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CHAPTER
1

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EXAMINING THE LEVEL OF KNOWLEDGE ABOUT APITHERAPY AND ITS USES

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INTRODUCTION

Apitherapy, which consists of the words "Apis" meaning bee in Latin and "θεραπεία" meaning to serve in Greek, is the naming of various natural methods such as acupuncture, bee products and apicultural practices that provide medical care to humans (Habryka vd., 2016; Zhu, 2008) In terms of nutrition therapy, apitherapy is the use of bee products such as honey, pollen, propolis, royal jelly, perga and beeswax to prevent or treat diseases(Hellner, Winter, Von Georgi, vd., 2008) Bee colonies have been inhabiting the world for 125 million years. Behind the success of their long existence are certain substances such as honey, propolis, pollen, perga and royal jelly that they produce (Ahuja & Ahuja, 2011)

Historically, apitherapy has its roots in ancient Egyptian medicine, which is more than 6,000 years old. Later on, the Greeks and Romans also used bee products for medicinal purposes, like the Egyptians. In addition, Hippocrates, Aristotle and Galen, who were prominent names in medical science, prescribed the use of honey and bee venom for treatment of baldness (Molan, 2006; Moolenaar vd., 2006) It is maintained that apitherapy products can be used to treat most diseases, and it is asserted that the use of bee products in traditional medicine has an effect that is equal to or better than standard treatments (Lee vd., 2005). It is stated that propolis, which is one of the most known bee products, has an impact on many physiological, antibacterial, antiviral, antifungal, local anesthetic, antiulcer and immunostimulant activities (El–Gammal vd., 2018). Perga (bee bread), which is formed by mixing bee pollen, honey and bee

secretions, has higher nutritional value, better digestibility and richer chemical composition than pollen. It is a food rich in protein, vitamins, minerals, amino acids, fats and carbohydrates. Thus, bee bread is a complementary nutritional support that can be used when a nutrition problem occurs in the human body due to a monotropic diet or malnutrition (Habryka vd., 2016; Hellner, Winter, von Georgi, vd., 2008). Royal jelly, which is the main nutrient of queen bees, can improve health by preventing the emergence of age-related debilitating diseases and is used in the treatment of diabetes, cancer and cardiovascular diseases (Ali & Kunugi, 2020). Because of these effects, apitherapy has been included in modern medical practices today (Zhang vd., 2015). With the growing interest, the widespread consumption of bee products as dietary supplements is increasing (Sivertsen vd., 2018).

This study was carried out to analyze the knowledge, attitudes and behaviors of individuals towards bee products.

METHOD

This study was carried out between November and December 2021. With the G*Power package program, which is one of the sample calculation programs, the number of samples was calculated as 306 with a 5% margin of error and 95% confidence interval at medium effect size. Considering that there may be 10% data loss, 330 people were reached. Since the data of 16 people was invalid, the rest of the study was carried out with 314 people.

Statistical analysis

The data were obtained by the researchers who sent the survey form- created through Google Forms- to the students' e-mail addresses. The survey consisted of 27 socio-demographic questions that investigated the consumption and knowledge levels of bee products. The data of the study were evaluated with SPSS 25.0 Windows software (SPSS Inc., Chicago, IL, USA). Descriptive statistics (percentage, frequency, mean) were used to evaluate the data(Saral & Yavuz, 2021). Before starting the survey, the participants filled out the voluntary consent form. The study was carried out with the permission of the ethics committee (decision dated 26.10.2021 and numbered E.23126).

RESULT

A total of 314 respondents, including 195 men and 119 women, participated in the study. When the educational statuses of the participants were examined, it was seen that 180 participants had undergraduate degrees and 67 of them were on postgraduate level. Approximately 75% of the individuals participating in the

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study resides in a city, 22% in a district and 3% in a village. When the body mass indexes (BMI) of the participants were checked, it was observed that approximately 35% of the participants had a normal weight and 60% were overweight or obese (Table 1).

Table 1. Demographic information

Features	**n**	**%**
Gender		
Male	195	62,1
Female	119	37,9
Education		
Primary School	10	3,2
Secondary School	5	1,6
High School	52	16,6
Undergraduate	180	57,3
Post Graduate	67	21,3
Residence		
City	234	74,5
Town	68	21,7
Village	12	3,8
Monthly income		
Less than 2000 TL	24	7,64
2000-4000 TL	69	21,97
4000-6000 TL	91	28,98
More than 6000 TL	130	41,41
BMI		
18,5 and under	11	3,50
18,5-25	112	35,67
25-30	119	37,90
30 and over	72	22,93

Most of the participants stated that the brand is important in bee products and they give importance to the brand when choosing. Again, the similar number of participants of the survey expressed that when using bee products, they pay attention to whether they are water or alcohol-based. While 65% of the participants asserted that apitherapy products are good for the Covid-19 disease, 35% reported that they are not good for Covid-19. Most of the participants articulated that they began using bee products not based on advice but with their own initiatives, and the rest specified that they started using bee products with advice received from dieticians, doctors, friends or social media. While 232 participants affirmed that they had a budget of 100 TL (monthly) or less for bee products, 82 participants reported that they allocated a budget of more than 100 TL (Table 2).

Table 2. General information

Features	**n**	**%**
Brand Preference		
Yes	213	67,83
No	101	32,17
Water or Alcohol-Based		
Yes	163	51,91
No	151	48,09
Good for Covid-19		
Yes	206	65,61
No	108	34,39
Paying Attention to Brand		
Yes	282	89,81
No		
Advise to Use	32	10,19
Doctor	9	2,87
Dietitian	14	4,46
Friend	29	9,24
Social Media	14	4,46
Myself	248	78,97

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Budget for bee products (monthly)		
Less than 50 TL	119	37,90
50-100 TL	113	35,99
100-200 TL	60	19,10
More than 200 TL	22	7,01

In Table 3, apitherapy consumption statuses and other parameters were compared. Consumption statuses of bee products by gender were also examined in contrast. No significant difference was found in the consumption of bee products in terms of gender ($p>0.05$). Also, no notable difference was encountered in the educational statuses of the participants in terms of their consumption of bee products ($p>0.05$). Likewise, there was substantial difference in the educational status of the participants who did and did not recommend the use of bee products to their circles ($p<0.05$). In addition, there was no meaningful difference between the educational levels of the participants according to their consideration of bee products to be good for Covid-19 ($p>0.05$). Similarly, no serious difference was discovered between the educational statuses of the participants with regards to their paying attention to the brand and the content of bee products ($p>0.05$). Moreover, according to the participants' monthly income, the characteristics of giving importance to the brand and the content of bee products were analyzed comparingly. Significant difference was found between the monthly incomes of the participants who did and did not give importance to bee products' brand and content ($p<0.05$).

Table 3. Comparison of some parameters

	Yes	**No**	
	n(%)	**n(%)**	**X^2**
Gender and Consumption of bee products			0,292NS
Male	172(88,2)	23(11,8)	
Female	100(84)	19(16)	
Paying attention to product brand and Co-vid-19			0,181NS
Yes	145(68,1)	68(31,9)	
No	61(60,4)	40(39,6)	
Income and Paying attention to product			0,023**

brand			
Less than 2000 TL	23(95,8)	1(4,2)	
2000-4000 TL	63(91,3)	6(8,7)	
4000-6000 TL	87(95,6)	4(4,4)	
More than 6000 TL	109(83,8)	21(16,2)	
Education and Recommendation of Bee Products			0,834*,NS
Primary S.	9(90)	1(10)	
Secondary S.	5(100)	0(0)	
High School	43(82,7)	9(17,3)	
Undergraduate	155(86,1)	25(13,9)	
Post Graduate	60(89,6)	7(10,4)	
Education and Covid-19			0,012*,**
Primary S.	8(80)	2(20)	
Secondary S.	5(100)	0(0)	
High School	42(34,1)	10(65,9)	
Undergraduate	114(63,3)	66(36,7)	
Post Graduate	37(55,2)	30(44,8)	
Educational status and Paying attention to product brand			0,521*,NS
Primary S.	8(80)	2(20)	
Secondary S.	5(100)	0(0)	
High School	49(94,2)	3(5,8)	
Undergraduate	161(89,4)	19(10,6)	
Post Graduate	59(88,1)	8(11,9)	

*: Fisher-Freeman-Halton exact test, **: p<0,05, NS: Not signifcant

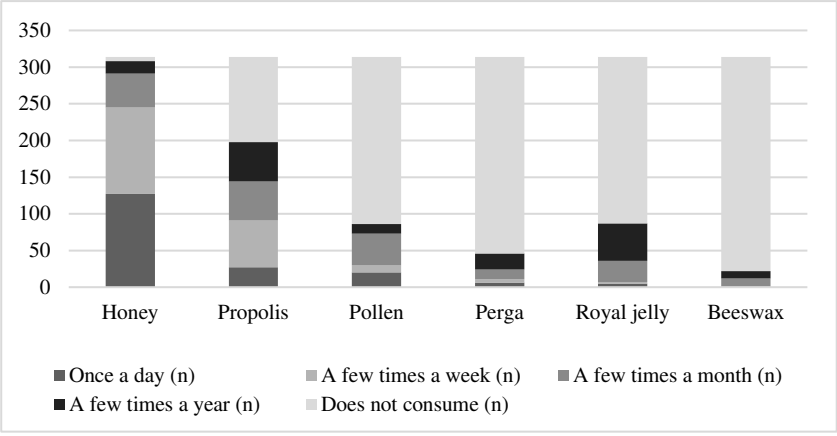
In Table 4, the participants' consumption frequency of bee products was examined. When honey consumption was analyzed, most of the participants stated that they consume honey once a day or once a week. 17 participants reported that they consume honey several times a year and 6 participants mentioned that they do not consume honey at all. When propolis consumption

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was reviewed, 116 participants asserted that they do not consume propolis and 54 participants stated that they consume it a few times a year.

When the frequency of pollen consumption, which is one of the various apitherapy products, was examined, it was seen that most of the participants do not consume pollen. Similar to pollen, the majority of the participants do not consume bee bread, royal jelly and beeswax. The minority stated that they consume it once or several times a day, a week, a month, or a year.

Table 4. Bee products consumption frequency



88.5% of the individuals participating in the survey stated that the consumption of bee products is good for existing symptoms of diseases. Similarly, 97 of the participants reported that they consume bee products because they have a chronic disease. 13 participants mentioned that they consume apitherapy products for diabetes, 9 participants for cancer, 12 participants for blood pressure, 6 participants for infertility, 12 participants for neurological diseases and 45 participants for different chronic diseases (Table 5).

Table 5. Apicultural products and chronic diseases

Type of disease	n	%
Good for Chronic Diseases		
Yes	278	88,5
No	36	11,5
Using Due to A Chronic Disease		
Yes	97	30,9
No	217	69,1
Diabetes		
Yes	13	4,1
No	301	95,9
Cancer		
Yes	9	2,9
No	305	97,1
Hypertesion		
Yes	12	3,8
No	302	96,2
Infertility		
Yes	6	1,9
No	308	98,1
Neurological Diseases		
Yes	12	3,8
No	302	96,2
Other		
Yes	45	14,3
No	269	85,7

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DISCUSSION

Although inadequate and unbalanced nutrition is a significant problem in our country in general, university students are one of the groups in which nutritional problems are most common (Saral & Yılmaz Yavuz, 2020). Bee products are valuable in terms of health due to their nutritional and energizing properties, as well as the biological active substances they contain (Pasupuleti vd., 2017). As a result of the study, it was seen that the majority of the participants pay attention to the brand of apitherapy products. This attention to brand may be due to a demand for high quality and the Covid-19 pandemic (Ali vd., 2021). It was observed that the consumption rates of bee products of each gender were similar. The reason for this may be due to the widespread consumption of bee products, especially honey, in our country. When the percentages of attention to brand of bee products are examined according to the participants' income, it is observed that the importance given to the brand increases as the income level increases. Individuals who participated in the study expressed that bee products are good for the Covid-19 disease, which may be the reason for the growing use and frequency of bee products (Pasupuleti vd., 2017). Although the rate of social media usage and consultation with a medical expert is high nowadays, the majority of the participants who use bee products stated that they had started consuming bee products on their own. In addition, it is recognized in the study that as the level of education gets higher, the recommendation for apitherapy increases. A development in the average level of education can help increase the knowledge about bee products. Similarly, it has been observed that the level of education of people who pay attention to brand of bee products is high. Furthermore, in the study conducted by Saral and Yavuz on university students, it is ascertained that the amount of consumption of bee products has been growing (Saral & Yavuz, 2021). In the study, the most consumed bee product was stated as honey and was followed by propolis, royal jelly, pollen, perga and beeswax. Similarly, in a different study conducted on university students, it was found that honey consumption is higher than the consumption of other apicultural products (Saral & Yavuz, 2021). The reason for this similarity may be because honey is the most well-known and produced bee product (Marangoz & Tayçu Dolu, 2019).

CONCLUSION

Honey bees provide natural products with biological and medicinal functions in a number and variety that cannot be obtained from any other animal or plant in nature. In order to benefit from bee products, which have many pharmacological activities, different production techniques should be applied according to the purpose of use. These products, which are obtained after production, must be prepared by undergoing quality tests and subjected to different processes according to standards suitable for the purpose. At the same time, these products should be analyzed and quality and residue tests should be routinely followed. Honey bee products produced in accordance with medical use should be applied by specialist physicians and dietitians. As a result of the study, it was seen that the knowledge and consumption levels about different types of apitherapy products were not sufficient. More studies are needed on apitherapy products, and the amount of consumption should be increased by increasing the promotion and variety of these products.

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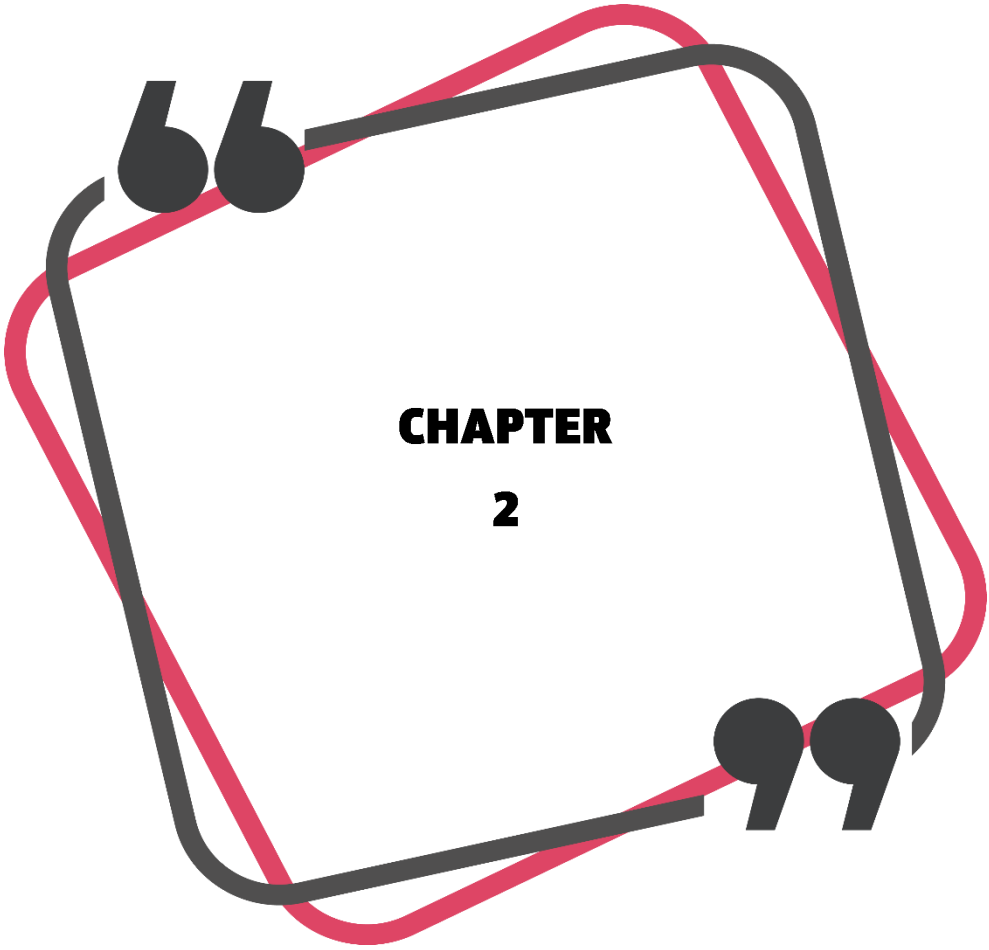
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CHAPTER
2

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SUICIDE AMONG DOCTORS

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It is well known that being a doctor is a profession that brings with it many difficulties since medical education, in addition to the material and moral values that it adds to a person. In recent years, there has been an increase in the interest in mental health and suicides of doctors after the suicides of doctors, some of which were talked about for days. Health administrators have also taken some steps to regulate the working conditions of doctors after accidents caused by harsh working conditions, mental distress and suicide cases (McKinley et al, 2019; Riley, 2004).

Burnout is a situation that negatively affects those who live in the struggle to adapt to today's world and those who work from all professions. Burnout is a psychological syndrome that can be defined as “emotional and exhausting conditions related to working environment”. The three domains of burnout are emotional exhaustion, depersonalization, and low personal achievement, and these seem to be more prevalent in the doctors (Patel, Sekhri, Bhimanadham, Imran, & Hossain, 2019)

Health workers are exposed to more occupational burnout than other occupational groups because they have to provide continuous and quality service to people who need help due to the nature of their working environment. Studies have reported that burnout syndrome is between 25-50% in physicians. (Fitzpatrick, Biesma, Conroy, & McGarvey, 2019)

Recently, it has been emphasized in many publications that burnout syndrome is more common in both doctors and other healthcare professionals, especially during the Covid-19 epidemic.(Ghahramani et al, 2021)

It was reported that factors associated with physician burnout and it's consequences affecting a physician's overall health, and patient outcomes and

healthcare system. This represents a public health crisis with negative impacts on patient care, professionalism, physician's care and safety issues such as mental problems and accidents, and healthcare systems and organizations (Yates 2020; West, Dyrbye, Erwin, & Shanafelt, 2016)

Mental disorders seen in society can also be seen in doctors. Studies have shown that mental disorders such as depression, anxiety disorders and PTSD are more common in doctors than in the general population (De Jong, Nieuwenhuijsen, & Sluiter, 2016; Petrie et al, 2019; Harvey et al, 2021).

The risk factors for mental disorders in the general population also apply to doctors. In addition, it was thought that intense working conditions, difficulties in balancing work and family life could be considered as additional stress factors on doctors (Hick & Biddinger, 2020; Harvey et al, 2017). In addition, during the recent Covid-19 epidemic, pressure on doctors has increased in many countries and working conditions have become more difficult (Hick & Biddinger, 2020). In the Covid-19 epidemic, doctors were worried about their own health and the health of their family members, and it was shown that sleep problems and depressive symptoms increased. (Sahebi et al, 2021)

Along with the bureaucratic work that has increased compared to previous years, the decrease in the autonomy of the doctors and the decrease in the sense of control are also stated as another factor that increases the risk of mental disorders (Edwards, Kornacki, & Silversin, 2002). Finally, it has been shown that the frequency of mental illness among doctors varies on a country basis. It is a fact that the workload of doctors is higher, especially in developing countries. Developing countries also have difficulties in career planning and self-development of physicians, and income levels are generally lower. All this means that doctors working in these countries are at greater risk for mental disorders and at the same time, it is more difficult to access treatment (Saxena, Thornicroft, Knapp, & Whiteford, 2007).

In some studies comparing the symptoms of mental illness in doctors with other occupational groups, it has been concluded that the rate of mental illness in doctors is similar to other occupational groups, but not higher than them. Therefore, it was thought that the stressful conditions brought by professional life itself may have triggered mental distress in many different professions including doctors. (Goodwin et al 2013)

Compared to the general population, physicians have lower overall mortality rates (Torre et al, 2005) Studies have shown that suicide rates are higher in physicians compared to both the general population (Dutheil et al 2019; Duarte

et al, 2020) and other occupational groups (Agerbo, Gunnell, Bonde, Mortensen, & Nordentoft, 2007). It has been thought that physicians' better knowledge of suicide-related tools, better knowledge of toxic and lethal doses of drugs, and easier access to them are associated with an increased risk of suicide (Irigoyen-Otiñano et al 2022; Hawton, Clements, Simkin, & Malmberg, 2000). It has also been reported that doctors have some difficulties in seeking help when they have mental problems. (Byrow, Pajak, Specker, & Nickerson, 2020). It has been reported that reservations about registration, time constraints, reservations about stigma, cultural beliefs and beliefs that this situation will reflect negatively on their careers are important obstacles for doctors to seek treatment and receive support for their mental problems (Harvey et al, 2021; Dutheil et al, 2019).

In addition, it has been observed that there is a connection between the specialty and the risk of suicide. In a review, it was seen that anesthesia, psychiatry, general practitioners and general surgeons had higher suicide rates than other branches (Harvey et al, 2021). In a meta-analysis study examining physician suicides, it was shown that suicide rates in female physicians are higher than female physicians in the general population, but suicide among male physicians is less than in males in the general population (Dutheil et al, 2019). Many studies have shown that advanced age is a risk factor for physician suicide (Petersen & Burnett, 2008; Lindeman, Läärä, Hirvonen, & Lönnqvist, 1997).

Being divorced, widowed, single and relationship problems are also reported sociodemographic risk factors for doctor suicides. (Kõlves, & De Leo, 2013; Gerada 2018; Palhares-Alves, Palhares, Laranjeira, Nogueira-Martins, & Sanchez, 2015)

Poisoning, firearms and suffocation were the most frequently reported suicide methods used by doctors. (Harvey et al, 2021). While suicide with firearms is prominent in America and Africa, poisoning is the most commonly used method in Europe, New Zealand and Australia. Therefore, it can be said that the method used varies according to the geographical region and culture (Harvey et al, 2021).

