designed immunosensor. These immunosensors are preferred clinically, especially since they signal by monitoring the changes in antigen-antibody complex formation [16,17,18].

Immunosensors produced by immobilization of antigen or antibodies on the sensor surface are generally classified according to the measurement principle. A common situation encountered with immunosensors is that these sensors are irreversible. Therefore, an immunosensor can often be used only once. Recently, some researchers have been working towards the development of a renewable sensor surface.

2.1. Optical immunosensors

The basis of these sensors is the detection of the changes created by the desired recognition elements in an optical field. These widely used sensors can detect without or using tags. In the label-free principle, the sensor generates a signal by directly analyzing the optical changes in the analyte. Labeled optical sensors also use light or colorimetric labels. The signal obtained as a result of interaction with the transducer is produced by the luminescence produced by this label.

Optical sensors are devices with a complex structure with a biomolecule and an optical transducer (Figure 1). In these devices, it is to analyze and display by generating signals proportional to the concentration of the analyte to be measured. The biological elements used in the modification can be hereditary material, enzyme, cell, antibody, antigen or receptor. The use of biological elements such as these is the reason for their selective specificity [19].

The most widely used in optical sensors are SPR-based immunosensors. In SPR, the layer is modified with antibody. The light absorption change that occurs when antigens in the environment are attached to the antibody layer coated on the metal surfaces of these immunosensor devices is analyzed [20].

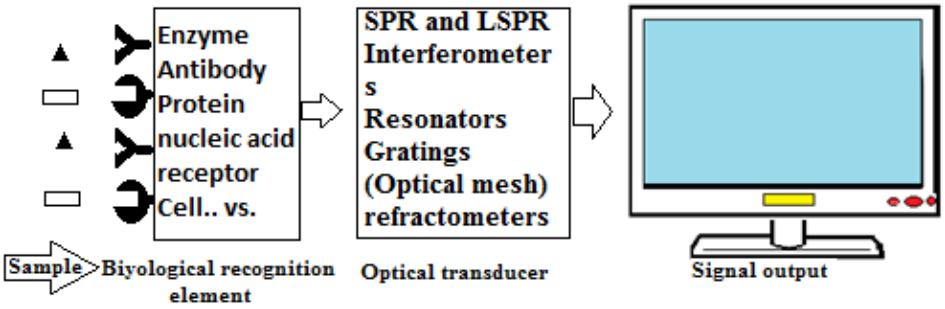

Figure 1. Schematic representation of optical sensors [19].

2.2. Piezoelectric immunosensors

Piezoelectric biosensors are analytical devices that work on the affinity principle. Piezoelectric immunosensors are biosensors that contain antibodies as a biorecognition element, and the specificity of the antibody significantly affects the specificity of the entire immunosensor. When the antibody used is specific, it is specific in the piezoelectric immunosensor [21].

2.3. Thermal (calorimetric) immunosensors

Most chemical and biochemical processes involve the absorption and generation of heat. This heat exchange is related to the amount of matter present. The device is coated with the bioreceptor, and when it interacts with the analyte, an exothermic reaction occurs, which is recorded as a temperature change [20].

Most chemical and biochemical processes involve the absorption and generation of heat. This heat exchange can be measured with either a thermistor or a thermophile and is related to the amount of matter present. A thermistor contains a metal oxide, a thermophile a silicon-gold material. The device is coated with the bioreceptor, and when it interacts with the analyte, an exothermic reaction occurs, which is recorded as a temperature change. They determined that enzymatic reactions of different molecules are possible using a calorimetric-based biosensor. The most obvious advantage of this technology is that it can be easily miniaturized and can be used directly in the system. Despite these advantages, there are few studies on calorimetric immunosensors in the literature [22].

2.4. Electrochemical immunosensors

As with optical sensors, these sensors are often preferred in medicine, environmental monitoring, food industry and quality control applications. Most of the research on the development of biosensors is on electrochemical immunosensors. The reason for focusing on them is that they are suitable for high efficiency, cost, precision and miniaturization.

Commonly used electrochemical processes to measure the amount of a relevant analyte are cyclic, square wave voltammetry, potentiometry, and spinning disk methods. The focus is on the effective use of nanomaterials in electrochemical sensors and thus on high-sensitivity electrochemical immunosensors as a result of innovative applications [23].

It is seen that electrochemical-based sensor studies are used more commonly than others in literature research. Researchers state that the popularity of electrochemical immunosensors in clinical analysis is increasing, and this is mainly due to the development of sensor design. In the bioreceptor layer, in an

electrochemical immunosensor system where antibodies are immobilized, there are other non-specific substances in the sample solution besides the antigen specific to the immobilized antibodies. The complex formation reaction takes place in the bioreceptor layer between antibodies and antigens specific to these antibodies only. Due to the complex formation, a change occurs in the bioreceptor layer between the state before binding and the state after binding. This change is converted into a measurable signal by the electrochemical transmitter and evaluated [24]. The working principle of an electrochemical immunosensor is schematized in Figure 2.

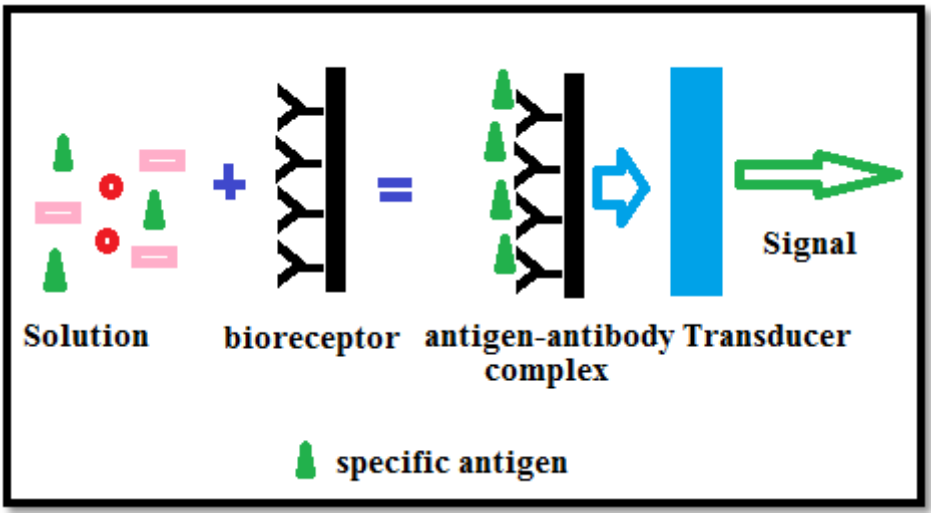

Figure 2. The working principle of the immunosensor is schematized.

2.5. Amperometric immunosensors

It is based on amperometric measurement of the current occurring between species with reducing and oxidation properties at a constant potential. It is an electrochemical immunosensor. Since these sensors can only be applied to electroactive analytes, they have not been developed in large numbers. This is not surprising, especially given that most of the antigens and antibodies are not electroactive. If analyte evaluation is to be made, an intermediary electroactive label may be used.

Of course, it is a disadvantage that a sensor cannot take measurements directly. Since these biosensor types use the tool, their use and development has been limited. However, its efficiency and sensitivity in the determination of electroactive materials is excellent. This biosensor, which is generally used for toxin concentration, is an important development in biosensing. [20, 25].

Of the amperometric biosensors, oxygen electrodes are the most commonly used sensors. Oxygen-permeable membranes used in these sensors are one of the most important developments. Various membrane materials such as Teflon, polyethylene and silicone rubber have been used for permeability. In these membranes, the selectivity should be selected according to the nature of the application conditions. [26].

2.6. Conductometric immunosensors

These sensors measure electrical conductivity at constant voltage. The current that occurs as a result of ion exchange that takes place in specific reactions (such as enzyme reactions) is measured. Conductometric based biosensors use the relationship between conductivity and biorecognition phenomenon. A conductometric biosensor has two metal electrodes located at a certain distance. These electrodes are mostly paltin or silver electrodes. Normally, an alternating current voltage is applied to the electrodes, which allows the current flux between them to be maintained. During a biorecognition event, the ionic composition changes and an ohmmeter (or multimeter) is used to measure the change in conductivity between metal electrodes. Compared with other electrochemical methods, the biggest disadvantage of the technique is its low sensitivity [24, 27].

2.7. Potentiometric immunosensors

Potentiometric systems measure the potential change in the ion-selective electrode due to an ionic reaction. In systems where the current passes little or no current, the electrochemical determination method in which the changing potential depending on the concentration change, which the indicator electrode shows against the reference electrode, is measured, is called potentiometry. The sensors used in this method are called potentiometric sensors. A potential change occurs depending on the concentration of the ions or ions present in the environment, and since this change is related to the concentration of the ions, their concentrations can be determined [28].

3. Antibodies

An antibody is a biomolecule that has the ability to recognize a specific antigen and is symbolized by “Ab”. An antigen is any molecular substance that causes an immune response and is recognized as foreign by the body, and is symbolized by “Ag”. Antibodies are highly preferred as a biological recognition element, as they have a high degree of specificity allowing recognition of a suitable antigen in the presence of interference effects. Antibodies are capable of recognizing antigen

over a wide range. Heterogeneous antibodies that respond to the same antigen are called polyclonal. Monoclonal antibodies are antibodies that only react against one epitope.

There are immunosensors used for disease detection and clinical purposes. For example, it is possible to multiply samples such as cancer biomarkers in serum (biomarker carcino-embryonic antigen), TGF-β1 (kidney disease) in urine, alpha fetoprotein (hepatocellular carcinoma), low-density lipoprotein (LDL) levels (cholesterol) [29; 33].

4. Results

The industry has increased the preference of biosensors in determining the analyte concentrations desired to be determined in the environment and medicine. The most important reason for this situation is that they are fast and low cost. Today, a large number of designed biosensor methods are still seen. These designs are enzyme, cell, tissue, DNA or antigen/antibody based. Since working in many fields increases the value of sensors economically, they are among the devices that are tried to be developed academically. Immunosensors, which have not been developed much commercially, will make significant contributions with the opportunities offered by today's technology (nanotechnological). There are studies on the development of sensitivity, stability and similar qualities of biosensors designed by considering the unique physical and chemical properties of nanomaterials.