So what can be done about these under-studied doctors' suicides and what kind of precautions can be taken? Maybe we can divide this into three headings: what can be done at the individual level, what can be done about the working environment and systemic precautions. It is thought that theoretical and practical applications to increase the awareness of doctors about mental problems/disorders, starting from medical education at the individual level, and applied training to reduce stigma may be beneficial. In addition, the implementation of practices that will increase the coping skills of doctors in this

stressful career journey since the education period will increase the level of coping with stress of doctors. This is an initiative that is expected to have positive repercussions not only in the professional sense, but also in the personal lives of doctors. As a matter of fact, it has been shown that physicians who develop skills to cope with this type of stress show less depression and anxiety symptoms and report less suicidal thoughts in the long run. (De Kock et al, 2021; Ruotsalainen, Serra, Marine, & Verbeek, 2008; Scheepers, Emke, Epstein, & Lombarts, 2020)

In addition, the measures that will facilitate the individual support of doctors who have mental difficulties in the working environment will also help doctors overcome their mental problems. In many cases, it has been observed that physicians refrain from seeking psychological/psychiatric help due to privacy concerns. It was emphasized that it is important to provide individual support to help overcome this barrier. In this respect, it is important to ensure that the seniors and trainers are supported not only in terms of professional development but also in terms of spiritual development, and that they receive support when necessary, especially in order to support the doctors in assistantship education. (Havermans et al, 2018)

Finally, systemic changes that can be made in the field of health are also important. In this sense, it is necessary to review and reduce the risk factors related to the working environment of doctors and to increase protective measures. It is thought that reducing the bureaucratic workload on the doctors, arranging the intensive working hours and increasing the rest hours, putting into practice applications that will compensate for the financial losses during the leaves, and organizing the physical conditions of the hospitals considering the working and resting hours of the doctors will reduce the burnout of the doctors and reduce the mental distress. (De Hert 2020; Epstein, Marshall, Sanders, & Krasner, 2022)

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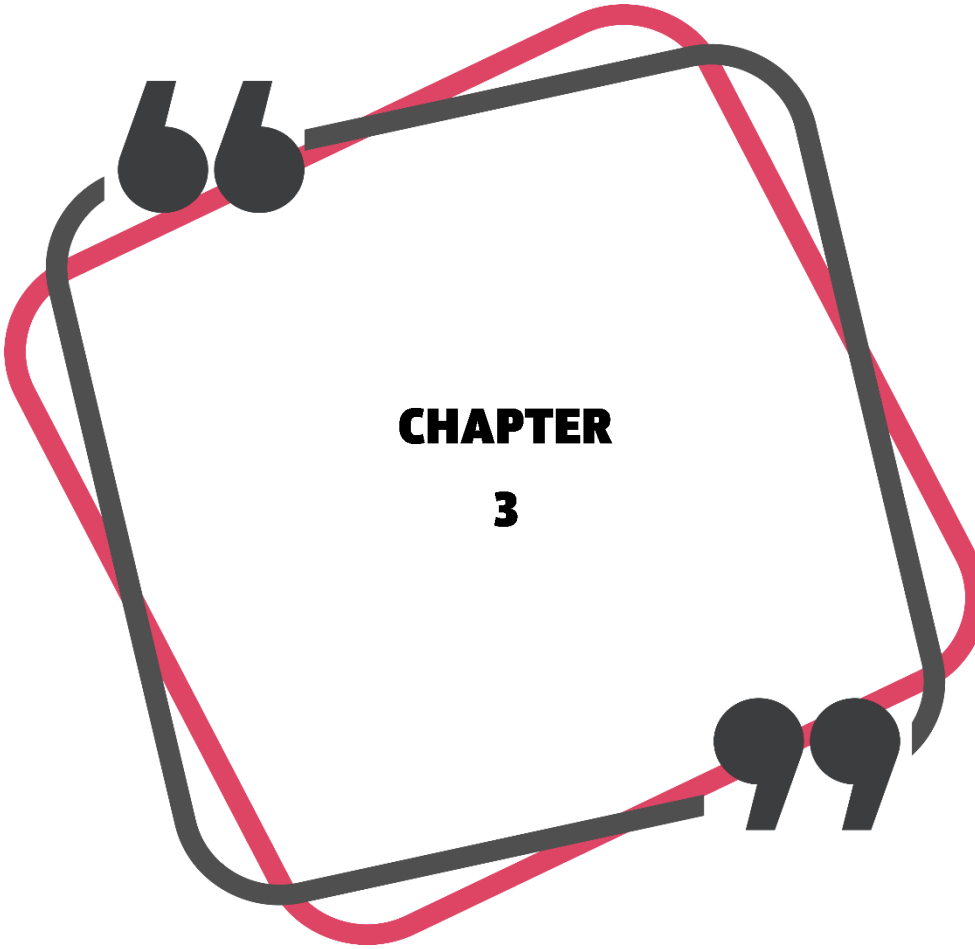
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CHAPTER
3

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PROPRIOCEPTION IN CHILDREN

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INTRODUCTION

Proprioception is the musculoskeletal system's spatial and mechanical condition being aware of both consciously and unconsciously (1). In the early developmental period, children experience the world through their sense. As they perceive and feel the outside world, they discover to move and explore. According to Ayer's theory of sensoryiintegration; It is assumed that three senses, tactile, vestibular, and proprioceptive, have an important effect on motor development. Disturbance in one of those sensoryxsystems can induce difficulties in establishing motor abilities in kids (2). The proprioceptive sense, its assessment, and education in children are the main topics of this review.

PROPRIOCEPTION SENSE

Proprioception encompasses the static and dynamic aspects of position sense. Static sense provides the conscious harmony of body parts with respect to each other, while dynamic sense provides notification about the speed and direction of movement through the neuromuscular system. Thus, proprioception is a neuromuscular process that includes afferent and efferent stimuli and ensures that the body is in balance and harmony during static and dynamic movements (3). The senses carried by the fasciculus gracilis and fasciculus cuneatus reach the cortex, taking the name of the conscious proprioception sense. The senses carried by the pathways ending in the cerebellum without reaching the cortex, which carry the same senses, are called subconscious proprioception sense (4). Conscious proprioception; It activates proper joint function in activity, sports and occupational tasks. Unconscious proprioception adjusts muscle function and initiates reflex stabilization of the joints via muscle receptors (5).

COMPONENTS OF PROPRIOCEPTION

Proprioception; includes subconscious or conscious awareness of kinesthesia, joint positionxsense, force perception, and velocity change (6). Senses from somatosensory sources are summarized below.

The ability to passively or actively repeat the determined joint angle is named as *Joint Position*x*Sense*. It forms the static component of proprioception. Muscle spindles are the main proprioceptors in charge of determining the position of joints. Musclexspindles are crucial for the perception of speed and strength. (7).

The sense of kinesthesia forms the dynamic component of proprioception. kinesthesia; It is defined as the ability to perceive features such as direction, duration, speed, amplitude and timing of joint movements (8). It is examined as the threshold of feeling passive movement, that is, "sensitivity to movement", and "acuity of movement", which is called the threshold of feeling the difference between two movements (8).

Although some studies in the literature (9, 10) consider joint position sense and kinesthesia separately, since information from joint position also gives information on the direction and speed of movement, it is challenging to discriminate between kinesthesia and static position awareness. In current studies, this distinction is no longer made (1).

Force perception is the ability to perceive and analyze the force exerted to any joint or its surroundings, and to test the muscle strength required to maintain or move the joint in a particular position (11).

Perception of velocity change can be defined as awareness of the displacement ratios (velocity) of the extremities (12).

PROPRIOCEPTIVE PATHWAYS

Peripheral Pathways

Afferent stimuli related to the proprioceptive sense are generated through receptors and provide afferent proprioceptive feedback to the cortex. Thanks to receptors, joint position and movement are regulated (13). Mechanoreceptors play an important role in the formation of reflex responses, the sense of joint position and the regulation of muscle tone. Proprioceptive sensory receptors are found in joints, muscles, tendons, ligaments, and skin (14).

Muscleispindle andiGolgi tendon organ (GTO) forming muscle receptors are crucial structures for proprioception. Spindles in the muscle provide information about the muscle's length. They are located in the origin and insertion of GTO

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skeletal muscle. In case of excessive tension, it informs the cortex to stop the muscle contraction. These muscle receptors are stimulated by stretching the muscle. Group 1a and group 2 afferents originating from the muscle spindle and group 1b afferents originating from the GTO reach the spinal cord via the dorsal root. From here it reaches the cerebulum via the spinocerebellar pathways. (15).

Ruffini endings and pacinian corpuscles, which are joint receptors, participate in the proprioceptive impulse transmission to the upper centers (16). Ruffini endings are stimulated by joint movement and provide information about intra-articular pressure, static joint position, speed and amount of joint motion. Pacinian corpuscles, which are dynamic mechanoreceptors, are sensitive to resistance and rapid movement, so they are not stimulated in constant speed movements and static conditions in the joint (17).

Free nerve endings are activated when any damage occurs in the articular tissues that may cause mechanical deformation. Found in joints, capsules and ligaments (18).

Peripheral musculo-ligament reflexes are also important in peripheral proprioceptive regulation. The upper level responsible for motor control are these mono- and poly-synaptic spinal reflexes. They regulate the movement of the ligaments and muscles around the joint and are transported from the posterior horn of the spinalicord to the upper centers (19).

The monosynaptic reflex is in charge of the transmission of protective data from the joint, tendon, ligament, soft tissue and skin to the dorsalihorn and direct stimulation of the anterior horn to create immediate/appropriateimuscle contraction. Monosynaptic reflexes that occur early or delayed cause uncontrolled joint movement and injuries (20).

The polysynaptic reflex is a muscle stretch reflex that occurs with the synapses of many interneurons between motor and sensory neurons (21). Reflexes from peripheral stimuli are regulated by feedback inhibition, while reflexes at the cortical level are regulated by feedforward inhibition. Inhibition systems have a part in determining the onset, speed and limits of movement. Spinal level reflexes are engaged in the control of muscle agonist and antagonist activity affected by these systems (22).

Cortical Pathways

Peripheral proprioceptive information is transported to higher cortical levels through synapses formed by afferent neurons. The major somatosensory region in the parietal lobe of the cerebral cortex interprets somatosensory data acquired

from peripheral receptors via sensory nerves and pathways (23). The somatosensory circuit has three neurons. The dorsal root ganglion of the spinal nerve houses the first neuron. The secondineuron's ascendingiaxons cross the spinalicord to the contralateral side and terminate in the cerebellum, reticular system, or thalamus. The third neuron terminates in the postcentral gyrus of the parietal lobe in the thalamus (24).

The corticospinal pathway is an efferent connecting mediator between alpha and gamma motor neurons and the motor cortex. (25). *The posterior column* carries kinesthetic information from the upper extremity muscle afferents to the cortex. *The dorsal spinocerebellar pathway* and *Clarke's column* provide the transport of kinesthetic information from lower extremity muscle afferents to the cortex. Information coming from the periphery via ascending pathways including the medial lemniscus, dorsalicolumn, and ventralispinothalamic tract first synapses in the reticular formation and thalamus and then reaches the cortex. Dorsal/ventral and spinocerebellar pathways carry information about balance and posture control (24). Spinocerebellar pathway regulates muscle spindle activity via gamma motor neuron, and in this way, the cerebellum conducting coordinated motorxmovement designs and changes motor activity (26). Spinocerebellar pathway; It carries peripheral information from muscles, joints and skin to the motor and sensory areas via the cerebellum, medulla, and dorsal column. Kinesthesia and jointiposition sense are formed by the cerebellar interpretation of smooth and appropriate information from stretch receptors and muscle spindles (27).

LOSS OF PROPRIOCEPTION SENSING

In posterior funiculus lesions, position sense and kinesthesia are lost at the level and lower part of the lesion. These sensory losses are more pronounced in the distal parts of the extremities. Loss of position sense and kinesthesia causes voluntary movements to be extremely flawless and irregular. In this case, patients can control their body position through the visual system and can stand and ambulate, although it is not very stable, but they have great difficulty in the dark. In addition, although there is no loss of tactile sensation as a result of Funiculus posterior lesion, patients have distinctive tactile disturbances (4).

THE ROLE OF PROPRIOCEPTIONIIN SENSORIMOTORICONTROL

The main task of the sensorimotor system is to create appropriate motor responses and adaptive changes through sensory inputs. The CNS requires a body

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diagram of the spatial and biomechanical properties of body parts, mainly provided by proprioceptors, to generate the appropriate motor response. Throughout the movement; proprioception has an important place in balance, coordination, joint stability, regulation of muscle tone under feedback and feedforward control. Joint stability; ıt occurs through the activation of agonist and antagonist muscles as well as inputs from the vestibular, visual and somatosensory systems to the CNS. Motor control is achieved by evaluating the stimuli from these systems in the spinal cord, brain stem and higher centers of the brain (28).

PROPRIOCEPTION IN HEALTHY CHILDREN

The proprioceptiveisystem is an important element of motoricoordination and planning. This structure is effective in correcting slow movements and providing information before movement begins in fast movements (1). As children acquire a motor skill, movement performance demands a great deal of conscious effort based on all visual, tactile and proprioceptive information. The proprioceptive system senses information regarding limb position, strength, and speed. The visual and tactile systems regulate the quality and accuracy of movement, and the systems interact. The movement also improves with practice, and the control process becomes quicker and subconscious. Proprioceptive information now serves as an subconsciousifeedback mechanism to make sure that efferent motor orders are carried out correctly (1).

The developmental stages of proprioceptive pathways in normally developing children are not yet clear (29, 30). Studies have reported that the proprioception sense develops until adolescence and is more conscious (31). Many studies have shown that the development of proprioceptive ability is rapid between the ages of 5-8, but slows down in late childhood and adolescence and reaches a plateau. In other studies, it has been reported that proprioceptive sense increases in a nearly linear slope between the ages of 4-13 and the developments continue until the age of 24 (32, 33).

PROPRIOCEPTION IN CHILDREN WITHICEREBRAL PALSY

In the third trimester, since the thalamocortical somatosensory projections are completed, they are not affected by lesions occurring in the periventricular or postcentral gyrus. However, middle cerebral artery lesions that occur later and often affect the postcentral gyrus are more likely to affect the somatosensory system. For this reason, sensory disturbances are seen especially in children with unilateral spastic CP who have middle cerebral artery infarction, and this causes motor skills to be affected. Impairment of the proprioception sense, light touch,

discrimination, and stereognosy is observed with the involvement of afferent sensory/motor pathways. However, the relation between sensory and motor abilities is not clear yet. An association has been found between motor function and stereognosia, but conflicting results have been shown for other senses (34). Children with autism and developmental coordination disorders with proprioceptive dysfunction often bump into them because they have difficulty grading strength when interacting with people and objects (35). Studies have shown that the decrease in proprioception is correlated with difficulty in writing and poor coordination (36). However, correlations were found between motor delays and movement disorders in children with proprioceptive delays (1).

Studies examining proprioception in patients with unilateral CP have often focused on the upper extremity. Studies have showed that children having unilateral CP have impairments in their proprioceptive abilities and the affected upper extremity has weaker proprioception (37). It has been shown that proprioception of the affectediupper extremity in patients with unilateralxCP correlates with a decrease in muscle spindle activation. Muscle shortness and stiffness caused by spasticity-induced stiff muscle fibers and short cercomere impair muscle spindle sensitivity by affecting the muscle-joint relationship, and joint position sense is affected (38).

Looking at the current literature, few studies investigating lower extremity proprioception were found in this group. In a study, it was shown that individuals with good lower extremity proprioception have less postural sway and walk faster, and there is a correlation between motor and sensory performance (39). Children with impaired proprioception walk slower and with smaller steps because they do not know where their feet are. The impact of poor proprioception on children's developing motor systems can be detected, even if the majority of children with weak proprioception do not completely lose proprioceptive feeling (1).

In a research involving kids who had quadriplegic CP, passive joint motion sensation was evaluated and it was seen that these children did not have any problem in passive joint motion sensation (40). In another research involving children with diplegic CP, they were asked to repeat a movement made with the index finger with the other extremity to evaluate proprioception, and it was reported that there was no difference in proprioception when these children were compared with the control sample (41). In the research comparing the sense of joint position and kinesthesia in participants with diplegic and hemiplegic CP with the healthy control group, it was found that both CP groups made more errors in joint position mapping in all extremities except the dominant upper extremity than in healthy subjects. In addition, it was determined that there were kinesthetic deteriorations in both lower extremities of hemiplegic CP (9). In a similar study by

Damiano et al., it was reported that proprioceptive deficits decreased in the dominant and nondominant lower extremities in individuals with diplegic and hemiplegic CP (39).

PROPRIOCEPTION IN THE BRACHIAL PLEXUS

In the upper extremity, the inputs transmitted to the CNS by the brachial plexus via efferent neurons are recorded by the CNS. The recorded data is transformed into a motor response through the brachial plexus. Joint stabilization and motor coordination may be affected by deafferentation of proprioceptive receptors (42). The traction of the brachial plexus during delivery causes obstetric brachial plexus injury (OBPY). The most often impacted roots are C5, C6, C7, and occasionally C4 roots, that form the middle and upper trunks of the brachialiplexus and are in charge of innervating the anterior and lower shoulder tissues. Suprascapular and axillary nerves innervate the capsule, ligament, and synovial membrane of the shoulder and glenohumeral joint and takes part in shoulder stabilization. In upper trunk OBPY injuries, disruptions in the proprioceptive network are seen due to deafferentation of receptors. Sensory and motor deficits in the shoulder reduce proprioceptive input with weak dynamic and static stabilizers (43). In a study on proprioception in children between 7 and 12 years of age with OBP, proprioceptive sensation was evaluated in both extremities and it was found that proprioceptive sensation was less in the affected extremity. At the same time, it has been shown that the decrease in proprioception may cause functional losses by affecting motor coordination (44).

EVALUATION OF PROPRIOCEPTION IN CHILDREN

It's crucial to evaluate the child's capacity for using and processing proprioceptive information for motor control in a thorough evaluation. Thus, various intervention methods can be offered for improvements in development. Assessment can be done as a direct proprioception evaluation, an indirect evaluation of proprioception, and an evaluation of the proprioceptive reflex (1).

Direct Evaluation of Proprioception

A common clinical test that is used to evaluate children's sensory abilities directly includes the proprioception-related subtests of **the sensoryxintegration and praxisxtest**. The test has been standardized in children aged 4-8 years. Requirement of a special certificate for the test can be seen as a limitation. The kid's capacity to return the finger to a certain test position without seeing it is evaluated

by the Kinesthesia subtest, which directly evaluates proprioceptive function. It provides details on proprioceptive functionality in other subtests. The static and dynamic balance, vestibular function, and proprioceptive function of the child are all evaluated during the standing and walking balance subtest. The postural praxis subtest measures how well the kid can organize their movements using proprioceptive information. The child's capacity to integrate tactile and proprioceptive information linked to body awareness is evaluated using the subtests of finger identification, graphicesthesia, and localization of tactile stimuli (45).

The Kinesthetic Sensitivity Test evaluates joint position sense and motion perception. It is suitable for use after the age of 5 years. Its use is limited due to equipment requirement (46). In the tactile acuity subtest, the participant's eyes are closed, They are asked to decide which of two ramps is steeper while their hands are pushed up and down them. Participants must recall the route their hand has traced on a stencil with a pen while employing kinesthetic perception and memory, without being able to see their hand or the stencil (47).

Direct assessment of proprioceptive function provides the most accurate information about each proprioceptive sense. However, its limitations are that it requires laboratory equipment and most of them are research protocols. Therefore, position, movement, sense of force and balance, which are other techniques that evaluate each proprioceptivexsense and related motorxfunction, can be used to measure proprioception (1).

Joint position sense can be evaluated by moving an extremity and asking the children to determine where the extremity has been transported without seeing it. Positioning can be done through verbal identification (48), contralateral limb pairing (49), or pointing to position (50). The most accurate way to assess the sense of position is with laboratory equipment without providing additional tactile information to move the extremities (51). However, the method of testing by manually moving the extremity is still used.

By moving the joint segment up or down, the position senseisubtest of the Fugl-Meyer test standardizes joint position sense testing (48). Placing the child's hand in the gap and asking them to find the thumb without looking at the other hand is another way to test their sense of jointiposition. The capacity of the youngster to hold the limb in isometric contraction without seeing it is the other option to assessing their sense of joint position. Largeideviations from the maintained posture could be a sign of inadequate motor control or proprioception. Additionally, the youngster can be assessed by matching the position in which the extremity is held with the contralateral extremity(1).

Sensation of movement is often tested with laboratory equipment similar to sense of joint position. It is tested by determining the displacement detection

threshold by moving the joint slowly by the device until the user can recognize the direction of movement. (52). In the absence of elaborate equipment, sense of motion may be examined by manually movingxthe limb, inquiring about the direction of motion, or physically moving the opposing limb to match motion in the absence of vision.

Force perception can be assessed using one's ability to replicate forces (53). The participant is requested to repeat the forces on the contralateralxlimb or a force previously generated and matched to the target force. It has been reported in a study that load perception is not associated with position sense (54). However, it is crucial to take into account in motor control since proper motions depend on the capacity to precisely perceive and create pressures (1). In addition, without using elaborate equipment, the child's force perception can be assessed by placing a weight on each hand and asking which one is heavier (1).

Balance can be tested with clinical tests including the Romberg test, Fugl-Meyeritest, and neurological examinations. The vestibular system has a primary function in balancing without vision. However, the proprioceptive system plays a greater role in dynamic balance, which requires ankle strategies to maintain balance on uneven floors. The amount of rocking and the time it takes to maintain balance under various conditions will give an idea about the child's proprioceptive and vestibular skills (48, 55).

Indirect Evaluation of Proprioception

Clinically, indirect assessment of proprioception is usually made with a clinician observation checklist or parent report. Indirect assessments provide information about behavioral patterns related to proprioceptive function within a short test period. In this context, there are 3 tests that examine proprioception (1).

A questionnaire called **the SensoryiProfile** looks at the sensory processing patterns of the kid as reported by the parent. Two subsections of eight items examining the child's ability to move around, how well he follows movement, and postural control focus on movement and body position-related processing in relation to how proprioceptive processing influences engagement in everyday routines (35).

A related parent survey that emphasizes sensory practice and processing is **the Sensory ProcessingiScale**. The three proprioception-related elements of the scale are bodily awareness, balance and movement, and planning and ideas. These chapters emphasize the capacity to assess strength and bodily motions, move around, plan, and carry out exercises (56).

The ComprehensiveiProprioception Observation is a scale for clinician observation that focuses only on actions and movements connected to proprioceptive processing. (57).

Proprioceptive Reflex Evaluation

Evaluation of proprioceptive reflexes is a validation method of proprioceptive afferents. Although proprioceptive reflexes do not support the proprioceptive system's sensory function, they do enable the isolation and assessment of proprioceptor activity before proprioceptive processing impulses are sent to the motor cortex. There are two approaches to assess proprioceptivexreflexes: passivextendon tapping (58) and perturbations of actively maintained joint positions (52).

An abnormal result from reflex assessments is not used to confirm proprioceptor-level dysfunction because the abnormalximotor response may be the result of any of the proprioceptive, neurological, or motoric mechanisms. On the other hand, the normal proprioceptive reflex can be employed to exclude dysfunction in theiproprioceptors.

By locating the tendon of the muscle to be examined and rapidly tapping it with the reflex hammer while the limb is motionless, **the Stretch Reflex** is assessed. It is considered that the live reaction is normal based on the observed muscle contraction and extremities movement.

Perturbation in the actively held joint position requires sophisticated tools to elicit distortions and assess minor corrections from sudden deterioration, but can be modified for more straightforward clinical applications, in which participants maintain the tested limbs in a preset posture, allowing doctors to watch for reactions to manual perturbations. The typical proprioceptive reaction is to swiftly restore the limb to its original position after correcting the impairment (59).

The use of technology in the evaluation of proprioception has also become widespread in recent years. Joint position sense and kinesthesia in the upper extremity were evaluated with a robotic device in 43 children with unilateral hemiplegic CP and it was shown that these senses were affected in hemiplegic children (10). In a study involving 24 children with hemiplegic and diplegic CP aged 7-13 years, joint position sense of the dominant leg, knee and ankle matching errors were evaluated with the “TiltMeter” mobile application. It has been reported that children with CP have deficiencies in proprioception when compared to the healthy control group (60).

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PROPRIOCEPTIVE REHABILITATION

The aim of proprioceptive rehabilitation is to improve the sense of joint motion by training the afferent pathways. Deficiencies in the function of proprioceptors can cause impairments in function and movement. Therefore, retraining of proprioceptors is extremely important. To increase functional capacity, proprioceptive input must be provided. Proprioception-mediated neuromuscularicontrol of joints within the CNS is provided at three different levels of motoriactivation (61):

Reflexes controlled at the spinalxlevel provide a reflex response in providing joint stabilization in case of excessive stress around the joint. To improve this neuromuscular mechanism, exercises used to improve dynamic joint stabilization are used (61).

In the second level of motorxcontrol, balance and posture, joint mechanoreceptors receive input from the vestibular and visual systems and are regulated in the brainstem (61).