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CHAPTER 16

THE ROLE OF TECHNOLOGY READINESS IN THE USE OF DIGITAL APPLICATIONS BY HEALTHCARE PROFESSIONALS

Zehra ANTEP[1]

Hasan Sadik TATLI[2]

Gokten ONGEL [3]

1 Ph.D., Dr. SiyamiErsek Training and Research Hospital, Department of Management, mail: zehra.antep@hotmail.com OrcID:0000-0003-1349-4401

2 Research Assistant, Beykent University, FEAS, Department of Business Administration (English), mail: hasantatli@beykent.edu.tr, OrcID: 0000-0003-1918-3188

3 Ph.D./ M.D., Istanbul Education Research Hospital, Children's Health and Diseases Clinic mail: goktenkorkmaz@yahoo.com OrcID No: 0000-0002-4165-3601

Introduction

It is observed that technological advancements in every field in recent years have affected the health sector as well as all sectors and have made change necessary. Technology and information systems that have begun to be used in the field of health aim to provide the quality delivery of health services with the objectives such as cost control and patient and employee safety, as well as increasing the efficiency and effectiveness of health services.

HIMSS[1], a non-profit organization that provides guidance on using information and technologies for better and safer delivery of health services in hospitals on a global scale and develops methods and algorithms, established the EHRAM[2] accreditation system to create the international standards of hospitals in the process of digitalization. This standardization system, which is represented in more than 200 countries worldwide, has also begun to be used in hospitals in our country. HIMSS is an organization that consists of the members of health stakeholders, who aim to use health informatics and management systems more efficiently for the benefit of the health sector, and that aims to create international standards in hospitals (Kılıç, 2016).

In today's world, where information technologies are transferred rapidly, the stunning spread of technological systems in the field of health has increasingly made healthcare service providers and recipients dependent on technology. However, it is observed that people's adaptation to technology use does not occur at the same speed. Individuals' attitudes, experiences, and skills with regard to technology differ. As there are individuals who love technology and have no difficulty using it, there are also individuals who abstain from technology and do not have the skills to use technology effectively. In the Technology Readiness Index (TRI) developed by Parasuraman (2000) based on these differences, it is argued that personal factors such as optimism, innovativeness, discomfort, and insecurity owned by individuals affect their adoption and use of technological systems. According to Parasuraman, the dimensions of optimism and innovativeness enable individuals to have a positive attitude toward technology and easily use and accept new technologies; however, insecurity and discomfort may cause individuals to exhibit a negative attitude toward technology and, therefore, not to be ready to accept and use technology. In the literature, there are many studies on how effective the level of individuals' readiness to use technology is on their level of using a technology/application (Lin et al., 2007; Lin and Chang, 2011; Acheampong et al., 2017; Bakırtaş and Akkaş, 2020). Unlike other studies, this study aimed to determine whether there was a relationship between technology readiness levels and technology usage levels of employees in health institutions in the process of digitalization. The study results are important in terms of developing and maintaining sample applications that are applicable for healthcare professionals in the development and digitalization of the health system and accessible for healthcare service recipients. Within the

[1] HIMSS (Healthcare Information and Management Systems Society)
[2] EHRAM (Electronic Medical RecordAdaption Model)

scope of the study, information about the technology readiness level and the theoretical framework is first presented. Then, the relationships between the concepts are determined, and the results are interpreted. Finally, the study is concluded by presenting recommendations for increasing the adaptation of healthcare professionals to technology within the scope of digitalization.

1. Conceptual Framework of the Study

In the current digital age, companies have increased their use of technology for reasons such as not losing their competitive advantages, increasing their productivity, increasing employee and customer satisfaction, increasing their flexibility and being permanent. However, it is observed that differences between individuals lead to differences in terms of technology readiness and new technology acceptance.

Many approaches and measurement models on new technology acceptance have been developed. The theory of reasoned behavior, technology acceptance model, theory of planned behavior, integrated technology acceptance, and usage model are the most common among them. The theory of reasoned behavior emphasizes the individual's positive and negative value judgments, the presence of social pressures, and the readiness of users to use new technologies (Fisbein and Ajzen, 1975). The technology acceptance model (and its expanded versions) focuses on the usefulness of new technologies, the ease of use of technology, attitudes toward usage, and intention to use in individuals' acceptance of new technologies (Davis, 1985;1989). The planned behavior approach is based on the theory of reasoned behavior and focuses on the competence of individuals to use technologies (Ajzen, 1991). The integrated technology acceptance model emerged as a result of the development of the technology acceptance model. The model focuses on factors such as social impact, effort, performance expectation, and ease of use in the individual's acceptance of new technologies.

Within the scope of the study, it was aimed to determine factors affecting individuals' use of new technologies based on the theory of planned behavior. The reason why the theory of planned behavior is the main approach in the study is that it focuses on individual factors in individuals' use of new technologies. The theory of planned behavior was derived from the theory of reasoned action (Ajzen and Fishbein, 1980). The theory of reasoned action is based on the use of will in an individual's behaviors (Özer et al., 2015). Nevertheless, individuals also act against their will while performing certain behaviors. Ajzen (1985) states that some behaviors occur apart from routines and that unplanned parts of behaviors are difficult to realize.