The highestxlevel of the CNS (cerebellum, basal ganglia, motor cortex) gives the perception of movement and body position in order to initiate the conscious and voluntary movement that occurs with motor commands. The use of cortical pathways allows the unconscious realization of movements that are previously stored in our memory. Kinesthetic and Proprioceptive exercises allow to improve this function (61).

To correct proprioceptive disorders, it is necessary to activate three levels of motor control in the earliest stages of rehabilitation (62). In order to achieve reflex joint stabilization, the focus should be on activities that require reflex neuromuscular control and cause sudden changes in joint position. Balance and postural activities should be exercised with eyes open or closed in order to regulate motor function at the level of the brain stem. Programmed training from conscious to unconscious, from easy to difficult, at the same time, joint positioning movements should be exercised at the end point of the rangexof motion (63).

Upper extremity proprioceptive exercises are used less than lower extremity in rehabilitation programs. However, shoulder joint positioning and repositioning is extremely important. Position sense exercises, joint repositioning exercises in the presence and absence of visual input, PNF patterns stimulate cognitive level processes. Dynamic stabilization exercises can be given to enable the activation of antagonist and agonist muscles together, and plyometric exercises can be given to combine cognitive and reflex levels. Function-specific activities are more successful if dynamic muscle-joint stabilization and joint sensitivity are improved (62). Balance exercises are also an important part of proprioceptive training. Isokinetic exercises help neuromuscularicontrol by developing the joint position

sense. These exercises may be conducted with eyes open and closed, or with eccentric or concentric loads or independently (61).

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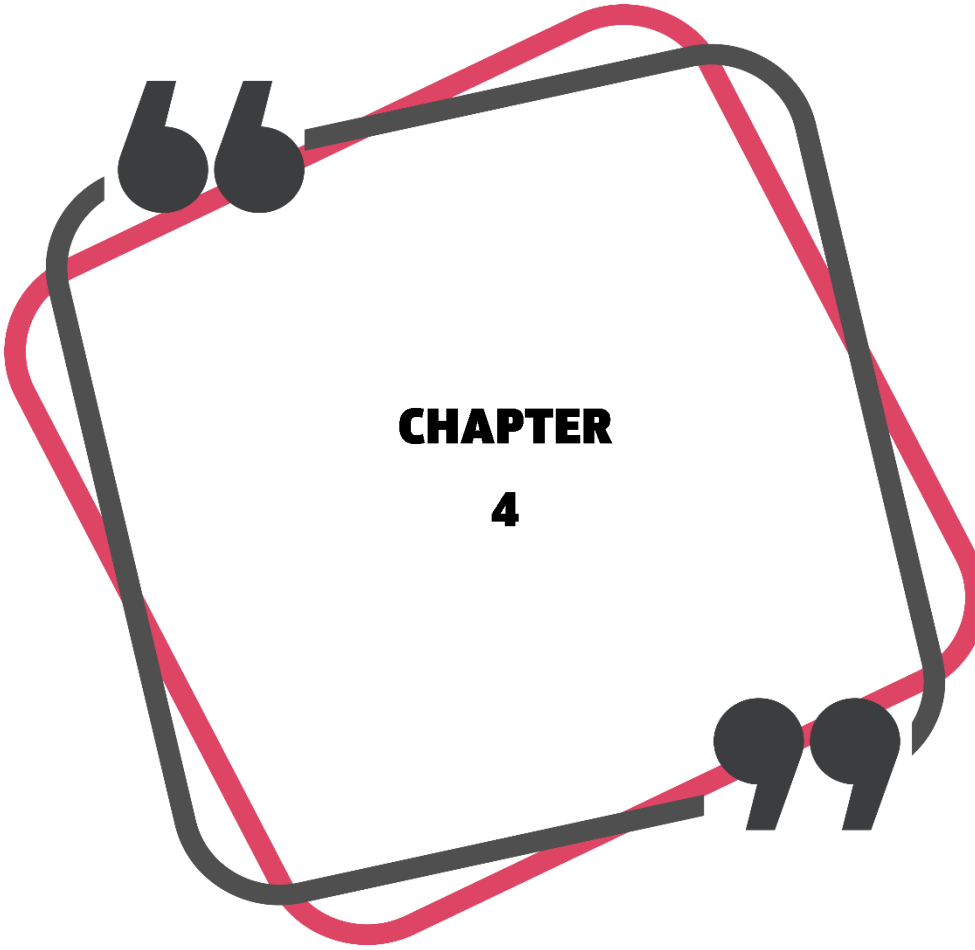
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CHAPTER
4

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INFANTILE HYPERTROPHIC PYLORIC STENOSIS

Mehmet SARIKAYA

Introduction:

Infantile Hypertrophic Pyloric Stenosis (IHPS) is the most common cause of gastric outflow obstruction in infants within the first month of life (1, 2). The disease typically manifests itself in healthy 3-6-week-old infants, first several times a day, with an increasing number of projectile and bile-free vomiting. It is accompanied by severe nutritional disorders, dehydration, and acid-base imbalances if not noticed and treated in a timely manner, and may result in death if neglected. The exact diagnosis of the disease was made by Hirschsprung (1888) even though Hildanus (1627) and Blair (1717) described hypertrophy in the circular layer of the pylorus muscle in autopsy studies (1, 2). The incidence of the disease is reported to be between 2 and 5 per thousand live births in the western world even though the disease is very rare in developing countries, Africa, and Asia (3). Therefore, it is considered a western disease. It is more common in males compared to females (5:1) (3). This disease is 4-15 times more common in children with a family history of IHPS (3). The disease is 200 times more common in monozygotic twins and 20 times more common in dizygotic twins (4).

The disease was referred to as congenital hypertrophic pyloric stenosis from the years when it was first described until recently but it was thought to be an acquired disease rather than congenital since no disease was encountered in the autopsy series and the majority of patients consisted of infants older than 2-3 weeks (1). Cases with ultrasonographic diagnosis in the antenatal period have also been reported although rare (5).

Etiology:

The etiology of the disease has not been fully elucidated even though more than a hundred years have passed since the diagnosis of IHPS. The disease is thought to occur due to genetic and environmental factors. Compensatory hypertrophy, neurological degeneration, or immaturity, and abnormal endocrine signals

are noteworthy theories trying to explain etiology are considered (6). The pyloric canal is obstructed by milk clots and hypertrophy develops as a result of excessive contraction of the pyloric muscle trying to open this obstruction according to compensatory hypertrophy theory (7). It was observed in some studies conducted in relation to this theory that the risk of developing IHPS was 4 times higher in bottle-fed infants compared to breastfed infants. However, it has not been fully understood whether the increased risk of IHPS is caused by bottle or formula since it has been observed that the vast majority of bottle-fed babies are fed with the formula (8). There are also publications associating pyloric stenosis with maternal stress factors such as premature delivery, cesarean delivery, maternal hyperthyroidism, maternal smoking, nalidixic acid use, and intranasal decongestant use (3, 4, 9). Prematurity, erythromycin, and azithromycin use are also additional risk factors (10).

Genetic inheritance of IHPS is a multifaceted and complex condition. No definite genetic predisposition has been determined yet even though different gene loci are associated with IHPS. Apolipoprotein A1 (APOA1), MBNL1, NKX2-5, and FOXF1 gene loci were associated with IHPS in one study (11).

There are numerous neuroendocrine hormones and glycopeptides associated with the etiology of IHPS. Dodge managed to create hypertrophy in the pyloric muscle by administering pentagastrin to dogs in his study in 1970 (12). Spitz and Zail also stated that babies with IHPS had high plasma gastrin levels in 1976 (13).

Another important substance associated with IHPS is Nitrite Oxide (NO), a cellular signal molecule produced from the endothelium. NO is a molecule that causes relaxation in smooth muscles. Kusafuka and Puri have revealed Nitric Oxide Synthase (NOS) gene defects in patients with IHPS (14). Huang et al. produced rats lacking the NOS gene and found that pyloric muscles of rats were hypertrophic (15). Irnak M. Barbosa et al. found thickening of pyloric muscle in newborn rats thanks to NO inhibition formed by giving L-NAME (enteral hormone) to pregnant rats (16). Boybeyi et al. found thickening in the pylorus of rats after NO inhibition with L-NAME (17). Other substances associated with IHPS include hormones such as Substance P, Neurotensin, Enteroglucagon, Somatostatin, Vasoactive Intestinal Peptide, Secretin (17).

Clinic:

The disease often manifests itself in 3-5-week-old infants with projectile, bile-free vomiting. The disease occurs very rarely (4%) after 12 weeks (1). Vomiting may suddenly start to be severe or gradually increase in severity. It is difficult to diagnose it in infants with premature and neurological problems because the severity of vomiting may be milder. Different patient groups ranging from mild

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dehydration to severe dehydration may be encountered depending on the time of admission. The baby is constantly hungry and consequently, cries constantly. She sucks the bottle with an appetite because she is hungry. Some patients start vomiting 2-3 hours after feeding and others start vomiting 2-3 hours after feeding. Hematemesis may be observed in vomit content. Gastric peristalsis can be seen visually, especially in weak and dehydrated patients, and palpation of the pylorus to hypertrophy is easier in these patients at the same time. The palpable hypertrophic pylorus is called the 'olive sign' and is diagnostic for the diseas (1, 2).

Vomiting stomach contents leads to the loss of sodium, potassium, chloride, and hydrogen ions and eventually to the development of classical hypochloremic, hypokalemic metabolic alkalosis. The ability of the kidneys to maintain a normal pH by releasing bicarbonate is impaired by chloride depletion. Instead, excess bicarbonate is reabsorbed to maintain electrochemical neutrality. This will exacerbate the alkalosis. Kidneys secrete hydrogen ions to maintain potassium, forming paradoxical acidity, which exacerbates alkalosis (2). Today, patients are diagnosed early and treatment is started thanks to the development and spread of diagnostic methods. Therefore, severe dehydration is rarely observed.

Diagnosis and Imaging:

A good anamnesis and physical examination play an important role in the diagnosis of IHPS as with many other diseases. The patient's bile and projectile vomiting are important symptoms that support the diagnosis. However, the different way of vomiting does not rule out disease. One of the cheapest and most precise methods for diagnosis is palpation of the hypertrophic pylorus. Palpation of the pylorus has a positive predictive value of 99% for diagnosis (2). Palpating the hypertrophic pylorus is not as easy as it is thought. The palpation rate of pylorus varies between 50-90% (18). The patient should be calm, in the supine position, knees should be pulled towards the abdomen in order for the examination to be performed properly. The pyloric mass is searched between the belly and the arcus costarum on the lateral side of the right rectus muscle with the fingertips of the right hand, and the mass is tried to be compressed by supporting it from the posterior abdominal wall with the other hand. Another physical examination result is the observation of gastric peristalsis.

Ultrasonography is the most widely used and reliable diagnostic tool in the diagnosis of the disease today even though direct radiography and gastric duodenal radiographs are also used in the diagnosis of the disease. The sensitivity and specificity of ultrasonography in experienced hands is over 95% (19, 20). It should be kept in mind that 5-10% false-negative results were obtained in ultrasonography (20). Pyloric muscle thickness, pyloric canal length, and diameter of

the pyloric canal are measured ultrasonographically in the diagnosis of IHPS. Pyloric muscle thickness (pyloric single wall thickness) is 3-4 mm, pyloric canal length is 5-14 mm, and the diameter of the pyloric canal is 10-14 mm in a healthy newborn (21). Pyloric muscle thickness above 4 mm, pyloric canal length above 18 mm, and pyloric canal diameter above 15 mm are supportive of the diagnosis even though it differs slightly according to clinicians (1). Endoscopy can also be used in patients who cannot be diagnosed with these methods or who are suspected of another disease. Endoscopy shows thickened antrum and pylorus mucosa (22).

Treatment:

There are also alternative treatment options such as atropine treatment and balloon dilatation, which have been applied in small patient groups, whose treatment effectiveness has not yet been fully proven even though Ramstedt pyloromyotomy is still the definitive treatment option for IHPS today (23).

The main electrolyte disorder in IHPS patients is hypokalemic, hypochloremic metabolic alkalosis, and patients have paradoxical acidity. The first step in the treatment of pyloric stenosis is to correct the biochemical abnormalities and dehydration of the patient even though severe dehydration is not observed much nowadays due to the widespread diagnosis methods.

IHPS was tried to be treated with nonoperative methods for a period of time. The patients were hospitalized in the semi-sitting position and their stomachs were first washed 3-4 times a day for at least 48-72 hours. Patients were then given methylscopolamine nitrate or metoclopramide 1mg/kg 20-30 minutes before feeding. Then, babies were gradually fed by increasing their nutrients (24). Atropine sulfate was also used for the same purpose (24, 25). Today, atropine sulfate is used by many centers in the treatment of pyloric stenosis. Atropine is parasympatholytic due to its reversible antagonistic binding to muscarinic acetylcholine receptors. It inhibits peristalsis and therefore reduces muscle spasms thought to be caused by IHPS muscle hypertrophy. It is also a very potent inhibitor of food-induced gastric acid secretion. Atropine can be administered intravenously or orally in this approach. The obstructive process decreases, and patients are fed a nasoduodenal tube for several months as patients gain weight. Atropine treatment has been described in a limited number of small case series (25). Takeuchi et al. demonstrated an overall success rate of 78.9% (142/180) in the series of 188 patients initially treated with atropine (25). The median number of days reported for successful medical treatment is 12-14 days and the most recommended dose of the drug is 0.06 mg/kg/day administered intravenously (25). It is recommended to administer atropine treatment in selected patient groups at

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high risk for surgical and anesthesia-related complications since studies on atropine treatment are inadequate, the treatment process takes a long time, and surgical treatment is safe and effective.

Fredet-Ramstedt pyloromyotomy is the most effective treatment method that has been valid for more than a hundred years even though alternative treatments have been tried for IHPS (art1). The hypertrophic muscles on the anterior wall of the pylorus are cut longitudinally and descended to the mucosa and the mucosa is expected to prolapse from the incision in this examination. This procedure allows the lumen of the pyloric canal to expand. The incision on the pylorus should extend from the pyloric vein to the circular fibers of the gastric wall at the duodenal end for an adequate pyloromyotomy. Muscle fibers on the duodenum side should not be overexerted and 1-2 mm should be advanced towards the gastric tissue on the stomach side due to the risk of perforation during the procedure (1). The classic incision is a transverse incision of approximately 2.5 - 3 cm in length above the right umbilicus. Abdominal midline vertical and supraumbilical half-moon incisions are also used as an alternative to this incision. However, neither has many advantages over the other.

Pyloromyotomy has also been performed laparoscopically in many centers in recent years. Alain JL et al. introduced the laparoscopy technique in IHPS to the literature first (26). Advocates of the laparoscopic method argue that the procedure lasts shorter, patients switch to full enteral nutrition in a shorter time, and patients are discharged earlier (27). It was observed in another study that the risk of missing surgery and mucosal perforation were slightly increased in the laparoscopic method compared to the open method (28). Developing techniques such as single-incision laparoscopic pyloromyotomy (29), microlaparoscopic pyloromyotomy (30), and endoscopic pyloromyotomy (31) are also available in addition to laparoscopic pyloromyotomy.

Postoperative Management and Complications Include:

The main issues in the postoperative follow-up of infants with IHPS are the patient's monitoring, analgesia, and nutrition. There are differences between clinics between postoperative feeding times. It is recommended to start feeding with sugary water as the baby tolerates and to switch to full enteral feeding with breast milk or formulas at the end of 24 hours due to the risk of vomiting and aspiration (2). In addition, there are also those who advocate the diet of feeding the baby as much as she wants (32). Advocates of this diet say that the duration of discharge of the patients is shorter with ad libitum feeding started at the 4th hour after the operation (32). On the other hand, Adibe et al. argue that there is no difference in

length of hospital stay between patients receiving protocol nutrition and those receiving ad libitum feeding (33).

Postoperative vomiting is quite common. Vomiting in the first 5 days is generally considered normal due to physiological reflux or impaired coordination in gastric peristalsis. Vomiting that continues after the 5th postoperative day should suggest inadequate surgery or perforation. However, vomiting should continue for at least 2-3 weeks in order to be able to say inadequate surgery (1).

Pyloromyotomy is usually associated with low morbidity and mortality. The most important postoperative complications are missing pyloromyotomy, mucosal perforation, and wound infection. General complication rates vary between 4.6% and 12% (1, 2). Perforation is almost always on the duodenal side. Small perforations are repaired primarily and omentum is placed on them. The mucosa is repaired, and the muscle layer is repaired full layer, then pylorus is reversed and pyloromyotomy is performed posteriorly in large-scale perforations (1). If the perforation is noticed, it is repaired, and then nasogastric decompression is recommended for the first 48 hours. If the perforation is not noticed, a series of events leading to tachycardia, tachypnea, abdominal swelling, and sepsis is observed in the postoperative period. Mortality from pyloric stenosis has been greatly reduced and is currently close to zero in most centers (34). There are significant differences in mortality rates after pyloromyotomy between developed countries and developing countries. It was found in a study conducted in Tanzania that the mortality rate was as high as 4.9%. The authors think that this is due to severe dehydration and electrolyte disorders due to late admission to the hospital (34).

Conclusion:

IHPS remains a common and important cause of vomiting during infancy. Early hospital admissions and increased use of ultrasound led to earlier diagnosis of infants with IHPS. Therefore, classical electrolyte disorders are rarely observed, but if seen, it is very important to treat them properly before the operation. The exact cause is not known yet even though more than 100 years have passed since the diagnosis of the disease. The disease is thought to occur due to genetic and environmental causes and NO and NOS are said to play an important role in etiology in recent years. Adherence to the pyloromyotomy technique described by Ramstedt is still ongoing even though developing techniques such as laparoscopy and endoscopy are used in the treatment of the disease.

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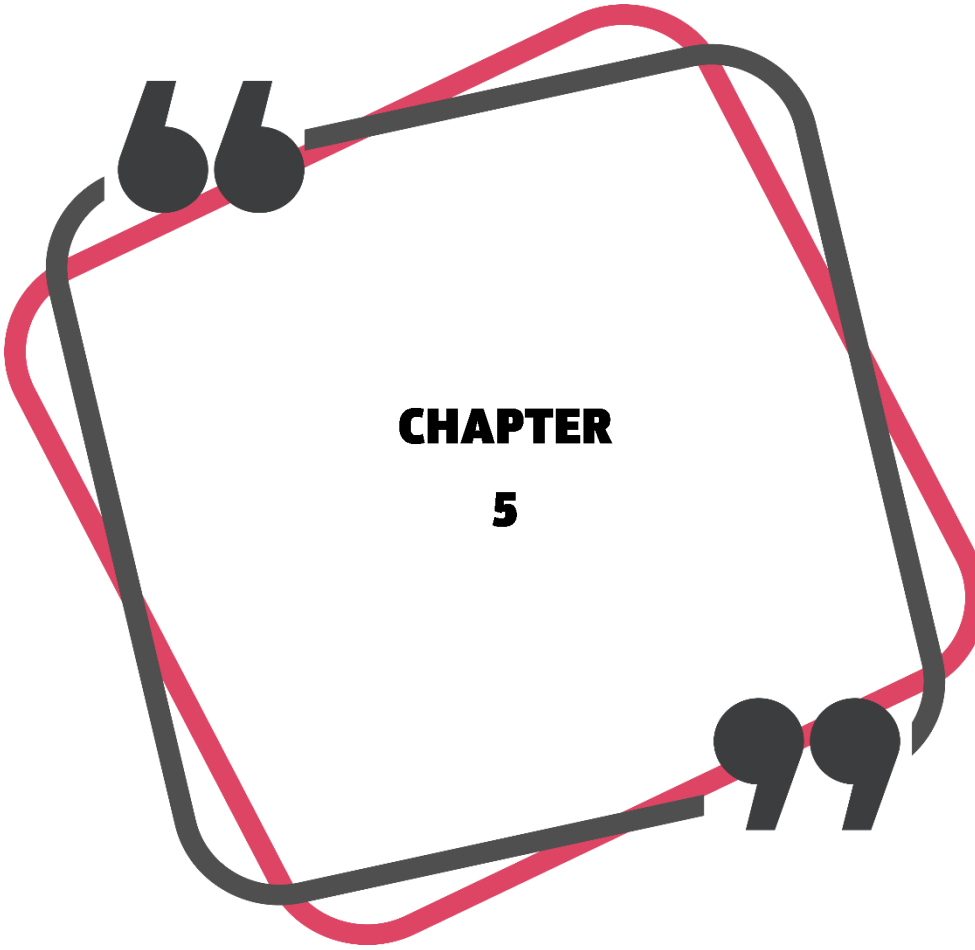
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CHAPTER
5

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CURRENT PROSPECTIVE OF MIGRAINE HEADACHE TREATMENT

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Introduction

Migraine is quite prevalent primary headache disorder with variable socioeconomic and individual-level burden[1]. After low back pain, chronic migraine remains the second most common cause of years spent disabled globally. Migraine still remains public health concern since about 21% of women and 10.7% of men are affected[2]. Migraine sufferers have a heavy burden, as 53.7% of them report their episodes leave them severely impaired. The disability caused by migraines affects performance at work, in the classroom, and in daily life. Additionally common complaints among people with chronic migraine sufferer are social impairments, emotional distress, and cognitive difficulty[3]. Migraine treatment fall into two main categories: pharmacological and non-pharmacological methods. Pharmacological management options for chronic migraine include abortive treatment and preventive therapy. This review discusses pharmacological management of both episodic and chronic migraine with regard to the current evidence of migraine treatment.

Acute Migraine Treatment

The primary objectives of abortive migraine treatment are to achieve relief of pain and other related migraine symptoms (e.g. nausea and vomiting), to reduce the need for adjunctive treatment, and improve functional ability of patients. Acute migraine treatment options include non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, migraine-specific triptans, antiemetics, and recently licensed antmigraine agents like lasmiditan, CGRP antagonists, and dihydroergotamine. The severity of the attacks, the occurrence of related symptoms (eg. nausea and vomiting), and individual patient-specific characteristics, such as the presence of contraindications and patient preference, all influence the pharmacologic method used to abort acute migraine [4].

Patients with mild to moderate migraine headaches (i.e. headache is not associated with vomiting or severe nausea), simple analgesics (NSAIDs, acetaminophen) or their combination forms are effective and less expensive than migraine-specific agents (e.g. Triptans). Combining an NSAID and a triptan seems to be more beneficial than using either drug class alone for attacks that don't respond to analgesics[5].

Moderate to severe migraine attacks:

Oral migraine-specific medications, such as oral triptans are first-line treatments for moderate-to-severe migraine attacks that are not accompanied by severe nausea and vomiting. Triptans are currently the most commonly used medications for acute treatment of moderate to severe migraine headache. Triptans are highly affine 5-HT1B and 5-HT1D selective 5-hydroxytryptamine (5-HT) receptor agonists. They work by lowering neurogenic inflammation, limiting meningeal vascular vasoconstriction, and controlling second-order neurons. Vasoconstriction is the effect of 5-HT1B activation, while 5-HT1D stimulation prevents the release of neurotransmitters in the dorsal horn that transfer nociceptive information to the thalamus. However, patients with underlying cardiovascular disorders or those who are at risk of specific adverse cardiovascular events should not be treated Triptans due to their vasoconstrictive effects[6]. Use of Triptans must be restricted to a maximum of 10 days a month to keep away from drug overuse headache. Based on current evidence, Triptans should still be avoided in individuals with hemiplegic migraine, basilar migraine, ischemic stroke, ischemic heart disease, patients with uncontrolled hypertension, and pregnant mothers. A CGRP antagonist or lasmiditan may be useful for people who are ineligible for triptans or who cannot tolerate them. Severe migraine attacks that are accompanied by

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vomiting or intense nausea may be treated with intravenous antiemetics, non-oral triptans such as nasal or subcutaneous sumatriptan, or parenteral dihydroergotamine[7].

Status migrainosus: Status migrainosus is defined as a debilitating migraine attack lasting that lasts more than 72 hours. Based up on latest guidelines intravenous fluids with parenteral medications such ketorolac and a dopamine receptor blocker including metoclopramide, prochlorperazine, and chlorpromazine). Other abortive agents are sodium valproate and/or dihydroergotamine. It is recommend that patients with status migrainosus be admitted to the hospital. Adjunctive treatment with IV or intramuscular dexamethasone or prednole have been shown to abort migraine headache and also reduce the risk of early migraine headache recurrence[14].

New pharmacological agents for abortive treatment of migraine

CGRP antagonists: These oral CGRP antagonists, also referred to as gepants are alternatives for the acute treatment of migraine in patients who don't respond to or have contraindication (for example, coronary artery disease) to triptan therapy. Recently randomized controlled trials for rimegepant and ubrogepant showed that they are effective for treatment of acute migraine attack[8]. A meta-analysis in 2021 found that pain resolution by two hours was likelier with either rimegepant (relative risk [RR] 1.8, 95% CI 1.5-2.1) or ubrogepant (RR 1.6, 95% CI 1.3-1.9) than placebo. Both Ubrogepant and rimegepant received US Food and Drug Administration (FDA) approval for the treatment of acute migraine in adults in December 2019 and February 2020 respectively[9, 10].

Lasmiditan: Lasmiditan is a selective serotonin 1F receptor agonist with no vasoconstrictor activity and hence is suitable option for patients who have contraindications to triptans. In a randomized trial involving more than 2200 episodic migraine patients, lasmiditan 200 mg were associated with higher rates of headache pain freedom at two hours (32 versus 15% with placebo; absolute risk difference [ARD] 17%; odds ratio [OR] 2.6, 95% CI 2.0-3.6). The US Food and Drug Administration (FDA) approved lasmiditan oral tablets for the acute treatment of migraine in adults In October 2019. The most frequent side effect of lasmiditan is dizziness, although paresthesia, somnolence, tiredness, and nausea are also somewhat common side effects[11, 12].

ERGOTS: Numerous ergotamine formulations were used to treat migraines adjuvantly, both alone and in conjunction with caffeine or other analgesics. When

administered intravenously, dihydroergotamine is almost always used in conjunction with an antiemetic medication. Dihydroergotamine is used in the treatment of chronic persistent migraine attacks or status migrenosus. Parenteral dihydroergotamine in combination with antiemetic agents appears to be effective in aborting acute migraine attack. This findings of 11 randomized controlled trials proved the efficacy of IV or IM dihydroergotamine treatment for acute migraine in adult patients[13].

Prophylactic Treatment of Migraine

The prophylactic treatment for migraine is aimed to decrease the migraine headache frequency and severity, to Improve effectiveness of abortive of acute migraine, to enhance the quality of life and reduce disability, and finally to prevent progression of recurrent migraine to chronic migraine.

Indications for prophylactic treatment: Preventive treatment of migraine are indicated for patients with frequent (recurrent) migraine attacks (more than four attacks per month), patients with severe migraine headaches that cause severe disability that decreases the quality of life despite receiving appropriate acute treatment, patients who do not respond to acute therapy or have contraindications to acute migraine treatment, patients at high risk for misusing migraine medications, and patients with menstrual migraine. Based on current evidence and expert opinion, prophylactic treatment is also indicated for specific types of migraine to minimize the risk of neurologic impairment. These migraines subtypes include hemiplegic migraine, migraine patients with brainstem aura, persistent aura without infarction and migrainous infarction [15].

In general, prophylactic agents of migraine should be considered for patients with four or more attacks every month; likewise the decision to start a prophylactic medications is influenced by the severity and duration of migraine attacks. Therapeutic dose of each of these agents vary among patients since they have different side effects and tolerability. The mechanism by which these agents prevent migraine attacks are not well understood, but it is believed that they modify the sensitivity of the brain that underlies migraine. Patients are usually started on a low dose of the selected medication. They are taken daily and started at low dose and titrated upward until effective dose is achieved. Reasonable effectiveness are achieved in 2 to 12 weeks in majority of patients.

The probability of success by these agents is nearly 50% to 75%. Once effective stabilization is achieved in tolerable dose, the drug is maintained for around

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6 to 12 months before being gradually reduced to determine whether continuation is required[16]. Prophylactic agents for migraine include beta blockers, anticonvulsants, antidepressants, and the newly approved 'CGRP antagonists. Individual patient features, coexisting diseases, drug side effect profiles, medication costs, and patient values and preferences should all be taken into account when selecting one of these prophylactic agents.

Anticonvulsants

Sodium valproate and Topiramate have proven to be more effective than placebo in reducing the frequency of attacks in migraine prophylaxis (Class A evidence). Gabapentin was also proved effective in migraine but not as effective as the other two anticonvulsants[17, 18].

Topiramate: Topiramate is an efficacious in migraine prevention according to numerous studies including several placebo-controlled studies, a systematic review, and a meta-analysis. In two clinical trials, Topiramate was found to be more effective than sodium valproate and gabapentin in preventing migraines. In most of these studies, the Topiramate dose was started at 25 mg/day and slowly increased by 25 to 50 mg/week to a maximum of 100 mg twice daily or the highest tolerated dose. Topiramate treatment-related side effects also included paresthesia, tiredness, anorexia, diarrhea, weight loss, concentration problems, nausea, and taste aberration. The majority of these side effects paresthesia is seen in nearly half of patients on Topiramate at doses of 100 or 200 mg per day [19, 20].

Sodium Valproate: Valproate is an effective drug in preventing migraine attacks even at doses lower than 1000 mg per day. Sometimes it may be necessary to increase up to 1500 mg per day to see treatment response, but it is not recommended as higher levels will increase side effects. Nausea, sleepiness, tremor, dizziness, weight gain, and hair loss are a few of these side effects. Due to its teratogenicity, it should not be used during pregnancy [21].

Beta Blockers

The efficacy of metoprolol, propranolol, and timolol in preventing migraines headache attacks have been demonstrated by numerous studies. Other alternative beta blockers are atenolol and nadolol but are not as strong as other beta blockers. Propranolol in divided doses starting at 40 mg daily are the most commonly used beta blockers for migraine; dose can be titrated from 40 to 240 mg daily. It may take several weeks to see the headache decrease with these medications; the dose

should be increased and used for at least three months before the drug is deemed to have failed in treatment. The use of beta blockers should be avoided in patients with erectile dysfunction, peripheral vascular disease, Raynaud phenomenon, and patients with bradycardia. Also they should be used with caution for patients with asthma, diabetes, and patients with cardiac conduction abnormalities [22, 23].

Calcium Channel Blockers

CCBs are other antihypertensive agents for migraine prophylaxis. Although their mechanism of action for migraine is not well defined, yet it is believed that CCBs induce intracranial vasoconstriction in migraine patients. Flunarizine, is nonspecific calcium channel blocker, is the prominent CCB for migraine prophylaxis. The dosage of flunarizine is 5 to 10 mg per day, according to various controlled research[24].

Antidepressants

Based on systematic review of prospective double-blind, randomized controlled trials, the tricyclic antidepressant amitriptyline was proved effective for migraine headache prevention. Amitriptyline is particularly preferred for patients with chronic migraine who have associated tension headache, depression, anxiety or insomnia. In clinical practice, amitriptyline dosage recommendations range from 10 to 100 mg daily[4,3,2]. Common side effects of tricyclic antidepressants include dry mouth, palpitations, orthostatic hypotension, constipation, weight gain, and urinary retention. Amitriptyline should be used with caution in elderly migraine patients. Venlafaxine (which is serotonin-norepinephrine reuptake inhibitor) is another antidepressant proved its efficacy for migraine headache prophylaxis. Venlafaxine should be started at a low dose and titrated slowly (starting at 37.5 mg once daily to a maximum of 150 mg). Venlafaxine is especially beneficial for patients with other comorbid panic disorder, generalized anxiety disorder, or social anxiety disorder [25].

Some Preventive Migraine Treatments

CGRP antagonists are recently approved agents for migraine treatment. These molecules mediate trigeminovascular pain transmission and neurogenic inflammation. For the prevention of migraines, large molecule preparations of monoclonal antibodies against the CGRP receptor are administered intravenously.

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Small molecule CGRP antagonists are oral therapy agents that have been shown to be effective in acute migraine attacks. Rimegepant and atogepant have been shown effective for migraine prevention. The U.S. Food and Drug Administration approved Erenumab, fremanezumab, and galcanezumab for use in the treatment of migraine in 2018, eptinezumab in 2020, and rimegepant and atogepant in 2021[26].

Erenumab is a human monoclonal antibody that is moderately effective in migraine attack prevention. It is applied subcutaneously to the upper arm, abdomen and thighs once a month. The most common side effects are injection site reactions and constipation.

Fremanezumab, Galcanezumab and Eptinezumab are other monoclonal antibodies that binds to both isoforms of the CGRP ligand. Randomized trials' results show that it is effective in preventing migraines. There are currently insufficient data on the use of CGRP antagonists in certain populations (children, the elderly, pregnant or breastfeeding patients). They should be avoided by women who are pregnant or who are planning a pregnancy, as well as by anyone who have recently experienced a cardiovascular or cerebrovascular ischemic event. Although these side effects were not seen in randomized trials, there has been concern that CGRP antagonists have potential for them.

Rimegepant is one of the small-molecule CGRP antagonists. It is taken orally and approved its efficacy for migraine headache prevention. A randomized clinical trial was conducted in 1591 migraine patients who received 75 mg every other day for 12 weeks with Rimegepant. As a result, it was observed that there was a greater reduction in migraine days compared to the placebo group (4.3 versus 3.5 days; difference -0.8 days, 95% CI -1.46 to -0.2). While nausea is the most often reported adverse effect, rimegepant has great appreciation safety and tolerability. Rimegepant does not have vasoconstrictive property, as demonstrated by earlier trials, and therefore is useful for individuals who cannot take triptans because of cardiovascular contraindications [27, 28].

Atogepant is another oral small-molecule CGRP receptor that has been shown its effectiveness in controlling migraine headache. A double-blind, multicenter, randomized clinical trial was conducted in the United States. It was administered to 834 people who experienced 4 to 14 migraine attacks per month for 12 weeks. In this study, the first group was given 10 mg of drug once a day, the second group was given atogepant regimens ranging from 60 mg twice a day, while another group was given a placebo. It was determined that chronic migraine headaches were reduced by approximately 50% in these subjects with Atogepant

treatment regimens. Higher dose of atogepant was associated with rimegepant-like nausea[26].

Botulinum toxin A for migraine

Botulinum toxin type A is an approved preventive chronic migraine (CM) treatment. Numerous clinical trials have shown that it is well tolerated and effective at preventing migraine headaches in people with chronic migraine who are not responding to other prophylactic medications. Botulinum toxin type A is injected in to particular head and neck muscles. It acts on the peripheral sensory nerve endings located in the trigeminal-occipital and cervical complex. Botulinum toxin type A ability to treat chronic migraines is mediated by a range of changes in pain activation mechanisms, including the down regulation of nociceptive neurons' receptors and a decrease in the level of released neurotransmitters and neuropeptides that can initiate pathways associated to migraine. According to PREEMT1 & 2, patients with chronic migraine who were given Botulinum toxin type A experienced fewer monthly headache days than those who received placebo [29, 30]. With Botulinumtoxin type A, improvements in secondary outcome indicators were also greater. Botulinum toxin A should be administered by an experienced clinician inly in in a clinical setting. Cost is relatively higher when compared to other migraine preventive agents and this is a limiting factor of its availability everywhere. Some insurance companies have policies that restrict Botulinum toxin A only allow for patients who are unable to take or are unable to tolerate other prophylactic drugs [31].

Conclusion

The choice of acute migraine treatment should be based on current evidence-based treatments. Non-oral agents should be preferred for patients with severe migraine attacks who have severe nausea and vomiting. Current evidence-based medications for acute migraine treatment include triptans, ergotamine derivatives, NSAIDs, and analgesic combinations. In addition, there are newer Calcitonin gene-related peptide (CGRP) antagonists. **Patients who experience frequent migraine headaches or persistent attacks that significantly impair quality of life or cause severe disability should be prescribed migraine prophylactic agents.** The choice of prophylactic agents should be based on patient's response to acute treatment, tolerability, patient preference, comorbidities potential for childbearing, as well as the current evidence of drug efficacy, and expert opinion.

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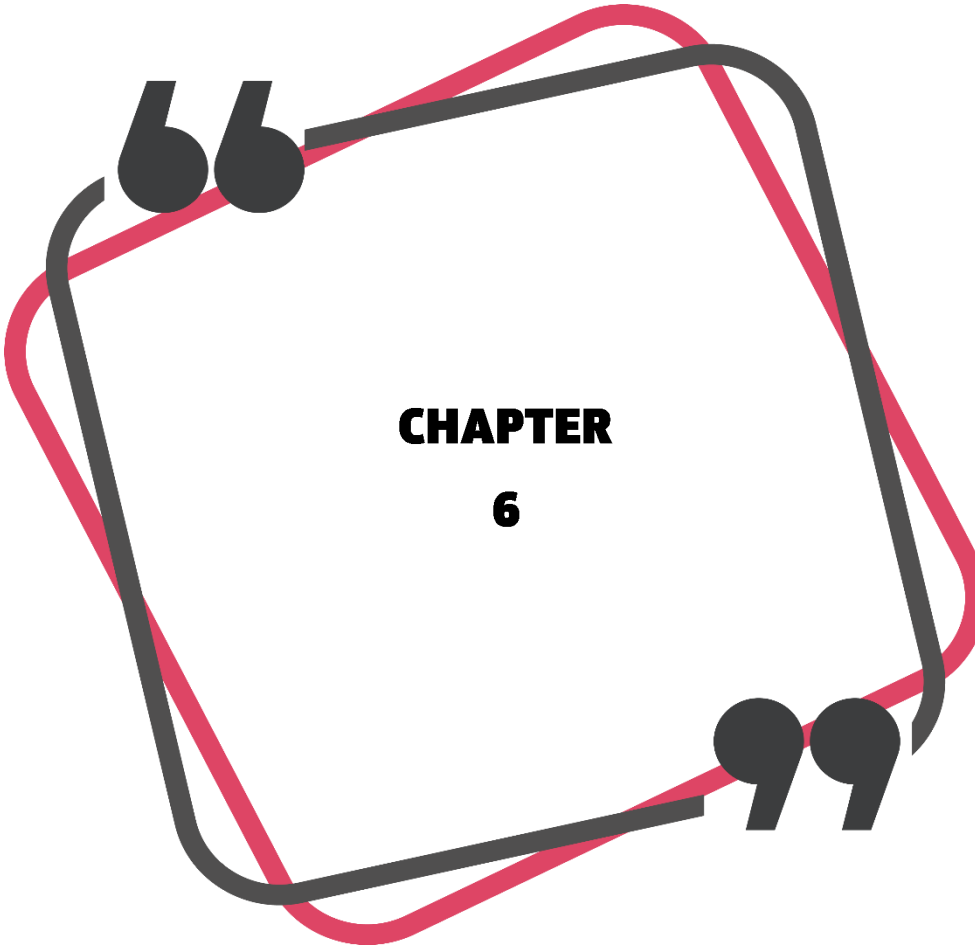
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CHAPTER
6

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PREVENTING EFFECTS OF MOSS EXTRACT (HOMALOTHECIUM SERICEUM (HEDW.) SCHIMP.) AND QUERCETIN ON PANCREATIC TISSUE IN DIABETIC RATS

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INTRODUCTION

Diabetes is a disease that poses serious threats to the future with the number of cases increasing day by day, and it is essential to use and illuminate different sources in the fight. The interest in alternative or supportive treatments is growing due to the high costs of health care and adverse effects of the drugs used to treat diabetes. Today, diabetic patients turn to herbal raw material sources with high

antioxidant and anti-hypoglycemic properties, as well as the drugs they use in the fight against hyperglycemia.

Bryophyte phytochemistry has long been neglected because bryophytes are morphologically very small and very difficult to obtain in pure form, considered nutritionally unhelpful for humans. No references to its use as food for humans have been found. In addition, bryophytes are a widely used medicinal plant in China to treat many diseases, from burns to poisoning animal bites, tuberculosis, and pneumonia. It has been reported that the biological activities of these species consist of especially terpenoids and aromatic compounds found in the oil masses of all species [1,2]. The production and use of herbal antioxidants are crucial in terms of reducing oxidative stress and inhibiting diseases accordingly [3-5]. Quercetin, one of the most widely used flavonoids, also shows an antidiabetic effect due to its strong antioxidant content [6-8]. Li et al. In their study with STZ-induced diabetic rats, they showed that ferroptosis resulting from iron accumulation causes dysfunction in pancreatic B cells [9].

It was aimed to associate the antioxidant properties of moss extract with diabetes and to determine the healing properties of pancreatic tissue in diabetic rats because of its combination with an effective antioxidant such as quercetin in this study.

MATERIAL AND METHOD

The study protocol was approved by the Çanakkale Onsekiz Mart University, Ethics Committee for Animal Research (Protocol number: 2022/09-06). 40 mg/kg STZ were injected into twenty-eight rats. Blood glucose levels of the subjects were measured before the administration of STZ.

Groups

Control (C) (n=7): 1cc saline was injected ip.

Diabetes (D) (n=7): STZ (40 mg/kg) were administered to the subjects by dissolving citrate buffer (0.01M, pH=4.5).

Treatment 1 (T1) (n=7): STZ-induced subjects received moss extract (150 mg/kg) for treatment by gavage for three weeks.

Treatment 2 (T2) (n=7): Commercially available quercetin (50 mg/kg) for treatment was given to STZ-induced subjects by gavage for three weeks.

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Treatment 3 (T3) (n=7): STZ-induced subjects received quercetin and moss extract for treatment by gavage for three weeks.

Histological and Biochemical Procedure

The subjects were sacrificed by ketamine/xylazine anaesthesia, tissue samples were placed in immunofix fixative and histological follow-up was performed.

Immunohistochemical staining

Polyclonal rabbit anti-nuclear factor-kappa B (NFκB p65, Abcam (ab16502) and interleukin-6 (IL-6 ab6672), Ki-67 (ab231172), Caspase-3 monoclonal antibody (Thermofisher, 74T2) were used as primary antibodies for other tissue samples.

ELISA assay

SOD (Superoxide dismutase), MDA (Malondialadehyde), Triglyceride, C-peptide insulin and glucose were analyzed with the ELISA assay.

Statistics

SPSS version 19.0 was used for the statistical evaluation. Kruskal-Wallis, one of the non-parametric tests, was used to determine the immunoreactivity between the groups.

RESULTS

The blood glucose values of the diabetes group increased significantly compared to the control group ($p<0.0001$). After the T1 and T2 procedures, the blood glucose values measured were significant compared to the diabetes group (Table 1).

Table 1 Glucose values of the experimental and treatment groups

Groups and glucose value	**C**	**D**	**T1**	**T2**	**T3**
Before Treatment	98 ± 2,6	404 ± 14,21	378 ± 18,41	476 ± 28,68	448 ± 20,24
After Treatment	101 ± 3,1	469 ± 11,14	264 ± 16,54*	225 ± 15,30**	211 ± 12,24***

*p<0.05 in the T1 group

**p<0.01 in the T2 group

***p<0.001 in the T3 group

Histological examining with routine Hematoxylin-Eosin (H&E) staining showed atrophy occurred in the islets of Langerhans, pycnotic cells increased, and vacuolization and mononuclear cell infiltration in the endocrine structure were detected in STZ-induced group. It was observed that the atrophic structure of the pancreas regressed in group T1, and both the appearance of the pancreatic tissue improved and the histopathological image began to disappear in groups T2 and T3 (Fig. 1).

It was determined that the inflammatory markers NFκB and IL-6 immunoreactivity were very severe in pancreatic tissues of diabetic rats (Fig. 2, 3) and decreased in the treatment groups ($p<0.0001$). It was a noteworthy difference between the control and treatment (T1 and T2) groups ($p<0.05$). In addition, Ki-67 was used to monitor the regenerative activity and the intensity of immunoreactivity, and the number of stained cells were light (Fig. 4). The increase in the number of cells progressing to apoptosis was relatively high in the diabetes group. The number of apoptotic cells decreased in the treatment groups. Caspase-3 positivity, which was used to mark apoptotic cells, was low especially in the T3 group (Fig. 5).