In the theory of planned behavior, it is argued that individuals are guided by behavioral intention to perform an action and that special norms and behavioral control are important factors for the realization of behavior (Ajzen, 1991). Attitude is a person's evaluation of behavior as positive or negative (Fishbein and Ajzen, 1975). Special norms refer to the external pressures applied to the individual for the realization of the behavior (Ajzen, 1991). In imperative or voluntary contexts, the effect of behavioral intention on the special norm may

become meaningless (Mathieson, 1991; Venkatesh and Davis, 2000). Behavioral control is difficulties and facilitators in the realization of the relevant behavior (Ajzen, 2002).

When these views are considered in terms of technology, individuals' behaviors of using new technologies are expected to be complex. There are motivational factors that direct individuals to use technology and internal and external factors that prevent usage. Claudi et al. (2015) state that the use of technology by people is not certain, although it has become widespread. Technology readiness plays a major role in individuals' tendency to use technology (Meuter et al., 2005; Parasuraman and Colby, 2014). Technology readiness is defined as individuals' tendency to adopt and use new technologies to achieve their goals in their lives. Technology readiness level is the individual difference variable in the use of technologies by individuals (Parasuraman, 2000). Technology readiness is evaluated on four dimensions: innovativeness, optimism, insecurity, and discomfort (Parasuraman and Colby, 2014; Blut and Wang, 2020). The innovativeness and optimism dimensions of technology readiness represent motivating factors that contribute to technology readiness, while insecurity and discomfort represent the inhibiting factors (Blut and Wnag, 2020). The optimism dimension of technology readiness refers to a generally positive view of technologies and the belief that technology gives people more flexibility and offers control and efficiency. Innovativeness refers to the tendency of individuals to be a pioneer and opinion leader in technology compared to individuals around them. Innovative individuals are pioneers in trying new technologies compared to the people around them. Discomfort refers to individuals' lack of control over technology and the state of being overwhelmed by technology. Discomfort represents prejudice against technology. Insecurity refers to being skeptical that technological elements will work properly (Parasuraman, 2000; Lin and Chang, 2011). Technology readiness is used to predict individuals' general attitudes toward technologies (Walczuchet al., 2007; Verhoefet al., 2009; Sun et al., 2020).

2. Literature Review and Hypothesis Development

The fact that individuals' relationships with technology lead to different consequences at the stage of using technology (Verhoef et al., 2009) causes the research framework on technology usage to be limited to certain variables. Due to the need for limitation, the study was conducted within the scope of "actual use" and "technology readiness." Within the scope of the study, it was aimed to determine to what extent healthcare professionals use the HIMSS program in the relevant institutions and the role of inhibiting and motivating factors in this usage.

According to Parasuraman (2000), optimism is a general structure that captures certain feelings of people and shows that technology is a good thing. Optimistic people generally use more optimistic strategies that are more effective in achieving the expected results (Tsikriktsis, 2004). In other words, optimists are less likely to focus on negative events, and they accept technology more freely (Walczuch et al., 2007). Therefore, optimists perceive technologies as more

useful and easier to use since they are less disturbed by the negative consequences of technology. Based on the specified views, the following hypothesis was developed.

H_1: Optimism positively affects healthcare professionals' use of HIMSS.

According to Parasuraman (2000), innovative individuals are people "who adopt technology early and tend to be opinion leaders." In the study conducted by Cruz-Cardenas et al. (2021), it was determined that innovativeness positively affected the actual behavior, which indicates that individuals with technological innovativeness have stronger intrinsic motivations to accept the new technology and even enjoy the feeling of trying new technology (Yi et al., 2005). People with high innovativeness generally have a positive impression of the usefulness of a new technology. Based on the specified views, the following hypothesis was developed.

H_2: Innovativeness positively affects healthcare professionals' use of HIMSS.

Insecurity is defined as skepticism about technology and the ability to function properly (Parasuraman, 2000). People with high insecurity are usually skeptical about the security of new technologies and usually ask for security (Parasuraman and Colby, 2001). In other words, they can feel that there may be some risk while using the new technology. The studies in the literature confirm that the perception of insecurity adversely affects the tendency to use technologies (Siegrist, 2000; Lu et al., 2005; Chiu and Cho, 2021). According to Tsikriktsis (2004), when people believe that they will obtain great advantages using the new technology, they are willing to take the risk of using such technology. Based on the views in the literature, the following hypothesis was developed.

H_3: Insecurity negatively affects healthcare professionals' use of HIMSS.

According to Parasuraman (2000), discomfort refers to the perceived lack of control over technology and a feeling of being overwhelmed by it. People who are uncomfortable with technology believe that they are controlled by it and that technologies are not suitable for common people. Various studies in the literature indicate that individuals who tend to have anxious feelings about the use of technology have a negative impact on technological innovations (Igbaria et al., 1994; Hackbarth et al., 2003; Godoe and Johansen, 2012). Based on the views presented above, the following hypothesis was developed.

H_4: Discomfort negatively affects healthcare professionals' use of HIMSS.