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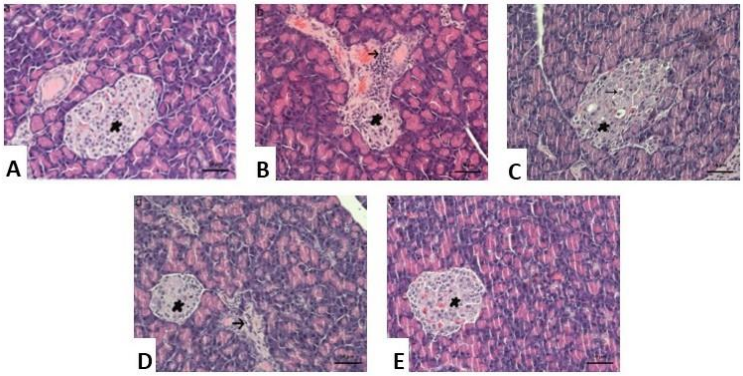


Fig. 1 H&E staining of control and experimental groups, X20 (**A:** Control, **B:** Diabetes, **C:** Diabetes+moss, **D:** Diabetes+ quercetin, **E:** Diabetes+moss+quercetin, Arrow: Immunoreactivity, Star: Langerhans islet)

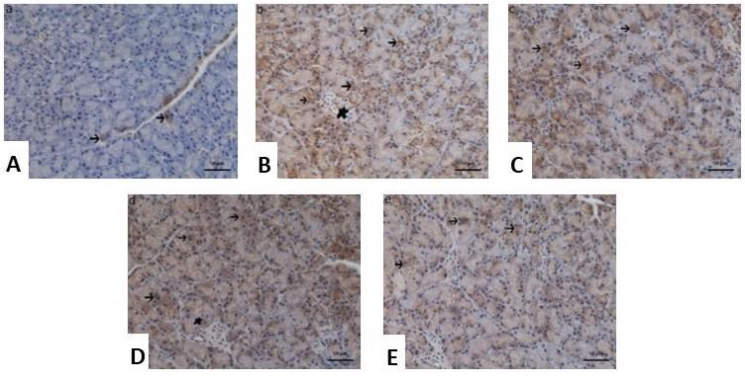


Fig. 2 Pancreatic tissue of control and experimental groups, NFκB staining, X20 (**A:** Control, **B:** Diabetes, **C:** Diabetes+moss, **D:** Diabetes+ quercetin, **E:** Diabetes+moss+quercetin, Arrow: Immunoreactivity)

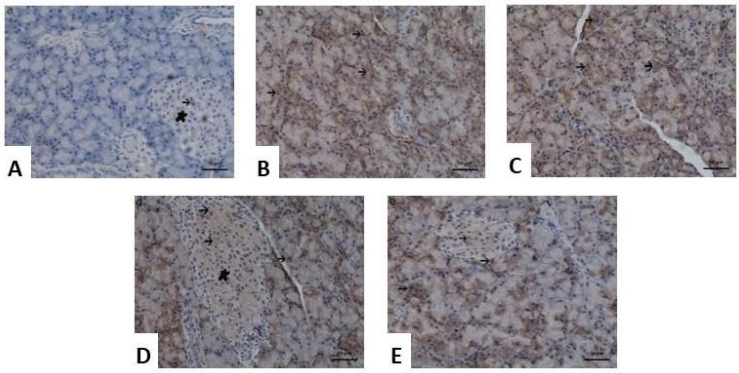


Fig. 3 Pancreatic tissue of control and experimental groups, IL-6 staining, X20 (**A:** Control, **B:** Diabetes, **C:** Diabetes+moss, **D:** Diabetes+ quercetin, **E:** Diabetes+moss+quercetin, Arrow: Immunoreactivity) of the groups given M+Q for treatment

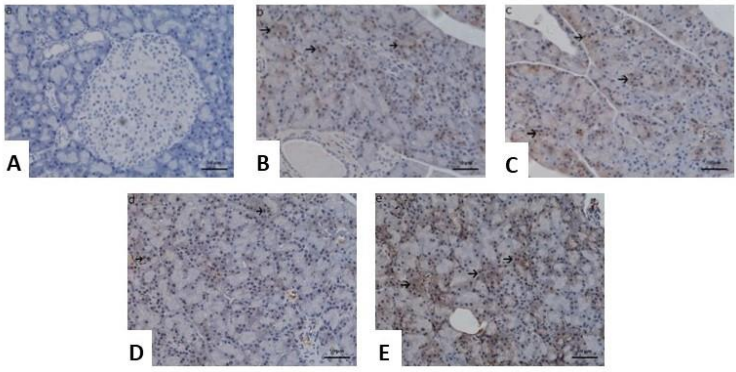


Fig. 4 Pancreatic tissue of control and experimental groups, Ki-67 staining, X20 (**A:** Control, **B:** Diabetes, **C:** Diabetes+moss, **D:** Diabetes+ quercetin, **E:** Diabetes+moss+quercetin, Arrow: Immunoreactivity)

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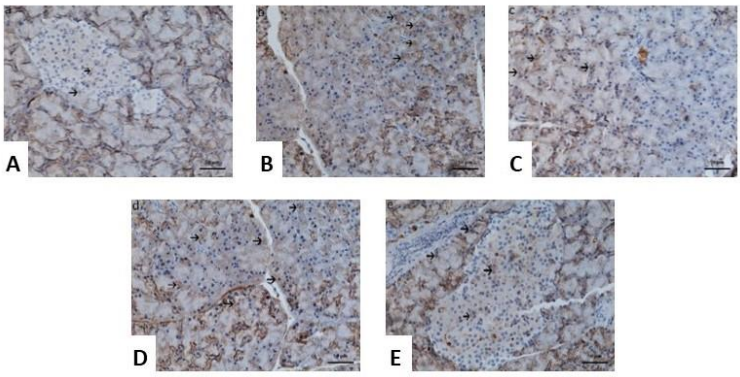


Fig. 5 Pancreatic tissue of control and experimental groups, Caspase 3 staining, X20 (**A:** Control, **B:** Diabetes, **C:** Diabetes+moss, **D:** Diabetes+ quercetin, **E:** Diabetes+moss+quercetin, Arrow: Immunoreactivity)

Biochemical Findings

Diabetic animals were showed a significant decrease in SOD activity compared to controls, and the T1 and T2 groups were showed a dose-dependent increase in SOD activity. The highest increase was observed in the T3 group. In addition, a significant decrease in serum C-peptide level was also seen in the D group compared to the control group. It was determined that serum C-peptide level increased significantly in T1 and T2 groups. The serum MDA level was doubled in the diabetes group compared to the control group. In the treatment groups, the MDA level decreased significantly compared to the diabetic group. IFN-γ, which was highest in the D group, decreased in the T3 group (Table 2).

Table 2 Biochemical analysis results

	Insulin	C-peptide	MDA	SOD	IFN-γ
Control	25.56 ± 3.21	46.12 ± 9.45	6.83 ± 2.21	224.12 ± 34.45	22.14 ± 8.51
Diabetes	15.24 ± 4.32[a]	28.54 ± 5.45[a]	13.2 ± 3.71[a]	148.42 ± 45.12[a]	30.06 ± 7.21[a]
Treatment 1	20.72 ± 3.40[b]	38.42 ± 6.23[b]	9.25 ± 6.21[b,c]	180.24 ± 28.45[b,c]	28.54 ± 5.26[b]
Treatment 2	22.31 ± 3.74[d]	42.65 ± 4.25[d]	8.05 ± 4.25[c]	189.24 ± 36.12[c]	27.79 ± 6.42[c]
Treatment 3	22.84 ± 3.52[d]	44.81 ± 6.85[d]	6.63 ± 3.27[d]	202.47 ± 42.21[d]	25.53 ± 3.66[d]

[a]$p<0.0001$ compared to the control group, [b]$p<0.001$ compared to the diabetes group, [c]$p<0.01$ compared to the control group, [d]$p<0.05$ compared to the control group.

DISCUSSION

In this study, antioxidant, and anti-inflammatory properties of orally administered moss and quercetin were determined to provide pancreatic regeneration in rats induced diabetes by STZ. Ahmed et al. reported that STZ increased free radical production in rats with diabetes. It has been stated that increased free radicals may deplete antioxidant defense components, lead to disruption of cellular functions and oxidative damage to membranes, and increase susceptibility to lipid peroxidation [10]. In our experimental model, STZ was preferred as a diabetic agent and diabetic effects developed in the subjects as stated in the literature.

Quercetin produced a significant positive effect in the treatment group given alone, as explained by the reduction in glucose and elevation in serum levels of C-peptide [11]. It has been reported that this effect may be related to the normalization of body insulin sensitivity by increasing the activity on the insulin receptor and its substrate, and the expression and phosphorylation of glucose transport-4 [12].

The study by Mukhopadhyay and Prajapati reported that quercetin could reduce hyperglycemia of pancreatic islets to release sufficient insulin. It is known that flavonoids trigger the regeneration of pancreatic islets of Langerhans to produce insulin and glucagon and secrete it into the bloodstream [13].

Quercetin inhibits the upregulation of IKK-α and IKK-β and reduces IκB-α degradation, preventing NFκB activation [14, 15]. IL-6 is produced in most cell types of the immune system, particularly endothelial cells, adipocytes, and islet β-cells [16, 17]. In our study, we observed that the expression of NF-kB and IL-6, which are inflammatory markers in the liver and pancreas, increases with diabetes. There is evidence that IL-6 contributes to the developmental processes of both types of diabetes and influences glucose balance and metabolism via pancreatic beta cells [18-20]. Fidan et al. determined that serum IL-6 levels in STZ-induced diabetic female rats for 6-8 weeks were like control subjects [21]. Altınova et al. showed no statistically significant difference in serum IL-6 levels between patient groups with and without microvascular complications of DM [22].

Considering the findings obtained in our previous studies, it was determined that the combination of quercetin, an effective antioxidant, with bryophytes [23, 24], which was determined to have a strong antioxidant property, was effective in diabetic rats. It was seen that diabetic complications could be prevented by using both products.

CONCLUSION

As a result, moss's antioxidant, and anti-inflammatory properties, one of the agents chosen for protection, were revealed for the first time. Furthermore, it was discovered that the combination of moss and quercetin could normalize glucose homeostasis, and that lipid profile and inflammation act retroactively with increased antioxidant power.

FUNDING

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CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest with respect to this article's authorship and/or publication.

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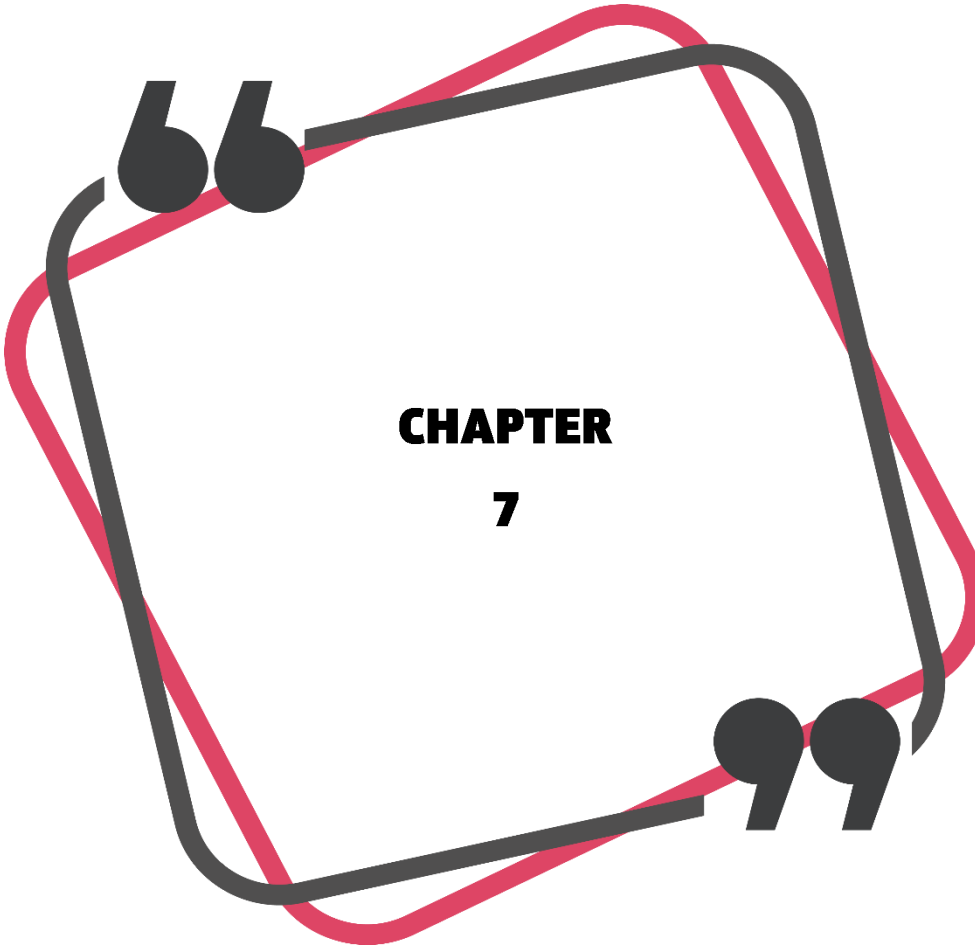
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CHAPTER
7

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BIOMARKERS FROM A CLINICAL AND APPLICATION POINT OF VIEW

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Definition

Biomarkers, also being used in the terms "surrogate marker" or "surrogate end-point" (Aronson & Ferner, 2017), are the biological indicators of normal biological or pathological processes and/or pharmaceutical reactions to a therapeutic action (Naylor, 2003). Besides from their biological or therapeutical significance, the definition of biomarkers is controversial. This is because biomarker term includes more enhanced research areas such as pathological and non-pathological outcomes as well as non-pharmacological interventions such as surgical procedures together with pharmacological responses (Aronson & Ferner, 2017).

Potential Applications

More than 100.000 biomarkers were described through 2015, reflecting an exceptional rise in biomarker studies (Holland, 2016). These molecules can be applied in a variety of ways including screening, characterization or staging of the diseases, informing prognosis, and predicting outcomes in response to drug treatments. Additionally, in drug discovery and development research, biomarkers can be used as potential targets, end points as measuring drug improvements and clinical trials, studying dose in pharmacodynamic studies (Aronson & Ferner, 2017).

The application areas includes the diseases such as heart failure (Braunwald, 2008) or myocardial infarction (Chan & Ng, 2010; Silva & Furie, 2009), neurological disorders (Hansson, 2021), or depression (Gururajan, Clarke, Dinan, & Cryan, 2016; Kore & Prabhavalkar, 2022), research fields such as psychiatry (Herron, Nerurkar, & Cavanagh, 2018; Singh & Rose, 2009), toxicology (Fitsanakis, Negga, & Hatfield, 2019; Gupta & Milatovic, 2014; Krishna, Goel, & Krishna, 2014), anti-aging therapies (Guest, 2019a, 2019b), pharmaceutical researches (Feuerstein, Alesci, Walsh, Rutkowski, & Ruffolo, 2021; Zhao,

Modur, Carayannopoulos, & Laterza, 2015) as well as environmental monitoring (Lam, 2009; Lam & Gray, 2003).

Biomarkers and Cancer

Biomarkers are being used more frequently and critically in cancer and associated studies. Biomarkers can be used in a variety of clinical stages of cancer, including risk assessment, screening, separating benign from malignant, determining prognosis and prediction, and tracking the disease's progression, either to detect recurrence or monitor the effectiveness of treatment (Henry & Hayes, 2012). The examples of biomarkers in those stages were given in Table 1.

Table 1: Potential uses for cancer biomarkers, *(Henry & Hayes, 2012)*

Use	Example	Reference
Estimate risk of developing cancer	BRCA1 germline mutation (breast and ovarian cancer)	Easton et al., 1995; Hall et al., 1990
Secreening	Prostate specific antigen (prostate cancer)	Lin et al., 2008
Differential diagnosis	Immunohistochemistry to determine tissue of origin	
Determine prognosis of disease	21 gene recurrence score (breast cancer)	Paik et al., 2004
Predict response to therapy	KRAS mutation and anti-EGFR antibody (colorectal cancer)	Allegra et al., 2009
	HER2 expression and anti-Her2 therapy (breast and gastric cancer)	Bang et al., 2010; Piccart-Gebhart et al., 2005; Romond et al., 2005
	Estrogen receptor expression (breast cancer)	EBCTCG 2011
Monitor for disease recurrence	CEA (colorectal cancer)	Locker et al., 2006

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	AFP, LDH, bHCG (germ cell tumor)	Gilligan et al., 2010
Monitor for response or progression in meta-static disease	CA15-3 and CEA (breast cancer)	Harris et al., 2007

Phases of Biomarker Development

In order to use biomarkers in several purposes, biomarkers have to go through many stages of development. The phases of biomarker development for offering a systematic and comprehensive method for creating, assessing, and validating biomarkers were suggested by Pepe et al. (2001) (Pepe et al., 2001) (Figure 1). In phase I, the differentially regulated biomarkers are identified in the comparison of cancerous and control tissues. In phase II, clinical assays are developed for validation. In phase III, the retrospective study, the putative biomarker screened in huge healthy population that susceptible to develop cancer. In phase IV, prospective study, in order to assess the use of determined biomarker in early detection of cancer, that biomarker is tested in individuals with high risk for cancer development. Lastly, phase V studies are intended to determine whether widespread screening reduces disease-related morbidity and mortality (Figure 1) (Kumar, Mohan, & Guleria, 2006).

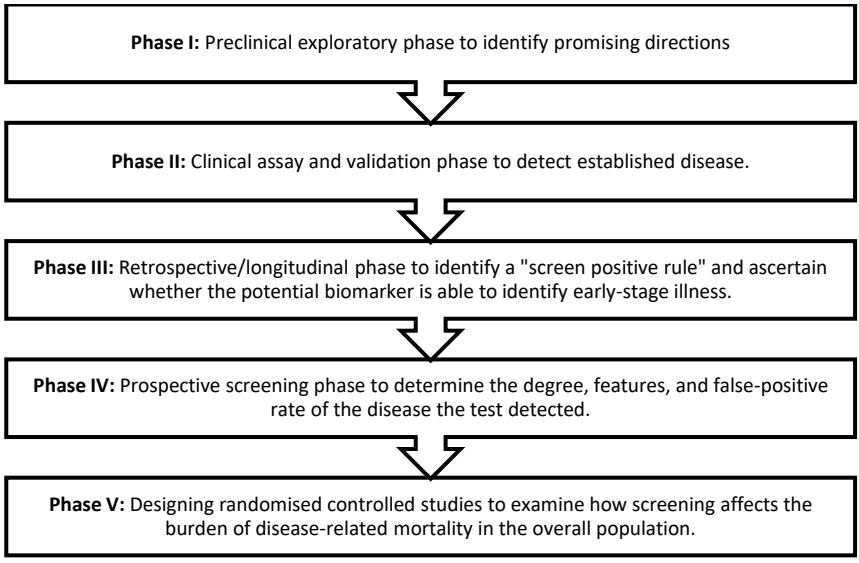


Figure 1: Developmental stages of biomarkers

Methodologies for Biomarker Discovery

It is necessary to first identify the type of biomarker to decide on the methodology for finding potential disease-related biomarkers. If the biomarker that is intended to be discovered is high density lipoprotein, that can be idendified using high performance liquid chromatography (HPLC) or nuclear magnetic resonance (NMR) microscopy or if it is microparticle, flow cytometry might be used. It biomarker is miRNA, polymerase chain reaction (PCR)-based arrays will detect. Mass spectrometry (MS) analyses are being used to detect biomarkers that are proteins, metabolites or lipids (Hoefer et al., 2015).

The development of proteomic techniques has improved studies on protein biomarker discovery and led to the discovery of novel biomarkers, which are still being found today. Proteomic technologies allow comparison of total protein in which thousands of proteins can be compared in diseased and control groups. MS based proteomic analyses differ based on biomarkers targeted or untargeted. In untargeted approach, proteins compared with any prior assumption and compared based on their abundance. The limitations of this method, due of the abundance of data, it is not easy to interpret and there is not current technology to resolve all human proteome. In targeted approach, pre-determined proteins measured with high precision. By using those proteomic approaches, biomarkers are being discovered from tissue, blood serum, cells, or other biological fluids like tear, sweat

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or urine. (Hathout, 2015; Hoefer et al., 2015; Wu, Liu, Baker, Rodland, & Smith, 2015).

Advantages and Disadvantages of Biomarkers

The use of biomarkers in clinical applications provides various benefits, such as the ability to perform many analyses quickly precisely. Additionally, biomarker usage is much more reliable than clinical endpoints since they can be validified. On the other hand, laboratory errors might be accomplished, using time is crucial for application as well as samples from which biomarkers detected has to stored properly in order to prevent misinforming. All other advantages and disadvantages were stated in Table 2 (Aronson & Ferner, 2017; Mayeux, 2004).

Table 2: Advantages and disadvantages of biomarkers, (Mayeux, 2004)

Advantages	**Disadvantages**
Objective assessment	Timing is critical
Precision of measurement	Expensive (costs for analyses)
Reliable; validity can be established	Storage (longevity of samples)
Less biased than questionnaires	Laboratory errors
Disease mechanisms often studied	Normal range difficult to establish
Homogeneity of risk or disease	Ethical responsibility

Conclusion

Using biomarkers in clinical application, therapeutical approaches, prevention studies, risk assessment, drug development, and/or individualized therapies is crucial and needs to be improved. Biomarker research have sped with the aid of established technology, yet they still have not realized their full potential. Their development should reach the rate of design of any clinical trial.

The use of biomarkers is significantly more reliable, accurate and feasible, despite some limitations. Therefore, it is necessary to promote the use of biomarkers in clinical applications.

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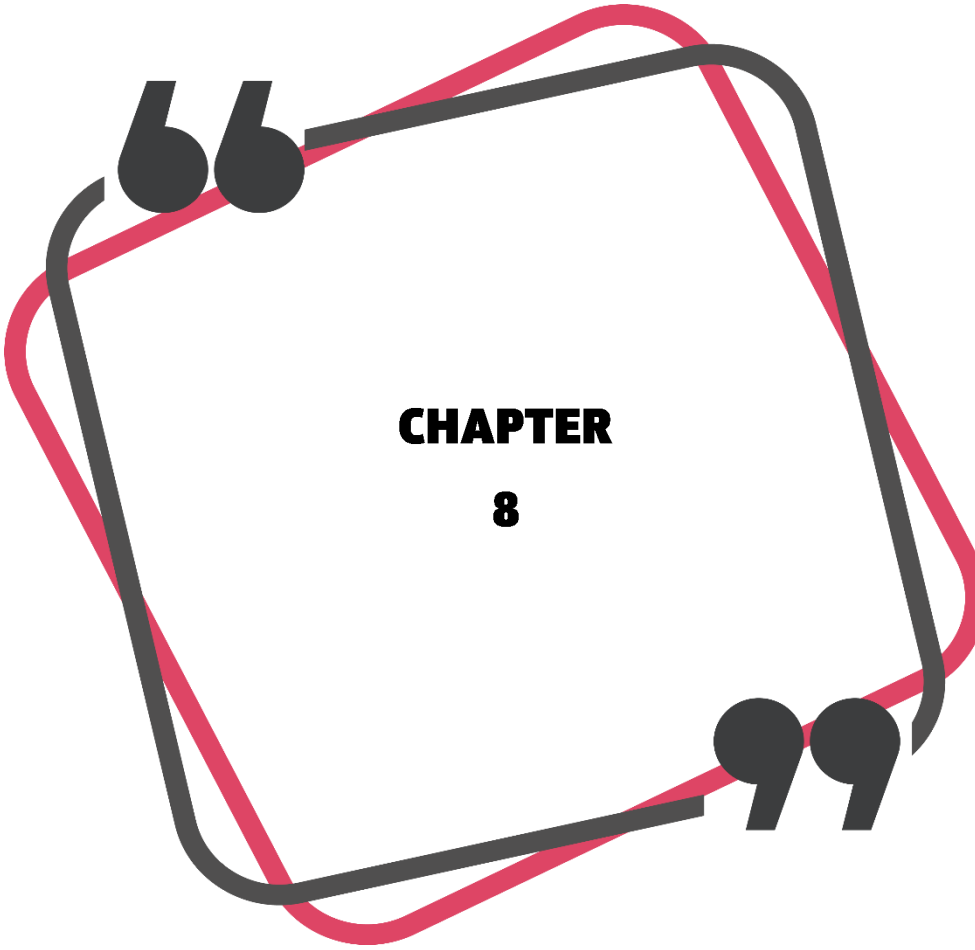
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CHAPTER
8

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ANTIMICROBIAL AND ANTIBIOFILM ACTIVITIES OF *TRIGONELLA MONSPELIACA* L.

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Introduction

Antimicrobial resistance is a global public health problem that prevents us from successfully treating infections [WHO, 2014; CDC, 2013]. Infections caused by antimicrobial-resistant microorganisms are more difficult and costly to treat than infections caused by microorganisms sensitive to antibiotics. Therefore, it causes more morbidity and mortality [Septimus, 2018]. Studies have shown that virulence characteristics of microorganisms are also important along with many factors in the development of antibiotic resistance [Ventola, 2015]. It has been reported that biofilms, which are considered as an important virulence factor causing persistent chronic and recurrent infections, are up to 1000-ford more resistant to antibiotics [Ventola, 2015; Fernández, 2011]. Antibiotics used in the treatment of biofilm-induced infections are insufficient today. For all these reasons, biofilm inhibition is considered the main drug target for the treatment of various infections [Bowler et al., 2020].

Medicinal and aromatic plants with various pharmacological properties are important sources of traditional folk medicine [Fierascu et al., 2021]. The genus *Trigonella* L. is considered a genus of traditional medicinal plants from the Fabaceae family. The most famous *Trigonella* species is *Trigonella foenum-graecum* known as fenugreek [Nagulapalli Venkata et al., 2017]. It contains about 135 species around the Western Asia, Eastern Mediterranean, Northern and Southern Africa, Southern Europe and Southern Australia. Some taxa of the genus are used as food and also in medicine. It is also used for different purposes, especially in Egypt and Greece, and has been known since ancient times [Akan et al., 2020]. The green leaves and seeds of many *Trigonella* species have long been used as foodstuffs, vegetables, fodder and medicinal plants in many countries [Kumar et al., 2019] and show various pharmacological effects such as anti-inflammatory, anti-diabetic, antioxidant, anti-fungal, hepatoprotective and hypocholesterolemic

[Zameer et al., 2018]. *Trigonella* L. genus is divided into 10 sections in Turkey. *T. monspeliaca* L. is a species belonging to the Bucerates Boiss. Section and is known as "Somçemenotu" in Turkey. *T. monspeliaca* is an annual herbaceous plant commonly found on oak groves, rocky slopes, empty plains, open lands in the forest, 0-1500 m [Akan et al., 2020].

Although there are many reports on *Trigonella* L. in the literature, there is no study on the antimicrobial and antibiofilm activity of *T. monspeliaca* yet. Therefore, in this study, it was aimed to investigate the antimicrobial and antibiofilm potentials of ethanol extracts obtained from *T. monspeliaca* seed, aerial part and seed coat parts.

Materials and Methods

Plant Material

The studied species *T. monspeliaca* was collected in the Maraş province of Turkey (Çağlayancerit). The plant was identified by Prof. Dr. Ahmet İlçim. Herbarium samples of plant material are kept in Hatay, University of Mustafa Kemal, Arts and Sciences Faculty, Biology Department Herbarium (MKU1786).

Extraction Procedure

Ethanol extracts [1:20 (w/v); ×3; ethanol (%96)] from powdered seeds (MNS/1), aerial parts (MNS/2) and seed coats (MNS/3) were obtained by mixing at room temperature for 3 days, then filtered using Whatman Grade No.1 filter paper. The solvent was evaporated with a vacuum evaporator and the extracts were stored in the dark at 4°C until activity studies [Güzel Kara et al., 2021].

Antimicrobial Activity Study

Microbial Strains

Five reference bacterial strains (*Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 35150, and *Bacillus subtilis* ATCC 6633) and three reference yeast strains (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC 15126, and *Candida parapsilosis* ATCC 90018) included in antimicrobial activity studies.

Antimicrobial Activity

Antimicrobial tests were performed to the standard microdilution method as previously practiced by our study group, and the minimum inhibitory concentration (MIC) values were determined [Uras Güngör and Öksüz, 2022a and 2022b;

Woods et al., 1995; Jorgensen and Ferraro, 1998]. The MIC was determined visually and using a microplate reader (BioTek Inc., USA) at a wavelength of 630 nm. As a reference drug; Ampicillin (Sigma, USA) for bacteria and fluconazole (Sigma, USA) for yeasts were used. All experiments were repeated 2 times.

Biofilm Tests

Determination of Biofilm Formation Capacity

The biofilm formation of the strains was determined in the crystal violet (CV) staining method as previously practiced by our study group [Uras Güngör and Öksüz, 2022a and 2022b; O'Toole, 2011]. The biofilm formation was determined by measuring the absorbance at optical density (OD) at 550 nm using a microtiter plate spectrophotometer (BioTek Inc., USA). The OD values of the wells without microorganism inoculum were used as the negative control. Biofilm production capacity was calculated according to the critical OD value (ODc); Biofilm formation negative (-): OD≤ OD; non-biofilm formation (+): ODc < OD ≤ 2 x ODc; moderate biofilm formation (++): 2 x ODc < OD ≤ 4 x ODc; strong biofilm formation (+++): OD> 4 x ODc [Gomes et al., 2019]. All tests were performed in duplicate.

Biofilm-Prevention Assay

Biofilm prevention testing of extracts was performed to the CV staining test as previously practiced by our study group [Uras Güngör and Öksüz, 2022a and 2022b; Zhong et al., 2019]. The lowest extracts concentration at which biofilm formation was inhibited by at least 50% was defined as the minimum biofilm inhibition concentration ($MBIC_{50}$).

$MBIC_{50}$ value was calculated according to the following equation;

Biofilm inhibition (%) = *(A control – A sample / A control) × 100*

A control: Absorbance value of wells containing only *P. aeruginosa* strains.

A sample: Absorbance value of wells containing extracts+*P. aeruginosa.*

Biofilm-Eradication Assay

The effect of extracts on preformed biofilm was performed to the CV staining assay as previously practiced by our study group [Uras Güngör and Öksüz, 2022a and 2022b; Zhong et al., 2019]. The lowest concentration of the extracts required to destroy at least 50% of the preformed biofilm was defined as the minimum biofilm reduction concentration ($MBRC_{50}$).

$MBRC_{50}$ value was calculated according to the following equation;

Biofilm inhibition (%) = *(A control – A sample/A control) × 100*

A control: Absorbance value of wells containing only *P. aeruginosa* strains

A sample: Absorbance value of wells containing extracts+*P. aeruginosa*

Results and Discussion

In this study, antibacterial and antibiofilm activities of ethanol extracts of *T. monspeliaca* species obtained from seed, aerial part and seed coat were investigated. The yields of seed, aerial part and seed coat extracts were determined as 7.4%, 3.75% and 4% (w/w), respectively.

Antimicrobial Activity

The antimicrobial activities of the extracts against the reference bacteria and yeast strains included in the study are shown in Table 1. Our findings determined that the extract inhibited the growth of the reference bacteria included in the study at concentrations ranging from 250-62.5 µg/mL. It also showed that it inhibited the growth of yeasts included in the study at concentrations ranging from 125-62.5 µg/mL (Table 1). The extracts showed moderate and low antimicrobial activity compared to fluconazole for yeasts and ampicillin for bacteria (reference antimicrobials). *T. monspeliaca* seed coat (MNS/3) extract showed the best antibacterial activity against *E. coli* (62.5 µg/mL). *T. monspeliaca* seed (MNS/1) and *T. monspeliaca* aerial part (MNS/2) extracts showed the best antifungal activity against *C. parapsilosis* (62.5 µg/mL). MNS/3 extract showed significant antifungal activity against *C. glabrata* and *C. parapsilosis* (62.5 µg/mL). All three extracts showed the lowest antifungal activity against *C. albicans* (125 µg/mL).

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Table 1. MIC values (µg/mL) of the tested *T. monspeliaca* seed, aerial part and seed coat extracts.

Extract/ Reference antimicrobials	*B. subtilis ATCC 6633*	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 29213	*E. faecalis* ATCC 29212	*E. coli* ATCC 35150	*C. albicans* ATCC 90028	*C. glabrata ATCC 15126*	*C. parapsilosis* ATCC 90018
MNS/1	125	125	250	125	125	125	125	62,5
MNS/2	125	125	125	250	125	125	125	62,5
MNS/3	125	125	125	125	62,5	125	62,5	62,5
Ampicillin	1,95	31,25	0,48	0,97	3,90	-	-	-
Fluconazole	-	-	-	-	-	0,12	8	0,24

MNS-1: *T. monspeliaca.*seed, MNS-2: *T. monspeliaca* aerial part, MNS-3: *T. monspeliaca* seed coat

T. foenum-graecum (fenugreek) is the most important economically and medically species of the genus *Trigonella* L., and antimicrobial studies on species belonging to this genus in the literature are mostly studies on fenugreek. In a study with fenugreek seeds, it was reported that aqueous ethanolic seed extract (50%) showed antimicrobial activity at various inhibition levels against *E. coli*, *K. pneumonia, B. cereus, S. aureus*, *P. aeruginosa* and *S. typhi* [Yakout et al., 2013]. In another study with fenugreek showed that ethanol and water extracts from the seeds exhibited moderate to high antimicrobial activities against the bacterial and fungal pathogens tested [Kadaikunnan et al., 2015]. The data in the literature are compatible with our current study. Akbaş et al., (2017), reported that aqueous-ethanol extract from fenugreek seeds showed to low antimicrobial activity against the tested bacterial and fungal pathogens. Al-Timimi (2019), have also demonstrated that the ethanol extract of fenugreek seed exhibited pronounced effect on *P. aeruginosa* and *S. aureus*. Also, a previous study by Singh et al. (2022) on fenugreek, it was shown that the seed and leaf extracts of fenugreek has significant antimicrobial activity against different microbial pathogens. In a study conducted by our group on ethanol extracts of *T. cylindracea* obtained from seed, aerial part and seed coat, it was determined that the studied extracts showed moderate to low antimicrobial activity when compared to reference antimicrobials [Uras Güngör and Öksüz, 2022a]. In another previous study, ethanolic

extracts obtained from *T. strangulata* seed, aerial part and seed coat showed moderate and low antimicrobial activity when compared with reference antimicrobials (fluconazole, ampicillin). In addition, when evaluated in terms of antimicrobial activity in general, it was determined that they had better activities on fungal strains compared to bacteria [Uras Güngör and Öksüz, 2022b].

When we examined the literature data, we did not find any antimicrobial activity studies on *T. monspeliaca*. For this reason, the results of the antimicrobial activity of the extracts we obtained using different parts of *T. monspeliaca* provide important preliminary data to the literature.

Determination of biofilm formation capacity

Biofilm-forming capacities of the reference bacterial strains included in the study were determined according to the crystal violet method.

Test results showed that *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213 strains strong (+++) and also *E. faecalis* ATCC 29212, *E. coli* ATCC 35150, and *B. subtilis* ATCC 6633 formed moderate biofilm (++). In this study, biofilm prevention and eradication tests were performed on the biofilm of *P. aeruginosa* that which is one of the strains with the best biofilm production capacity.

Biofilm prevention and eradication assays

Biofilm inhibition test determined that tested extracts inhibited biofilm formation by 34%, 37%, 38% and 31%, 30%, 26% and 23%, 18%, 21% at 1X, 0.5X, and 0.25X MIC, respectively (Figure 1). None of the extracts included in the study were able to prevent biofilm formation by 50% at the concentrations tested. Therefore, the extracts have no $MBIC_{50}$ value. In addition, the biofilm eradication test showed that tested extracts inhibited biofilm of *P. aeruginosa* at formation by 42%, 38%, 46%, and 38%, 34%, 34% and 32%, 28%, 27% at 2X, 1X, and 0.5X MIC, respectively (Figure 2). According to these data, all three extracts failed to eradicate 50% of preformed biofilm in the MICs tested (µg/mL). Therefore, the extracts have no $MBRC_{50}$ value. However, MNS/3 extract was able to destroy biofilm (46%) with a value close to 50% at 2X MIC (250 µg/mL). Overall, in this study, the rate of eradication of the preformed biofilm of *P. aeruginosa* was higher than the inhibition of biofilm formation (Figure 1, Figure 2).

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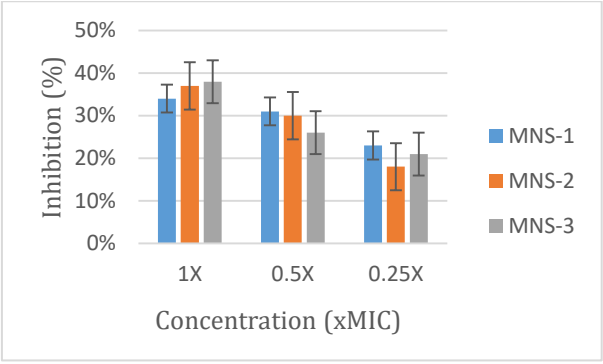


Figure 1. Inhibition (%) of *P. auriginosa* biofilm by *T. monspeliaca* seed (MNS-1), aerial part (MNS-2), and seed coat (MNS-3) extracts at 1X, 0.5X, and 0.25X MIC concentrations.

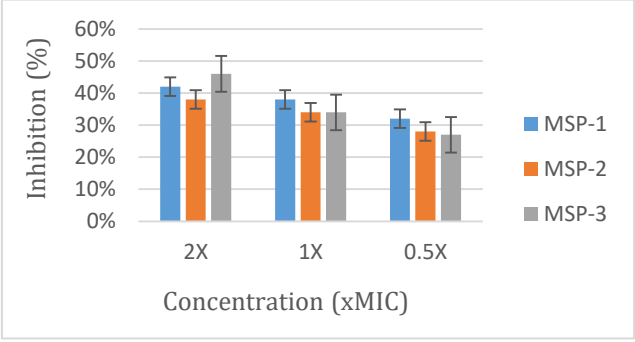


Figure 2. Preformed biofilm inhibition (%) of *T. monspeliaca* seed (MNS-1), aerial part (MNS-2) and seed coat (MNS-3) extracts at 0.5X, 1X, and 2X MIC concentrations.

As a result of the literature review, we observed that there are very few studies on the antibiofilm activity of species belonging to the genus *Trigonella* L. Husain et al. (2015) found in their study that fenugreek seeds inhibited Quorum sensing (QS) and biofilm formation in *P. aeruginosa* and *A. hydrophila* [Husain et al., 2015]. In our previous study with ethanol extracts obtained from seeds, aerial part and seed coats of *T. cylindracea*, we found that these extracts have antibiofilm potential. Biofilm tests have shown that the seed and seed coat extracts were able to reduce the preformed biofilm by 50% above the 2X MIC. It was also determined that seed coat extract can reduce biofilm formation by 50% at subMIC (0.5X) [Uras Güngör and Öksüz, 2022a]. As a result of our findings, none of the

extracts (*T. strangulata* seed, aerial part, and seed coat extracts) included in the study prevented biofilm formation by 50%. However, it was observed that all three extracts have the potential to destroy the preformed biofilm at different concentrations by 50%. This indicates that *T. strangulata*'s seed, aerial part and seed coat extracts have better potential to destroy preformed biofilm than to prevent biofilm formation [Uras Güngör and Öksüz, 2022b]. No study has been found in the literature on the antibiofilm activity of *T. monspeliaca*.

Conclusions

This study is the first attempt to investigate the antimicrobial and antibiofilm activities of *T. monspeliaca* seeds, aerial parts and seed coats. Therefore, our study provides important preliminary data to the literature about this species. As a result of our research, we observed that the antimicrobial activities of the extracts were low and moderate, but they had partial antibiofilm potentials. The use of natural compounds as antimicrobial agents and food stabilizers is increasing day by day due to their advantages such as low cost and high availability. Our study will lead to new research aimed at evaluating the potential of this species as an antibiofilm agent in the treatment of infectious diseases, together with further research such as animal experiments and toxicity tests. Moreover, it is envisaged that *T. monspeliaca* may be a promising resource for the development of new therapeutic agents for various industries.

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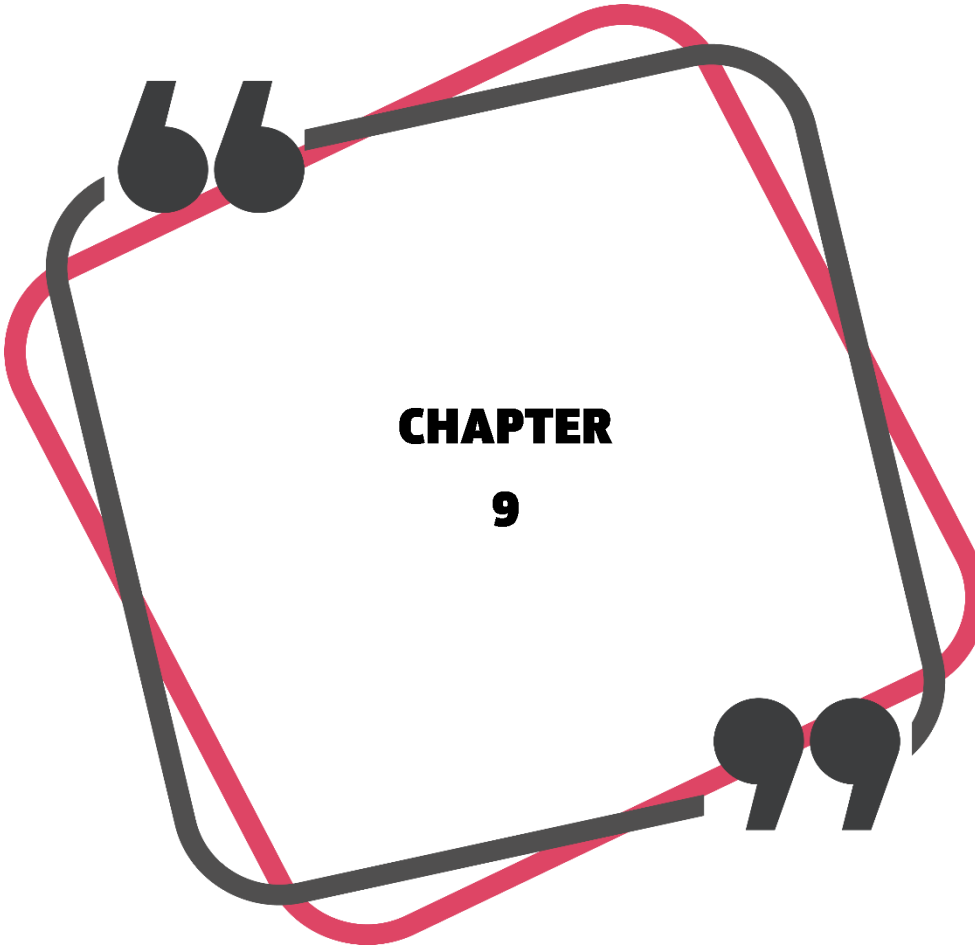
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CHAPTER
9

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NATURAL SOURCE DRUG MOLECULES TARGETING ALZHEIMER'S DISEASE

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Alzheimer's Disease and Related Hypothesis

Alzheimer's disease (AD) is a complex neurodegenerative disorder and the dominant form of dementia for which no definitive treatment has yet been found. Alzheimer's disease (AD) is a multifactorial neurodegenerative disease that affects millions of people. It is also common in our society and ranks first in terms of incidence compared to other neurodegenerative diseases. It has been reported that there are approximately 600,000 Alzheimer's patients in Turkey and an estimated 50 million worldwide. It is predicted that there will be an increase in the number of Alzheimer's patients due to the increasing elderly population in the society, and this number will reach approximately 150 million worldwide by 2050 (Gezici and Kocum, 2021).

Alzheimer's disease is becoming a financial and social burden, both for individuals and their families, and for society. Since there is still no definitive treatment for the disease, the treatments are either aimed at suppressing the symptoms or slowing down the course of the disease. Therefore, effective and non-toxic natural-sourced drugs to be developed are extremely important for the patient to have a better quality of life and not suffer from side effects (Gezici and Sekeroglu, 2022).

The prominent markers and mechanisms involved in the physiopathological findings of AD can be listed as follows (Koçancı and Aslım, 2016; Bukhari, 2022; Taliyan et al., 2022):

1) Overexpression of acetylcholinesterase (AChE)

AChE is a protein that hydrolyzes the neurotransmitter acetylcholine (Ach) into "acetate" and "choline" (Figure 1). Since ACh is required to connect the hip-

pocampus to the cerebral cortex, the post-synaptic activity of ACh is then inhibited by its degradation. It has been found that AChE levels are increased in brains with AD which makes AChE an important drug target for designing inhibitor molecules.

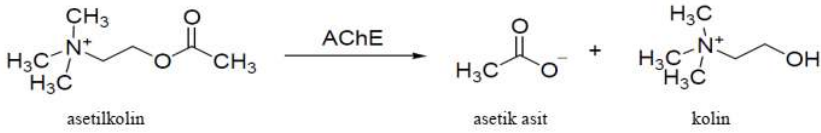


Figure 1. Reaction showing the degradation of acetylcholine (AChE) by its enzyme acetylcholinesterase (AChE) (Schumacher et al., 1986)

2) Accumulation of amyloid β (Aβ) protein in the form of amyloid plaques

Due to mis cutting processes of the molecule Amyloid Precursor Protein (APP), Aβ protein has been determined to accumulate in the form of extracellular neuritic plaques especially in the cortex that are called 'amyloid plaques'. Proteins that take role in the APP cleavage are beta and gamma secretases (β-secretase and γ-secretase)

3) Accumulation of phosphorylated tau proteins in the cell

Tau protein plays an important role in stabilization of microtubules, integrity of cytoskeleton and axonal transport. In AD pathogenesis, it has been determined that Tau protein is hyper phosphorylated via hyperactive kinases and/or hypoactive phosphatases leading to inability to bind to microtubules. This condition then results in accumulation in the form of intracellular neurofibrillary tangles (NFY).

4) Inflammation

NFY is accompanied by astroglial and microglial activation and plaque formation in AD. Inflammatory processes such as acute phase proteins, cytokines, complement elements and proteases has been detected which proves the effect of

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inflammatory processes in AD. Various hypothesis linked to AD and related target proteins are depicted in Figure 2.

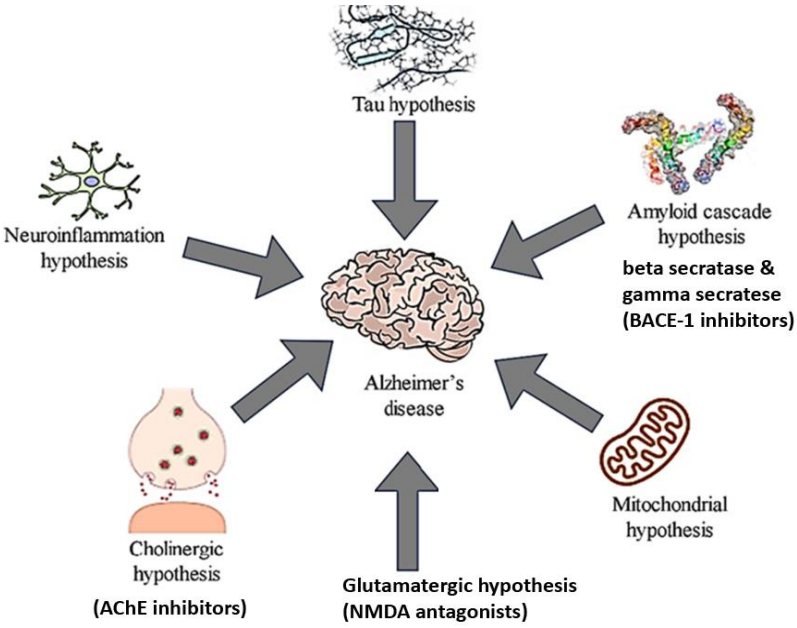


Figure 2. Alzheimer's disease hypothesis and the mechanisms involved (adapted from Taliyan et al., 2022).

5) Glutamergic

Another target may be *N*-methyl-D-aspartate receptor (NMDAR) which also plays a crucial role in AD development and progress. NMDA is activated upon Glutamate binding which then leads to Ca2+ influx activation in the a signaling pathway. Overstimulation of NMDARs results in an abnormal increase in the Ca2+ influx which causes excitotoxicity damage, synaptic dysfunction, and neuronal cell death (Wang et al., 2017) As a result, drug molecules that block NMDAR in order to limit Ca2+ influx would be another strategy. For this, NMDAR antagonists, such as Memantine, have been developed to treat AD (Folch et al., 2018) These drugs are used to relieve patients of Alzheimer's disease symptoms to some extent.

Cholinergic Mechanism: the most widely accepted one

The most traditional explanation of the mechanism of AD formation is the cholinergic hypothesis. The enzyme called acetylcholinesterase (AChE) can cause Aβ plaques to increase, hence emerges as a very accurate inhibitory drug development target both in reducing plaques and increasing the amount of acetycholin. In fact, the system of cholinergic abnormalities is also closely associated with other neurodegenerative disorders such as Parkinson's Disease, dementia with Lewy bodies, and vascular dementia (Müller and Bohnen, 2013). Decreased acetylcholine neurotransmitter level in patients causes irreversible neuronal loss, thus decrease in cognitive functions. Therefore, inhibition of the AChE enzyme causes ACh accumulation at cholinergic synapses that is expected to increase cognitive function (Colovic et al., 2013). It is aimed to decrease AChE activity with a potent AChE inhibitor to be developed, and in this way increase in ACh concentration in the synapse which would lead to increased cholinergic transmission and hence diminish AD symptoms. At this respect, both synthetic chemicals or natural compounds known to contain cholinergic antagonists can be designed as anti-Alzheimer's drugs (Breijyeh and Karaman, 2020; Gezici and Sekeroglu, 2022).