3. Methodology of the Study

3.1. Aim and Importance of the Study

With the renewed world, technology has continuously developed and changed. According to Parasuraman (2000), individuals' tendency to new technologies is encouraged by some personal factors (optimism, innovativeness). However, it can be prevented due to personal factors (comfort, insecurity). In many studies attempting to determine factors affecting the technology usage of individuals, evidence indicating that internal and external factors are effective, especially in the technology usage of individuals, was found (Ajzen, 1991;

Parasuraman, 2000; Kou et al., 2013). The factors affecting the use of health technologies are diverse, such as special norms (Holden and Karsh, 2010), behavioral control (Duyck et al., 2008), and usage skills (Schaper and Pervan, 2007). The motivating and inhibiting factors in the technology usage of individuals are observed to be effective on the use of the relevant technologies. The conclusions on how effective technology readiness is in the use of technology are still unclear. The aim of the study, which was designed based on the relevant views, was to investigate how the technology readiness of healthcare professionals and their perceptions of HIMSS digital hospital systems affected the use of the HIMSS system. The results obtained as a result of the study are important in terms of understanding how the newly developed applications in the field of health will be reacted and how employees will adapt to them. Furthermore, they are also important in terms of observing whether physicians execute treatment periods and nurses perform their daily care routines and record keeping more efficiently and effectively in the hospitals adopting digital applications. Moreover, most of the previous studies focused only on the intention to use technologies (Dünnebeil et al., 2012). Along with the increase in technology use (remote working, working from home, using digital tools, etc.), especially during the COVID-19 pandemic, people have further come into contact with technologies. In fact, there may be differences in the results of the studies conducted after the pandemic and the studies conducted during the pandemic. The results obtained will enrich the literature in terms of making periodic comparisons.

3.2. Research Model and Hypothesis Development

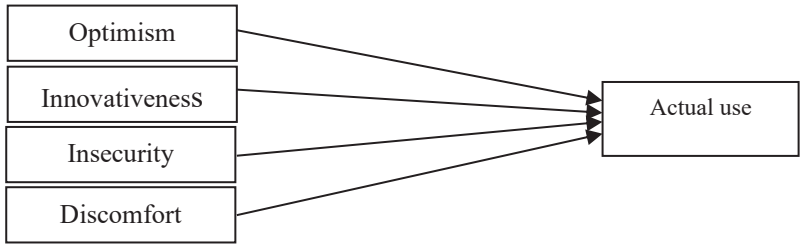

Figure 1. Research Model

According to Figure 1, the variables of optimism, innovativeness, discomfort, and insecurity constitute the independent variables of the study, and the actual use refers to the dependent variable.

3.3.Population of the Study and the Sampling Process

The study was conducted in a public hospital in Istanbul that has entered into the digitalization process with the use of the HIMSS-EHRAM model. Within the scope of the study, online questionnaire forms were distributed to 1200 healthcare personnel in the relevant hospital. Participation in the study was entirely

voluntary.The simple random sampling technique was used to determine the sample. It was attempted to reach all healthcare professionals, and the questionnaire forms were presented. Positive responses were received from 351 people to the online questionnaire forms. In the social sciences literature, it is indicated that at least 10 samples per item are required for the adequacy of the sample number (Hair et al., 2014). Considering that the measurement tool used in the study consisted of 19 items, 351 participants were considered to be sufficient since their number was greater than the minimum (19×10=190) number of 190. Furthermore, the sample size should not be very large since insignificant relationships acquire significance in the analyses performed with the data obtained from very large samples. Therefore, the number of questionnaires was stopped at 351. Thus, spurious significance (at $p<0.05$) that would occur depending on the number of samples was prevented.

The characteristics of the sample included in the study are as follows. Healthcare personnel consisted of physicians by 50%, nurses by 27.7%, administrative staff by 8.3%, and individuals with other titles by 14%. Healthcare professionals who participated in the study consisted of people with high school-associate degree education by 11.2%, people with a bachelor's degree by 30.6%, people with a master's degree by 30%, and people with doctorate education by 28.3%. Furthermore, the participants consisted of females and males by 55.4% and 43.7%, respectively. However, 0.9% of them did not specify gender. While 34.9% of the participants were 30 years old and younger, 32.32% of them were in the age range of 31-40 years, 22% of them were in the age range of 41-50 years, 9.7% of them were in the age range of 51 years and over. However, 1.1% of them did not specify their age. While 42.3% of healthcare professionals worked for 5 years or less in the same institution, 16.6% of them worked for 6-10 years, 14.3% of them worked for 11-15 years, 11.1% of them worked for 16-20 years, and 13.7% of them worked for 21 years and more. Furthermore, 2% of the participants did not provide information about their working time.

3.4. Research Method and the Creation of Scales

An online questionnaire form was prepared to determine the technology readiness levels of healthcare professionals and their levels of using HIMSS applications. The developed questionnaire form consists of 3 sections and includes 24 questions. In the first section of the questionnaire, there are 5 questions to determine the demographic characteristics of healthcare professionals. The Technology Readiness Index (TRI 2.0), developed by Parasuraman (2000) and reduced to 16 items by Parasuraman and Colby (2014), was used to measure the technology readiness level. The scale includes the dimensions of optimism (4 statements), innovativeness (4 statements), insecurity (4 statements), and discomfort (4 statements).