AChE is a 537 amino acid long peptide that can hydrolyze 250,000 acetylcholine molecules in one second.The structure of the acetylcholinesterase enzyme was first described by Sussman in 1991 in Torpedo californica (Sussman et al, 1991) determined by X-ray analysis. The AChE active site consists of two binding subunits. The first of these is called the "catalytic anionic site" (CAS) that contains the catalytic triad of Ser200, His440 and Glu327. These three residues are responsible for the hydrolysis of the ester bonds in ACh. The anionic subunit of CAS consists of amino acids Trp84, Tyr130, Gly199, His441 and His444.The indole side chain of Trp 84 makes a cation-π interaction with the ACh quaternary amino group (Ma and Dougherty, 1997). The amino acid Phe330 is responsible for the recognition of ligands. The second binding subunit, "peripheral anionic site" (PAS) contains the essential amino acid Trp279. This region also contains amino acids Tyr70, ASP72, Tyr121, Trp279 and Tyr334. Oxyanion hole formed by peptidic amino groups of amino acids Gly118, Gly119 and Ala201, is another important functional unit within the esteratic subunit (Wiesner et al., 2007; Koçancı and Aslım, 2016) (Figure 3). Ligands have to pass through a narrow cleft attached at the top of the AChE enzyme to reach to its active site. Amino acids in the gate region of the enzyme are important in interacting with drug molecules having aromatic structures. It is crucial that ligands carry a positive charge for binding to the enzyme (Radic and Taylor, 2001; Axelsen et al.,1994).

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Two independent study groups demonstrated that cholinergic markers in the cerebral cortex were severely lost (about 95%) in AD. Subsequent studies have shown that there were decreases at varying rates between in the cholinergic neuron counts values varying between 15% and 95% of in AD patients (Davies et al., 1976; Smith et al, 1976). In addition, cholinergic deficits and severity in AD has been found to be related to the progression and duration of AD (Arendt et al., 1985). Drachman and Leavitt demonstrated that blockade of cholinergic receptors in young and healthy individuals, causes a memory deficit similar to that seen in individuals with AD (Drachman and Leavitt, 1972; Koçancı and Aslım, 2016).

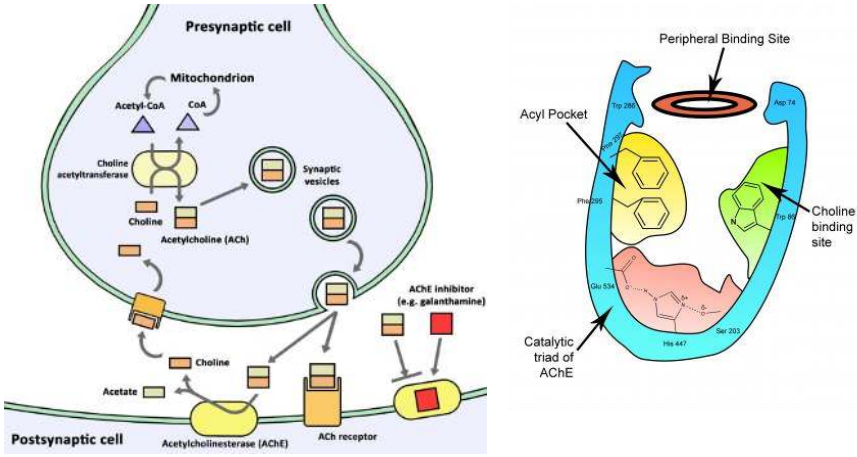


Figure 3: AChE function and binding sites

Being a a transmembrane protein amyloid precursor protein (APP) is metabolized and one of its products, Aβ, has also been shown to be multi-directionally related to AChE: While APP is formed, as a result of the activities of β and γ secretases, solid neuritic β-folded plaques (Aβ-42) that cause significant regional neurotoxic effects forms. Accumulation of Aβ-42 in brain tissues result in the formation of oligomers and amyloid fibrils, which are markers of AD that induce AChE levels as well (Sberna et al., 1997).

Although the relationship between AChE and tau hyperphosphorylation has not yet been fully explored, there are studies showing that tau hyperphosphorylation can trigger an increase in AChE expression (Silveyra et al., 2011). In transgenic mouse models, where phosphorylated tau protein is overexpressed, it has been determined that AChE activity is also increased. The results show that there was an increase in AChE expression around NFYs. There is evidence that boosted levels of AChE may be effective in tau hyperphosphorylation pathways by causing an increase in (GSK3β) protein (Garcia-Sierra, 2008).

AChE targeted FDA-approved drugs (AChE inihibitors)

In brains with AD, the activity of AChE increases in the cortex and hippocampus, and accordingly, the amount of ACh and activation was found to be decreased. The most specific approach in the drug treatment of AD today is acetylcholine esterase (AChE) inhibitors. Most of them are of plant origin. But so far full success has not been achieved in any of them. Therefore, in clinical, epidemiological and experimental studies for the treatment of AD, alternative drugs are of great importance. In recent years, strategies targeting the discovery and development of AChE inhibitors for the treatment of AD and discovery of new effective therapeutics having less side effects have increased (Koçancı and Aslım, 2016).

Although a drug that stops or reverses AD is not available today, the FDA (U.S.Food and Drug Administration) approved several drugs for AD treatment, most of them being AChE inhibitors, and one is NMDA receptor antagonist (Table 1).

Table 1. FDA approved AD drugs

AChE targeted FDA approved drugs	**NMDA targeted FDA approved drugs**
Tacrin (Cognex)	Memantine
Rivastigmine (Exelon)	
Galantamine (Razadyne)	
Donepezil (Aricept)	

Chemical structures are provided in Figure 4. Out of these, Galanthamine and Rivastigmine are natural source, while Tacrine and Donepezil are synthetic drugs. Tacrine was the first approved drug for AD treatment however has been discontinued since 2013 due to high liver toxicity (Herrman et al., 2011).

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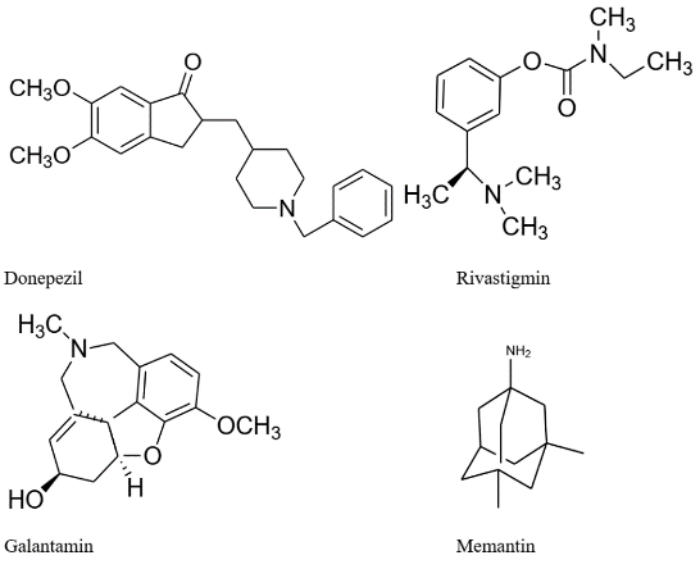


Figure 4. FDA approved drugs used in AD treatment (Özçelik et al., 2019)

Sometimes combined useage of drugs are preferred for dual activity. For instance, combined use of a low-affinity NMDA receptor antagonist memantine, with the AChE inhibitor donepezil has been found to be beneficial in cognitive functions (Marum, 2009).

While these few FDA-approved drugs on the market cannot go beyond slowing the progression of the disease to some extent, they also bring many serious side effects. It is thought that more and more people will suffer from this infection in the next few decades. For this reason, it is hoped that the recommendation of a FDA-approved drug for the treatment of AD, which has fewer side effects due to its natural origin, will make a great contribution to this disease for which there is no cure yet.

Natural drugs used in AD treatment

For years, plant-derived chemicals have been used as traditional medicine for the treatment of many diseases and disorders. Plant chemicals are divided into primary and secondary metabolites. Secondary metabolites are produced by specialized metabolic pathways that activate when the organism encounters any stress situation (Manach et al., 2004). The active compounds biologically synthe-

sized by fruit, vegetable and medicinal plants are mainly indole, steroidal-piperidine-alkaloids, furanocoumarins, xanthones, flavonoids and diterpene derivatives. This secondary metabolites play an important role in the pathogenesis of many neurodegenerative disorders such as AD (Houghton et al., 2005). There is an increasing trend for the use of neuroprotective natural compounds to prevent oxidative stress and neuroinflammation, which are distinctive features in the progression of neurodegenerative diseases (Hussain et al., 2018; 2019; Gregory et al., 2021; Gezici and Sekeroglu, 2022).

Physostigmine

The clinical application of AChE inhibitors started with the use of Physostigmine in AD in the early 1980s by the oral and intravenous administration. Physostigmine is an alkaloid isolated from Physostigma venenosum Balf (Fabaceae) plant. It is a short-acting AChE inhibitor and is associated with cognitive impairment in both normal and AD patients in vivo (Houghton and Howes, 2005). The carbonyl group of physostigmine in AChE interacts with serine and forms a covalent bond with the catalytic site of the enzyme, in this way hindering the enzyme activity. Rivastigmine, a semi-synthetic derivative of physostigmine discovered by Marta Weinstock-Rosin was the first product approved by the FDA for the treatment of moderate and mild dementia in 2000 under the name Exelon®.

Galantamine

Galantamine is an important amaryllidaceae alkaloid of great therapeutic value for the treatment of AD. It has been determined that it has an AChE inhibitory effect of 80-100%. Galantamine, enhances the effect of acetylcholine on nicotinic receptors. By acting on AChE, it increases the cholinergic efficiency, provides correction of functions and cognitive effects in AD (Mukherjee et al., 2007). Because of this feature, Galantamine (Razadyne™), was approved by the FDA in 2001, to be used for AH treatment.

Huperzine A

Among the compounds that has acetylcholinesterase inhibitory activity, huperzine A, which was discovered through research on plants, is the most important molecule today. In traditional Chinese medicine it is named as "Qing Ceng Ta" and is an alkaloid isolated from the plant Huperzia serrata (Lycopodiaceae). Huperzine A has been known and used for centuries in the treatment of schizophrenia, forgetfulness and memory loss. It is a reversible and selective inhibitor of the enzyme acetylcholinesterase, furthermore, it can prevent damage to the brain caused by glutamate by acting as a N-Methyl-D-Aspartic Acid (NMDA) receptor

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antagonist (Tang and Han, 1999). Because of these features, it has started to be used as a AD drug though it is not FDA approved drug it is sold as a supplement.

Curcumin

Curcumin, which is obtained from the *Curcuma longa* plant has long been suggested for treatment of many diseases owing to its anti-inflammatory, antibacterial, and anticarcinogenic effects Clinical studies based on the anti-dementia effect of curcumin are still ongoing. Researches on Indian people who heavily consume castor saffron and turmeric which also include Curcumin have demonstrated that they had less probability to develop dementia (Chandra et al., 2001; Vas et al., 2001). In a similar population-based study aiming to compare the incidences of Alzheimer's disease in the US population versus India, has shown 4.4-fold higher incidences of Alzheimer's disease in US when compared to the rate of incidences in India (ages vary between 70–79). Similarly, the reason for this was inferred to the common usage of turmeric and castor saffron (Chandra et al., 2001). Several scientific studies have pointed out curcumin as being involved in amyloid beta (Aβ) related cascade in which curcumin might assist in preventing the formation of Aβ aggregates (Hamaguchi et al., 2010). Owing to the role of antioxidative effect of Curcumin, it might also play a key role in beneficial effects seen after curcumin usage in Alzheimer's disease.

Quercetin

Quercetin is a flavonoid found in foods and some plants such as apples, onions, tea and red wine. It shows pharmacological effects such as vasodilation, anti-inflammatory, antioxidative, antithrombotic, antihypertensive and anticarcinogenic effects (Hussain et al., 2018). Quercetin consists of two crucial pharmacophores, namely hydroxyl and catechol that play a key role in its anti-oxidative potential by assisting in neutralizing reactive oxygen species (ROS). Quercetin improves learning and recognition memory, and reduces the production senile plaques, mitochondrial dysfunction and reactive oxygen bodies (Babaei et al., 2018). It prevents oxidative damage of neurons and provide support against endotoxicity. Quercetin is able to inhibit Aβ accumulation and reduces the apoptosis caused by Aβ aggregation processing through the inhibition of β-Secretase (BACE) activity (Bui and Nguyen, 2017). It protects neuronal cells from oxidative stress-induced neurotoxicity observed in Alzheimer's disease (Hussain et al., 2018).Competitive inhibition of Quercetin observed on AChE helps to boost the level of ACh which is responsible for enhanced cognitive abilities (Abdalla et al., 2014).

Epigallocatechin-3-Gallate (EGCG)

Epigallocatechin gallate (EGCG), is a catechin type present on green tea leaves. Polyphenols obtained from green tea has antioxidant, anticarcinogenic and anti-inflammatory effects (Hussain et al., 2018). This flavonoid can improve memory and can inhibit γ-secretase enzyme activity (Bui and Nguyen, 2017). EGCG can clean free radicals by several mechanisms such as: blocking free radical chain reactions directly or indirectly; stimulation of intracellular antioxidant enzyme activity; metal ion excretion; reduction of lipid peroxidation and intracellular calcium release. Antioxidant activity can reduce neuronal destruction and neurotoxicity caused by free radical attack. Free radical production mediates neurotoxicity and neuronal death (Hussain et al., 2018). This flavonoid is responsible for protecting neural cells against increased death and programmed cell death upon oxidative stress. This flavonoid can reduce the accumulation of Aβ as well as inhibit tau protein ab normal phosphorylation (Bakhtiari et al., 2017; Ayaz et al., 2019).

Tannic Acid

Another important polyphenolic molecule drived from plants is Tannic acid (TA) which has many beneficial effects. Basically it is found in strawberries, apple, barley, coffee and tea (Serrano et al., 2009). TA has a similar structure to EGCG hence similar modes of action is expected. In AD related cascades, they are both found to have an influence on the processing of APP through inhibiting the cleavage on the β-site by inhibiting β-Secretase (BACE-1) activity (Figure 5). This inhibition would finally result in a decrease in Aβ level which in turn boosts cognitive functions in AD (Oishi et al., 2011). Apart from its effects on the Aβ cascade, TA has also been discovered to influence tau protein related cascade by inhibiting the in vitro aggregation of tau peptide which then prevents the formation of intracellular neurofibrillary tangles (NFTs).

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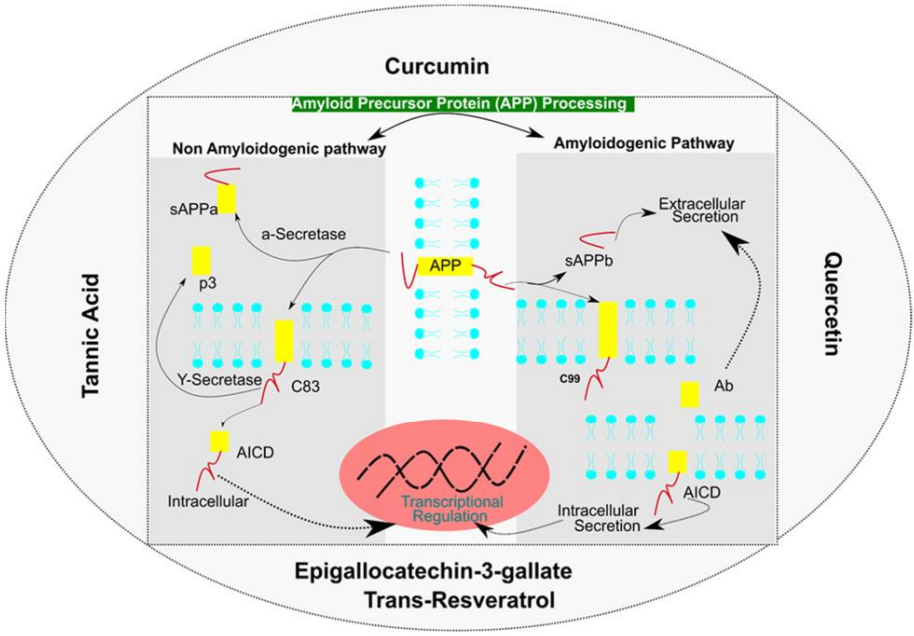


Figure 5. Some natural source ompounds effecting Amyloid Precursor Protein (APP) by modulating amyloidogenic pathway (Bukhari, 2022)

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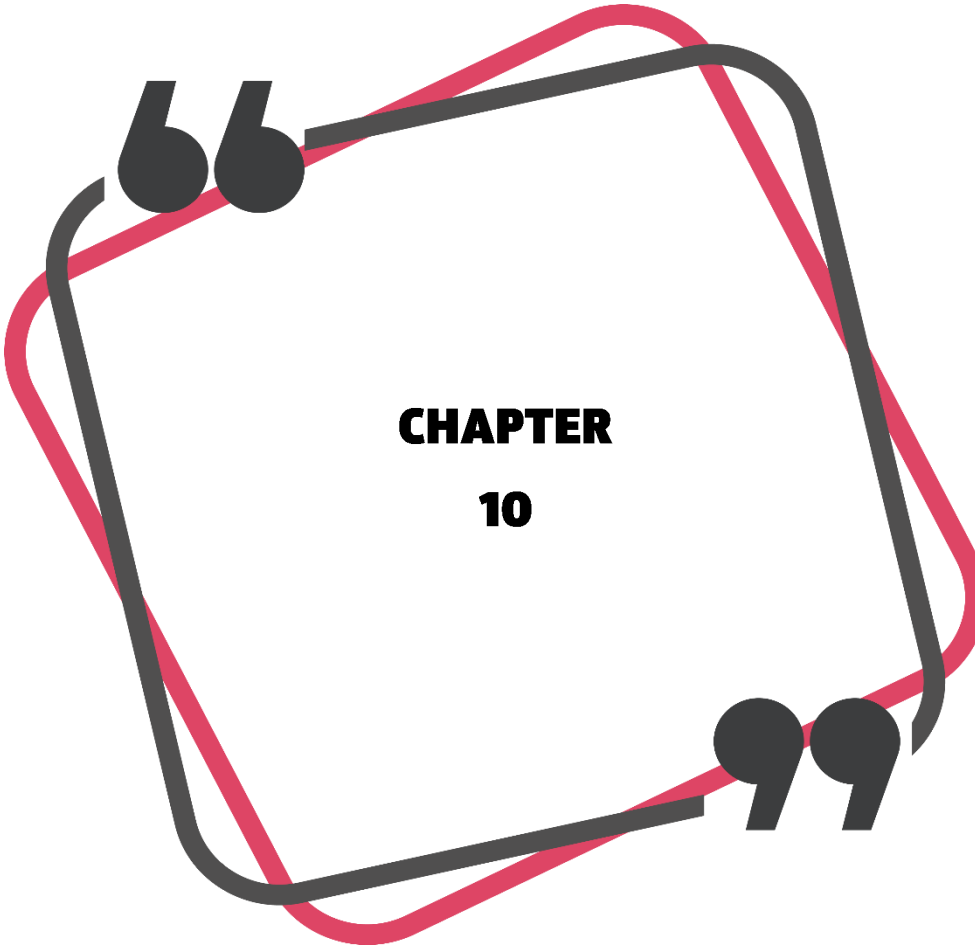
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CHAPTER
10

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LONG NON-CODING RNAS IN HUMAN BREAST CANCER

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Introduction

Breast cancer (BC) is the most frequently diagnosed cancer in women, with an estimated 2.3 million new cases each year, according to the BC Global Cancer Statistics 2020. As a result, it has recently surpassed the most commonly diagnosed lung cancer (1). While the development of screening and treatment methods continues, many molecular mechanisms that affect the onset and development of this heterogeneous cancer still remain unclear. Classical treatments are sometimes insufficient. Therefore, there is a need for targeted molecular therapeutic agents. Recently, the contribution of non-coding RNAs to cancer formation and whether they will be therapeutic targets have been the subject of many studies. These RNAs, which make up the majority of human transcripts, have broad mechanisms of action. Most non-coding RNAs have altered expression in BC tissues. In this review, the functions and effects of long non-coding RNAs (lncRNAs) in breast cancer and their effects on BC will be summarized.

Epidemiology

BC is the most common cancer in women. It was reported as the main cause of female cancer deaths in 11 of the 20 major global regions in 2018 (2). It was determined that there was an annual increase of 3.1% between 1980 and 2015, and the incidence continues to increase (3). The incidence in North America was 92 per 100,000 (4) and 144 per 100,000 in European Union countries (5). While it is diagnosed at the age of 50-70 in European countries, it is diagnosed at the age of 40-50 on average in Asian countries. Africa and Central Asia have the lowest incidence, but this may be due to inadequate screening and diagnostic programs (6). Although it is more common in developed countries because it is

diagnosed at an earlier stage, the overall mortality rate is lower. The highest death rate due to BC is found in Sub-Saharan Africa (7).

Pathology

In BC, there are various tumors with different histological, clinical, and molecular-biological manifestations (8)**.** BC tumors are histopathologically divided into invasive and pre-invasive types. Pre-invasive types include lobular carcinoma in situ (LCIS) and ductal carcinoma in situ (DCIS LCIS is considered a risk factor because the risk of progression is very low. Although this type is bilateral, it usually does not disrupt the lobular structure. However, DCIS is unilateral and spreads through channels. This type can be invasive, accounting for more than 80% of all preinvasive lesions (7, 8). Invasive BC subtypes are invasive lobular carcinoma, and the more common invasive carcinoma, formerly known as invasive ductal carcinoma (no specific type: NST). Non specific invasive ductal carcinoma makes up the majority of all BCs, followed by lobular carcinomas, and the rest are rare histological subtypes (9)**.** Women with DCIS have a 10-year risk of developing BC (20-53%) (10)**.** Molecular classifications can identify tumor characteristics, but these procedures are costly (11). BC is histologically examined in five groups: luminal A-like, luminal B-like HER2 (-), luminal B-like HER2 (+), HER2-enriched, and triple negative (8,12).

Non-coding RNAs

Although 52% of the human genome is copied, only 1.2% of transcripts encode proteins. Approximately 98% of the human genome consists of non-coding sequences (ncRNAs) (13). Once seen as "junk DNA", ncRNAs are important regulators of various biological processes. Many of the currently used cancer biomarkers and target therapies are determined by the genes coding for them. However, up to 90% of non-coding sequences are transcribed, thus producing large numbers of non-coding RNAs (14, 15). In genome-wide association studies (GWAS), it has been determined that one-third of the many genetic variants associated with diseases belong to non-coding regions (16). Non-coding RNAs are classified according to their length as microRNAs (18-22 nucleotides) and long non-coding RNAs (LncRNAs, ≥200 nucleotides). Considering the late diagnosis, poor prognosis, recurrence, and resistance of cancer cells to treatment, cancer remains the leading cause of death all over the world. Newer markers are required

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in addition to existing markers for the diagnosis, prognosis, and treatment of cancer. ncRNAs are good candidates for this.