The items of the Actual Use Scale (3 items), which was developed by Hu et al. (2003), Venkatesh et al. (2003), Yang and Yoo (2004) and is frequently used in the literature, were used to measure the level of using HIMSS applications by healthcare professionals. Within the scope of the study, the questionnaires were

used in the form of a 5-point Likert-type scale (1=Strongly disagree, 2=Disagree, 3=Neutral, 4=Agree, 5=Strongly agree).

3.5. Results of the Study

In the analysis of the data obtained from the study, factor and reliability analyses were first performed using the SPSS 25 package program. Then, descriptive statistics were used to identify the sample characteristics and variables. First, correlation analysis and then regression analysis were conducted to test the conceptual model of the study. The obtained results are presented below.

Factor and reliability analysis results

The validity and reliability analyses of the measurement tools used in the study were performed using the SPSS 25 package program. The factor analysis and reliability analysis were first conducted, and the results are presented in Table 1. The determination of the limit values of factor and reliability analyses was based on the values specified by Hair et al. (2014) and commonly used in the literature. These values were KMO sampling adequacy; 0.70, Bartlett's test of sphericity; $p<0.05$, total variance explained; 60% and Cronbach's alpha; 0.70.

Table 1. Factor and reliability analysis results

Transformed component matrix[a]					
Items	Component				
	Innovativeness	**Optimism**	**Actual use**	**Discomfort**	**Insecurity**
Innovativeness 2	.843				
Innovativeness 1	.842				
Innovativeness 4	.764				
Innovativeness 3	.716				
Optimism 3		.788			
Optimism 4		.785			
Optimism 2		.782			
Optimism 1		.761			
Au2			.901		
Au3			.882		
Au1			.868		
Discomfort 4				.756	
Discomfort 3				.739	
Discomfort 2				.723	
Discomfort 1				.614	
Insecurity 3					.783
Insecurity 2					.748
Insecurity 1					.669
Insecurity 4					.578
Variance explained (%)	14.475	14.360	13.195	12.244	11.282
KMO sampling adequacy = .788 Approximate chi-square =2675.700 Degree of freedom =171 Bartlett's test of sphericity significance =0.000 Total variance explained (%)s=65.556 Cronbach's alpha= 0.714 Number of items = 19					

Factor analysis and reliability analysis results are presented in Table 1. According to the results of the factor analysis, it was observed that the KMO value was 0.788, Bartlett's test of sphericity result was $p<0.05$, and the total variance explained was 65.55%. According to the reliability analysis results, it was observed that Cronbach's alpha coefficient was 0.714, and there were 19 items in the measurement tool. It was revealed that the technology readiness index was loaded on 4 factors and the actual use scale was loaded on a single factor. When the obtained results were considered together, it was possible to state that it was appropriate to use measurement tools measuring the technology readiness level and individuals' technology use levels.

Table 2. Relationships between the variables and descriptive statistics

	Mean	Std. Deviation	1	2	3	4	5
Actual use (1)	*3.6206*	*.93976*	1				
Optimism (2)	*4.0786*	*.77402*	**.308****	1			
Innovativeness (3)	*3.4398*	*.99728*	**.262****	**.433****	1		
Discomfort (4)	*2.8059*	*.91096*	**-.190****	-.075	-.071	1	
Insecurity (5)	*3.8930*	*.80112*	**-.213****	**-.120***	**-.144****	**.467****	1

***. Correlation is significant at the 0.01 level,*
**. Correlation is significant at the 0.05 level (2-tailed)*
N=350

The results of the correlation analysis are presented in Table 2. While the low level of significant relationships was found between healthcare professionals' use of HIMSS and their optimism toward technology use, low, positive and significant relationships at the $p<0.05$ level were found between their optimism and innovativeness. Furthermore, very low relationships were found between their levels of using HIMSS technologies and their discomfort with technology, and low, inverse relationships at the $p<0.05$ level were found between their levels of using HIMSS technologies and their distrust of technologies. Moreover, according to the descriptive statistics showing healthcare professionals' technology readiness levels and levels of using HIMSS technologies, healthcare professionals had a high usage level of HIMSS technologies. While healthcare professionals' general optimism about technology use was very high, their level of innovativeness and their level of discomfort were neutral, and their level of insecurity was high.

Table 3. Regression analysis results

Variables	R^2	Adjusted R Square	Durbin-Watson	Fixed (The dependent variable)	Standardized coefficient (Beta)	ANOVA[a] Sig.	Hypothesis
Optimism (x_1) → Actual use (y) (Model-1)	.095	.092	1.467	2.105	.308**	0.000[b]	H_1= Supported
Innovativeness (x_2) →Actual use (y) (Model-2)	.068	.066	1.493	2.731	.263**	0.000[b]	H_2= Supported
Insecurity (x_4) →Actual use (y) (Model-4)	.045	.042	1.465	4.589	-.213**	0.000[b]	H_3= Supported
Discomfort (x_3) →Actual use (y) (Model-3)	.036	.033	1.430	4.172	-.190**	0.000[b]	H_4= Supported
Total effect							
Optimism (x_1), Innovativeness (x_2), Discomfort (x_3), Insecurity (x_4) →Actual use (y) (Model-5)	.149	.139	1.486	2.914	x_1= .216** x_2= .146* x_3= -.113* x_4= -.115*	0.000[b]	
*= *coefficient significance at the 0.05 level* **=*coefficient significance at the 0.01 level*							

The results of the regression analysis performed to determine the role of technology readiness levels in the use of the HIMSS application by healthcare professionals are presented in Table 3. As a result of the regression analysis, healthcare professionals' optimism toward the use of technology increased the use of the HIMSS application by 9.2%. Furthermore, the fact that healthcare professionals were innovative about technologies increased the level of using HIMMS applications by 6.6%. It was observed that the factors of discomfort and insecurity that adversely affected technology readiness reduced the level of using the HIMSS application by 3.3% and 4.2%, respectively. In the model created to determine the effects of positive (optimism, innovativeness) and negative (insecurity, discomfort) factors that affect individuals' technology readiness levels together on the level of using the HIMMS application (model-5), the effect of technology readiness level on using the HIMSS application was 13.9%. In the total model (model-5), healthcare professionals' optimistic and innovative approaches toward technology increased the level of using HIMSS technologies. However, discomfort and insecurity reduced the level of using HIMSS. Optimism toward technologies was the technology readiness factor that mostly affected the use of HIMSS.

Conclusion and Discussion

In the study conducted to determine how the technology readiness of healthcare professionals and their perceptions of HIMSS digital hospital systems affected the use of the HIMSS system, healthcare professionals' technology readiness levels and levels of using HIMSS applications were first examined. It was observed that healthcare professionals' levels of using HIMSS technologies were at a level that could be considered high. It was observed that healthcare professionals had an optimistic approach toward general technologies. However, their innovative attitudes were not high enough. It was observed that their trust in technologies was low, and their discomfort with technologies was not very high. Optimism, innovativeness, discomfort, and insecurity levels of healthcare professionals affected the level of using the HIMSS application. While optimism and innovativeness (motivating factors) positively affected the use of HIMSS application, discomfort and insecurity (inhibiting factors) adversely affected it. Furthermore, while the most effective factor in using HIMSS applications by healthcare professionals was optimism, the least effective factor was discomfort.

When the results obtained as a result of the study are considered in general, the result indicating that the motivating factors that allowed healthcare professionals to be ready for technology increased the use of technology was an expected result. The obtained results are similar to the views presented by Ajzen (1991), Parasuraman (2000), Tsikriktsis (2004) and Walczuch et al. (2007). Furthermore, strengthening healthcare professionals' positive views on technology, increasing their self-efficacy about new technologies, and emphasizing the useful and amusing aspects of new technologies (Compeau and Higgins, 1995; Cruz-Cardenas et al., 2021; Rivers, 2021) will enable them to use new technologies more effectively. The results indicating that the inhibiting factors affecting technology readiness reduced the use of HIMSS technologies are consistent with the literature (Parasuraman, 2000; Godoe and Johansen, 2012; Chiu and Cho, 2021). Explaining the benefits of technologies to healthcare professionals and providing them with support for the solutions of potential problems may decrease their anxiety about technologies. As stated by Tsikriktsis (2004), employees with an idea about the advantages of technology will have a more positive tendency to use new technologies.

The results obtained in the study differ from the literature in some aspects. Although similar results with previous studies were found, the different context of the study paves the way for new discoveries in the results obtained. The obtained results differ from the literature in that the study was conducted during the COVID-19 pandemic and provided results on a new technology actively used in the health institution. The decrease in contact between people during the COVID-19 pandemic and the fact that many work procedures were done with technological tools increased the use of technology by employees and other segments of society. In this respect, new research agendas have emerged in the use and adoption of new technologies. Here, it is recommended that future studies be conducted in terms of institutional pressures, personality traits of employees, and organizational support. On the other hand, the results of this study have some limitations. For example, the increased use of technology as a result of institutional pressure was not examined within the scope

of the study. The use of technology may differ in imperative or voluntary contexts (Mathieson, 1991; Venkatesh and Davis, 2000). Since the techniques used methodologically express the views of healthcare professionals, they are limited in terms of reflecting real behaviors. Finally, since the study was conducted in a health institution, institutional and regional dynamics were not examined within the scope of the study.

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CHAPTER 17

APPROACH TO CARBON MONOXIDE (CO) POISONING

Nuray Kılıç[1]

1 Dr., Manisa Alaşehir Devlet Hastanesi

Carbon monoxide (CO) is formed during the combustion of carbon-containing fuels such as wood, coal, dung, natural gas, and heating devices used for heating purposes, especially in winter (table 1). Unfortunately, carbon monoxide, which comes out of combustible materials that are still used as a heating tool in cold winter days, has a very high killing potential. Carbon monoxide is also a colorless, odorless, tasteless gas that is difficult to detect. It is also known as the "silent killer" by the society due to these features.

Carbon monoxide poisoning is the most common cause of acute fatal poisoning and death by fire. According to the studies, 20 000 cases and 500 deaths per year are seen in emergency services in the USA, 400 deaths per year in Europe (1) and 10 000 cases per year in our country and 39% of the rate of death.

Smoke inhalation from fire is responsible for most CO poisonings. Non-fire CO exposure most often comes from the stove. Stove poisonings are usually caused by improper chimneys, closed air vents, poor quality fuel use, adding coal to the stove while lying down, closing the air inlets and outlets of the stove, and in addition to this, the CO rate in the house increases in southwestern and reverse winds(2)

Table 1. Sources of carbon monoxide

1. Wood or coal-fired ovens or heaters
2. Propane fired heaters
3. Building fires
4. Natural gas heaters/ ovens/ generators
5. Automotive exhaust
6. Motorboat exhaust
7. Gasoline-powered generators or engines
8. Forklifts/ frosted surface coating machines
9. Methylene chloride

CO has a greater tendency to bind to hemoglobin (Hb) than to oxygen (O2)(3). We are exposed to carbon monoxide through inhalation or oral intake. CO is about 10 ppm in the air but can be higher in the city. CO is also an endogenous substance and is produced in our body during the breakdown of Heme from its physiological circulation. Normal physiological levels of CO (as carboxyhemoglobin) in this event average 1% in healthy, non-smokers. CO production may be physiologically increased in hemolysis or sepsis. CO poisoning causes deep tissue hypoxia and the initiation of the inflammatory process. Unfortunately, this situation causes permanent degenerations in the cardiac, cerebral and, to a lesser extent, peripheral

nervous system(4).

If there is no history of exposure to stoves, fire fumes or CO-containing substances in patients admitted to the emergency department, the diagnosis of CO poisoning in the winter months is quite difficult. Emergency room physicians should be suspected in CO poisoning with a detailed anamnesis, exposure to a CO producing substance in the history, the presence of another affected individual in the same environment, and even pets in the same environment. The signs of CO are atypical and can be easily confused with many different clinical signs. Headache, nausea-vomiting, extreme fatigue, weakness, ataxia, seizure, syncope, chest pain, shortness of breath, tachypnea, palpitations, dizziness, confusion, hallucinations, agitation, vision loss, urinary and fecal incontinence, stroke respiratory arrest, Appearances such as cardiac arrest have been reported(5).

The physical examination of patients admitted to the emergency department is not definitive, just like the symptoms. Burn findings may accompany, as well as tachycardia, hypertension or hypotension, and cardiac arrest may develop. Spotting on the skin, cherry-colored skin, cyanosis, cherry red oral mucosa findings are typical, but rare (6).

Co-oximetry, which measures oxyhemoglobin, methemoglobin and COHG saturation as well as total hemoglobin, is the only accurate measurement tool for the measurement of CO poisoning, which we frequently encounter in our emergency services. Standard pulse oximetry, which is widely used in emergency services, is not reliable in the diagnosis of CO poisoning. (4).

Carboxyhemoglobin ratios are naturally found to be <1-2% in non-smokers and 4-10% in smokers. Detailed anamnesis and clinical suspicion are very important for the diagnosis of CO poisoning, which is difficult to diagnose in emergency services. A history of exposure, certain symptoms and signs, as well as a high rate of COHb are helpful in making the diagnosis. However, COHb measurement alone is an insufficient indicator to assess the severity of poisoning. When interpreting the COHb level, the duration of exposure to CO should be determined, if possible, and the source of exposure. If possible, high-flow oxygen therapy should be started on the way to the emergency room(6)

The management of CO poisoning, which has a wide spectrum of symptoms, should also be done carefully by the emergency physician. First, the patient's airway, respiration, and circulation should be evaluated. After vital follow-up, ECG, blood gas analysis and COHg measurement should be performed immediately for ischemic heart change. If CO poisoning is suspected, 100% oxygen (eg, high-flow oxygen, reservoir face mask) therapy should be initiated immediately. Indications for hyperbaric oxygen therapy should be reviewed (7).

Table 2. General Indications for Hyperbaric Oxygen Therapy for Referral

Unconsciousness/Syncope	Acute Myocardial Ischemia
Mental Status Change/confusion	Cardiovascular Dysfunction/dystritis
Watch	Hypotension
Coma	severe metabolic acidosis
Focal Neurological Deficit	Carboxyhemoglobin Level >25%
Pregnancy Carboxyhemoglobin Level > 15%	
Unconsciousness/Syncope	
Mental Status Change/confusion	

If patients have mild symptoms and there is no indication for Hyperbaric Oxygen therapy, an emergency room observation period of 6 hours is recommended with 100% oxygen until symptoms resolve and COHb is normal (8).

In patients with severe CO exposure, delayed neurological sequelae due to profound hypoxia may occur even after 4 days to 5 weeks. In a study consisting of 347 patients diagnosed with CO exposure, 24% were diagnosed with delayed neurologic sequela(9).

Infants may be more sensitive to the effects of CO because of CO poisoning, persistence of fetal hemoglobin during the first 6 months of life, and higher metabolic rates. In this case, the treatment and hyperbaric indication for children is the same as for adults. At the same time, pregnant women should be referred to the hyperbaric center with a CO level of 15%-20%, since CO is shown at lower than normal levels due to the high binding rate of fetal hemoglobin (10).

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