LncRNAs

LncRNAs, which are structurally similar to mRNAs, are formed by DNA transcription (17). LncRNAs are transcribed by RNA polymerase II and can undergo post-transcriptional changes such as closure, polyadenylation, and splicing (18). LncRNAs can be found in the cytoplasm, but they are especially concentrated in the chromatin of the nucleus (19). LncRNAs do not code for proteins, however, lncRNAs have many functionalities, such as transcriptional modulator, splicing, post-transcriptional processor, enhancer, complex traps for miRNAs, chromatin remodeler, protein-protein, protein-DNA, and protein-RNA interactions (20, 21). LncRNAs are often expressed in a disease, tissue, or developmental-specific manner. Thus, it has the potential to be used as a biomarker and therapeutic target (22). Dysregulation of the expression of LnRNAs may lead to malignant transformation by disrupting cellular homeostasis. However, the mechanisms of most lncRNAs are still not fully elucidated.

Mechanism of lncRNAs in cancer biology

An important link between cancer and lncRNAs was discovered in studies investigating the function of lncRNAs. Person cancer-associated biological detection of a large number of lncRNAs involved in processes has been done. Therefore, lncRNA mutations, dysregulation, or abnormal expression may cause cancer (23). Genome-wide, numerous studies on the human genome single nucleotide polymorphism (SNP) in intergenic or intronic regions exist; this is a lncRNA and may affect the function shown (24). LncRNAs, like protein-coding genes, can function as tumor suppressors and oncogenes. The mechanisms of this complex regulation can be summarized in four ways: signal, scaffold, trap, and guide.

1) Some lncRNAs are expressed at different levels in various cells. Thus, it can reflect the state of development or disease (25). For example, X chromosome inactivation.

2) LncRNAs can bind proteins and act as scaffolds in the formation of regulatory complexes (26).

3) LncRNAs regulate gene expression by inhibiting the binding of transcription regulators (27). Also called endogenous RNAs (eRNAs), long noncoding

RNAs (lncRNAs) bind miRNAs and prevent RNA degradation. This is a common occurrence in cancer.

4) LncRNAs can direct transcription factors to specific sites (28).

Areas of lncRNA use in cancer

Some lncRNAs are highly tissue-specific and are aberrantly expressed in cancer. It can be extracted noninvasively from the circulation (29). This feature allows them to be used for cancer diagnosis. Expression of lncRNAs can be used as prognostic markers in cancer patients as it correlates with overall survival, metastasis, tumor stage, or grade (30). Because of the aberrant expression of lncRNAs and their role in various cellular processes, cancer therapy is suggested as a target (31).

LncRNAs in BC

LncRNAs have been shown to be frequently upregulated in studies of various cancers. Numerous lncRNAs have been identified in BC studies that show different expression patterns in tissue compared to normal breast tissue (32). Yang et al. identified more than 1300 lncRNAs that showed significantly aberrant expression in the HER-2-enriched breast cancer subtype (33). Shen et al. showed that in triple BC, more than 1750 lncRNAs were differentially expressed. This suggests that the aberrant expression patterns of lncRNAs may play an important role in the pathogenesis of BC (34). Miano et al. showed 133 ERα-dependent lncRNAs that are promising for identification because they are specific for luminal-like BC (35). Jiang et al. defined an mRNA-lncRNA that categorized triple-negative BC patients as low-risk and high-risk groups in their study (36)**.** It was identified as a novel LINC02273 with higher expression in metastatic lesions than in primary tumors (37). Increased LINC02273 expression demonstrated BC metastasis in vitro and in vivo. Chi et al. reported that the survival analysis showed that Linc00959 was an independent prognostic marker in BC (38). LncRNA HOTAIR in BC has been shown to affect cell growth, metastasis, and apoptosis through the miR-20a-5p/HMGA2 axis (39).

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Conclusion

In recent years, many ncRNAs have been shown to be involved in a variety of physiological and pathophysiological events, including BC. LncRNAs as subtypes of ncRNAs have been extensively studied in BC. lncRNAs are closely associated with cell apoptosis, proliferation, migration, and drug resistance, resulting in BC initiation and progression. Due to the high mortality and recurrence rates, different treatment strategies should be developed for BC patients. Therefore, lncRNAs may be accepted as potential biomarkers for diagnosis, treatment, and prognosis.

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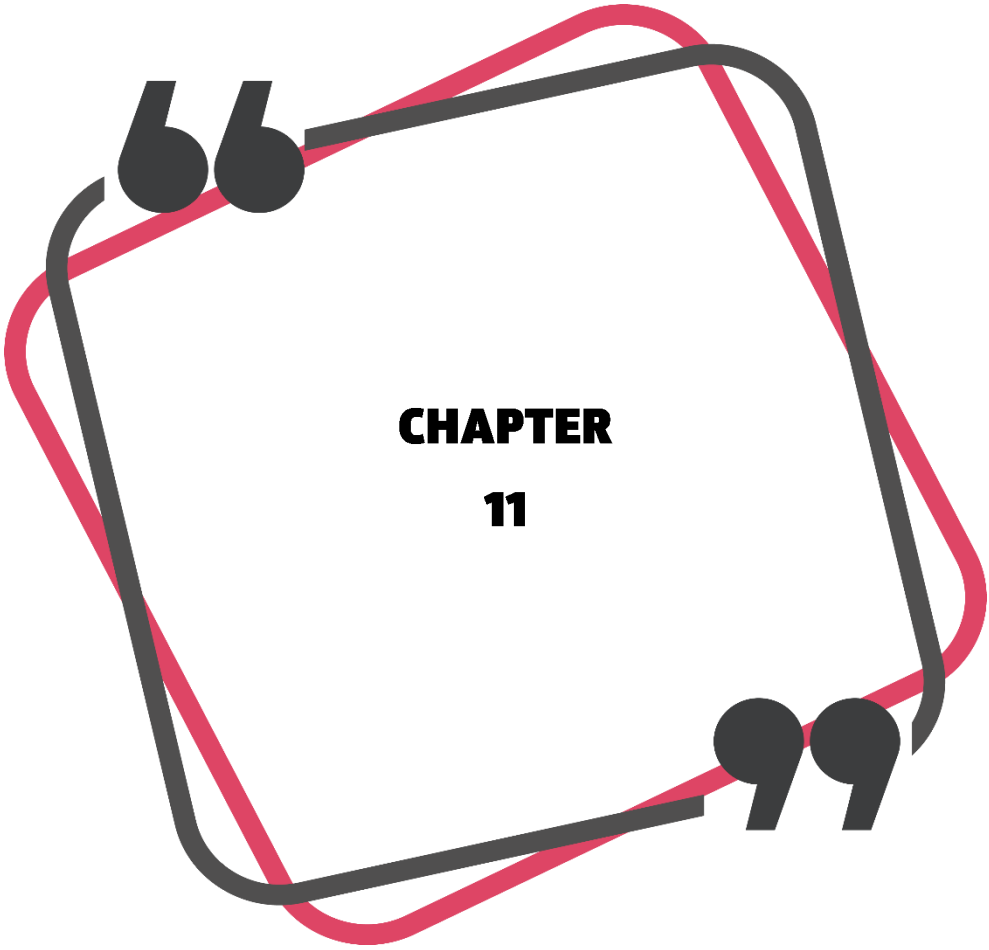
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CHAPTER
11

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COVID-19 INFECTION AND PREGNANCY

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1. Introduction

The severe acute respiratory syndrome coronavirus (SARS-CoV-2), a member of the Coronaviridea family, was discovered in China in 2019, and on March 11, 2020, the World Health Organization (WHO) proclaimed COVID-19 (Coronavirus 2019) a worldwide pandemic.

It is known that the disease is transmitted primarily through droplets. In addition, it is transmitted by the coughing and sneezing droplets of an infected person and by touching the mouth, nose, or eye mucosa with the infected hands of another individual. As determined by studies, the average incubation period of the disease was 5.85 days (1). As with other viral infections, the literature lists fever, cough, dyspnea/shortness of breath, and fatigue as the most common symptoms (2). In addition to these symptoms, patients may also witness a runny nose, myalgia, sore throat, headache, loss of taste and smell, and diarrhea (3). Although the disease's severity is highly variable, numerous clinical manifestations are observed, ranging from asymptomatic disease to pneumonia, severe upper respiratory infection, cytokine hernia, multi-organ failure, and even death (4). Due to the possibility of asymptomatic infection, it is essential to isolate patients, monitor contacts as soon as possible, and, if necessary, quarantine. Educating the public on the importance of personal hygiene and advising healthcare professionals to adhere to infection protocols are also crucial for controlling the infection.

It is known that COVID-19 disease can cause life-threatening complications and even death in the general population. Due to the physiological adaptation mechanisms and cardiovascular, immunological, and respiratory changes that develop during pregnancy, pregnancy is considered a high-risk process; therefore, numerous studies attempt to reveal the disparities between the clinical course of the disease during pregnancy and that in the general population. Due to physiological changes in the immune system and cardiopulmonary systems, pregnant women have a higher incidence of viral respiratory tract infections. Complications such as intubation, renal failure, intensive care stays, and death during pregnancy were observed in (Severe Acute Respiratory Syndrome) SARS and (Middle East Respiratory Syndrome) MERS outbreaks (5). In studies conducted during the COVID-19 pandemic, it was discovered that the disease experienced during pregnancy had higher rates of invasive ventilation, intensive care hospitalization, mortality, preterm birth, and cesarean section (6). All these factors impair pregnant women's mental health and facilitate the development of anxiety and depression (5). At the same time, pregnant women are concerned about how they will navigate this process and plan their delivery (7). Social restrictions and the fear of getting sick may lead to a decrease in prenatal care and a delay in pregnancy follow-ups. Although some studies indicate that pregnancy is a risk factor that adversely affects the clinical course of COVID-19, there is insufficient evidence to support this argument.

In pregnant patients with COVID-19 infection, there is a lack of data regarding fetal outcomes. The ACE-2 (Angiotensin Converting Enzyme-2) receptor's density and presence in the placenta are well documented. Utilization of this receptor by the SARS-CoV-2 virus during cell entry increases the likelihood of placental and secondary fetal involvement (8). COVID-19 infection has been shown in studies to increase the prevalence of decidual arteriopathy in the placenta and cause maternal vascular malperfusion (9). Its association with adverse perinatal outcomes was also mentioned in the same studies. Some studies examine the connection between COVID-19 infection and fetal circulation. Doppler ultrasound analysis of pregnant patients with 54 COVID-19 infections did not reveal any effect of infection on fetal circulation (10). In contrast, it was suggested in a different publication that Covid-19 infection deteriorated uterine artery and umbilical artery pulsatile index and resistance index parameters (11).

2. General Information

2.1. COVID-19 Infectıon/Dısease

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Coronaviruses are one of the most major human and animal infections. Individuals that attended the animal market in December 2019 in Wuhan, Hubei province, People's Republic of China, began to acquire pneumonia. On January 8, 2020, it was declared that a new coronavirus was responsible for the epidemic; the new virus was first termed the 2019 novel Coronavirus (2019-nCoV). Because of its remarkable similarities to SARS-CoV, the name was changed to SARS-CoV-2 on February 11, 2020. The COVID-19 pandemic was proclaimed on March 11, 2020, when the virus triggered a worldwide outbreak.

2.1.1. COVID-19 Virology

SARS-CoV-2 is a highly pathogenic coronavirus subgroup of the Coronaviridea family that produces COVID-19 disease. In 2019, it was first identified in China. The virus is transmitted between individuals via droplets. This disease is easily transmitted, and patients typically experience mild to moderate symptoms. Acute respiratory distress syndrome and pneumonia are two severe illnesses that some patients may get (12). Patients who have comorbidities such as diabetes, chronic lung disease, and underlying heart disease may experience a more severe disease progression (13).

The virus comprises nucleocapsid (N) proteins containing single-stranded RNA with a positive orientation and a lipid bilayer envelope. The virus envelope contains membrane (M), envelope (E), and spike (S) proteins. The coronavirus uses the ACE-2 receptor to enter the cell. ACE-2 is a membrane-bound protease. S proteins are located outside the virus envelope and permit coronavirus entry into the target cell. Two subunits, S1 and S2, make up the S protein. The binding of the S1 subunit, which is the surface unit of the S protein, to the cellular receptor on the target cell's surface ensures virus entry into the cell. The S2 subunit of the S protein mediates viral fusion. The modification of the M protein makes it possible for the virus to invade the cell and effectively acquire antigenic properties. The replication and transcription of the coronavirus are mediated by N proteins (14). In addition, the structure of SARS-CoV-2 contains six additional proteins. The ORF1ab, ORF3a, ORF6, ORF7a, ORF7b, and ORF8 genes encode these proteins. As a result of the virus' mutation, these genes undergo alterations. The severity of the disease and the contagiousness of the virus are directly affected by viral mutations (15).

The S protein of the SARS-CoV-2 virus has a high affinity for the ACE-2 receptor, a cellular transmembrane protein, binds to this receptor and then enters

the cell via fusion or endocytosis. After entering the cell, the virus's RNA-dependent RNA polymerase enzyme is synthesized in the cell's ribosome. The RNA-dependent RNA polymerase enzyme is used to replicate viral RNA. The virus' structural proteins are then synthesized and joined to the nucleocapsid. The virus, which matures in the Golgi, exits the host cell through exocytosis (16).

2.1.2. COVID-19 Epidemiology

The COVID-19 pandemic, which originated in December in Wuhan, People's Republic of China, began to spread globally in March 2020, and by August 2020, 32 million people were reported to be afflicted. It was responsible for 985 thousand of deaths on six continents (17).

According to the data of the Ministry of Health of the Republic of Turkey, a total of 251.805 people were infected with the disease, and 6016 deaths occurred (18). In the United States (USA), only a fraction of acute cases are diagnosed and reported, so it is estimated that the actual number of cases is likely to be significantly higher. According to seroprevalence studies conducted in the United States and Europe, and taking into account the possibility of false-positive and false-negative results, it is believed that the actual number of cases may be ten times greater than what has been reported.

2.1.3. COVID-19 Transmission and Pathogenesis

Data from case studies show that transmission is mostly by droplet, with ventilation and distance being the main determinants of risk. In the few cases where transmission by direct contact and touching a contaminated surface was assumed, respiratory transmission could not be excluded entirely. Contagiousness peaks about a day before the onset of symptoms and subsides within a week of the onset of symptoms. The virus has inhomogeneous transmission dynamics (19). The virus has also been detected in non-respiratory samples, including stool, blood, ocular secretions, and semen, but its relationship with transmission has not yet been determined (20). The transmission range of the infected person is uncertain. The potential for transmission begins before symptoms develop and is greatest early in the course of the disease. The risk of transmission is not likely after seven to ten days (21).

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2.1.4. Clinical Information and Symptoms

The SARS-CoV-2 virus-caused COVID-19 disease has been classified as a worldwide pandemic. The patients' clinical signs vary from moderate fever, cough, and malaise to severe pneumonia, acute respiratory distress syndrome, and multiple organ failure. Although the disease is considered to have a global death rate of roughly 2.2%, its mortality is impacted by numerous factors, including the patient's age, the severity of the disease, and the presence of co-morbidities (22). It is commonly known that the SARS-CoV-2 virus has an incubation period between three and seven days. Several studies have shown that the SARSCoV-2 virus may be detected in the throat and nose many days prior to the beginning of symptoms. According to studies published in the scientific literature, asymptomatic patients had the same viral load as symptomatic patients (23). COVID-19 is distinguished by its strong inflammatory response. The SARS-CoV-2 virus exacerbates inflammation by boosting ACE-2 receptor production. Proinflammatory cytokines IL-1, IFN-, IP-10, MCP-1, IL-4, and IL-10 were found to be higher in COVID-19 patients' blood levels. Significantly greater serum levels of IL-2, IL-7, IL-10, GCSF, IP-10, MCP1, MIP-1A, and TNF- are observed in COVID-19 patients hospitalized in the critical care unit as compared to patients who were not hospitalized in the intensive care unit (22). COVID-19 predominantly affects cells with a high production of ACE-2 receptors, including lung epithelial cells, alveolar macrophages, nasal goblet cells, vascular endothelium, renal tubular endothelium, and ileal cells. The SARS-CoV-2 virus affects lung tissue by infection of type 2 alveolar cells and lung endothelial epithelial cells. Arterial and venous endothelial cells are sensitive to SARS-CoV-2 infection because they generate the ACE-2 receptor; endothelial damage may result. Von Willebrand In the presence of endothelial injury, a factor stimulates the attachment of subendothelial cells and platelets, resulting in clot formation. Due to endotheliitis induced by the SARS-CoV-2 virus, macrophages and neutrophils simultaneously concentrate in the vascular region. In this instance, microthrombosis developed due to induced thrombosis and reduced fibrinolysis. Nevertheless, virus particles have also been detected in endothelial cells of the kidney, which explains why COVID-19 disease causes renal damage.High ACE-2 receptor synthesis in cardiac cells is associated with myocardial damage in COVID-19 infected patients. Nonetheless, it is believed that endothelitis and increased inflammation may also cause myocardial infarction (24).

In addition to the lungs, the ACE-2 receptor is also present in the heart, endothelium, renal tubular epithelium, intestinal epithelium, and pancreas. The SARS-CoV-2 virus is capable of invading all of these tissues. If the virus multiplies in

all organs and causes tissue damage, this can result in multi-organ dysfunction syndrome (MODS). Fever, malaise, cough, diarrhea, and vomiting are non-specific symptoms of COVID-19 infection, while dyspnea, hypoxia, multi-organ failure, and disseminated coagulation disorder are severe clinical manifestations. In a meta-analysis, COVID-19 patients exhibited 88.5% fever, 68.6% cough, 38.5% weakness, 12.1% dizziness, 4.9% diarrhea, and 3.9% vomiting (25). Neurological symptoms such as arthralgia, chest pain, rash, alopecia, sleep disorders, and memory impairment, and psychiatric symptoms such as depression have been reported in other studies. Due to weakened immunity, it has been reported that COVID-19 symptoms are more severe in people over 60 with comorbidities such as lung disease, hypertansion, and heart disease (26). The most prevalent COVID-19 disease symptoms and signs include cough, sore throat, fever, musculoskeletal symptoms (muscle and joint pains), weakness, headache, and loss of taste and smell (27).

2.1.5. COVID-19 Disease Severity Classification

Even though SARS-CoV-2 infection is asymptomatic in some patients, it necessitates intensive care support for others and, in some cases, causes death. The US National Institutes of Health (NIH) and Chinese Wu classifications define the clinic.

The United States National Institute of Health has divided the extent of the disease into five categories:

1. Asymptomatic or presymptomatic infection: patients with a positive virological test but no clinical symptoms.

2. Mild disease: Patients who exhibit any COVID-19 signs and symptoms (fever, cough, sore throat, weakness, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not exhibit shortness of breath or abnormal chest imaging results.

3. Moderate disease: Patients with evidence of disease on clinical evaluation or imaging and with a room air-inspired oxygen saturation ≥94% at sea level.

4. Severe disease: In patients with oxygen saturation in room air at sea level below ninety-four percent of the arterial partial oxygen pressure, the ratio of the oxygen fraction of inspired air (PaO2/FiO2) to 30 breaths/min and lung infiltrates above fifty percent.

5. Critically ill: Patients with respiratory failure, septic shock, and/or multiple organ failure (28).

The Chinese Wu classification consists of 3 subgroups:

1. Mild disease: Patients with no or mild symptoms

2. Severe disease: Tachypnea (respiratory rate >30 breaths/min) hypoxia (patients with pulmonary involvement with oxygen saturation in room air below 93% or PaO2/FiO2 above 50%.

3. Critical illness: Patients with respiratory failure, shock, or multiple organ failure (29).

2.1.6. COVID-19 Diagnosis

COVID-19 should be ruled out in individuals with suspected disease symptoms, particularly those in close contact with the COVID-19 case. All symptomatic individuals with a COVID-19 disease suspicion should be tested for COVID-19.

SARS-CoV-2 RNA is found from the upper respiratory tract as the primary diagnostic test for COVID-19 illness utilizing the nucleic acid amplification test (NAAT) using reverse transcription polymerase chain reaction (RT-PCR) assay, which is the most routinely used test (30). In rare circumstances, antigen testing may be the initial test, however antigen tests are less sensitive than NAAT, and negative antigen tests should normally be verified by NAAT.Antigen tests are helpful for serial infection screening in high-risk environments. Antigen testing may be the initial test in some instances, but antigen tests are less sensitive than NAAT, and negative antigen tests should typically be confirmed using NAAT. Antigen tests are helpful for serial infection screening in high-risk environments.

Blood tests reveal SARS-CoV-2 virus antibodies. In patients with a negative COVID-19 (Polymerase Chain Reaction) PCR test, serological testing after three to four weeks may offer new information but cannot be used for the acute diagnosis of the virus.Immunoglobulin G testing is recommended, and testing for total antibodies may be beneficial, but data are limited. In areas where COVID-19 disease is prevalent, all women can be tested prior to labor or within 72 hours of hospital admission if sufficient testing is available (31).

2.2. Pregnancy and COVID-19

During pregnancy, elevated progesterone levels cause an increase in vascularity and edema in the respiratory system, particularly on the mucosal surfaces of the upper respiratory tract. As the gestational week progresses, the increased abdominal pressure caused by the expanding uterus causes the diaphragm to rise 4-5 cm. Due to this change, the functional residual capacity decreases by 20-30%, the expiratory reserve volume by 15-20%, and the residual volume by 20%. Inspiratory capacity increases by 5-10% and tidal volume by 30% during pregnancy. Total lung capacity and lung compliance, however, remain unchanged. Additionally, during pregnancy, oxygen consumption increases by approximately 20%. During pregnancy, respiratory system diseases have a more severe clinical course due to increased closing pressure and oxygen demand. Due to pregnancy-related physiological changes, a pregnant woman's hypoxia tolerance is diminished. This may exacerbate the disease's clinical manifestations.

During pregnancy, viral infections may cause maternal and fetal complications. Viruses rarely cross the placental barrier, but when they do, they can cause severe morbidities such as microcephaly, intrauterine growth retardation, and even fetal death. Through the blood or ascending from the lower genital tract, the virus can reach the placenta and decidua. In addition, it has been observed that the placenta is a regulator rather than an obstruction to blood flow and that maternal-fetal bidirectional transmission may affect the fetus transplacentally (32). The involvement of the placenta and decidua varies based on the cellular receptor structure of tissues that permit virus entry into the cell and the maternal immune response. Virus-host interactions vary following cell type, gestational age, and the immune response of the pregnant. Infections with viruses such as COVID-19 can affect the fetus directly and indirectly by influencing placental development, which can impact fetal outcomes (33).

2.2.1. COVID-19 and Immune Response During Pregnancy

During pregnancy, the mother's immune system adapts to prevent the rejection of the semi-allogenic fetus and maintains a balanced defense against microorganisms. One of these changes is a shift from Th1 to Th2 (cellular to humoral immunity) in CD4+ T lymphocytes, which is a change in the immune response (34). The reduction of Th1 reactivity in the immune response to viral infections may change the clearance of infected cells. Concurrently, a Th1 and Th2 response to SARS-CoV-2 is observed in the pathogenesis of severe COVID-19 (35). NK cells are innate and responsible for virus elimination. Losing these cells can impair the immune system's capacity to eliminate viruses. However, the effect of reducing

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NK cells on COVID-19 remains unclear. Previous research has demonstrated that progesterone increases lung repair in influenza-infected patients with lung damage (36). In this instance, a high progesterone level during pregnancy may facilitate recovery from viral lung infections. More studies are needed to show the effects of changing progesterone and other hormones on the immune response in SARS-CoV-2 infection that develops during pregnancy. In addition, it is unknown if these mechanisms, which develop during the maternal immune response, are protective against COVID-19 or even cause the opposite susceptibility or morbidity (37).

2.2.2. COVID-19 and the Respiratory System in Pregnancy

Intra-abdominal pressure increases and the diaphragm rises by 4-5 cm due to the enlargement of the uterus during pregnancy (38). Progesterone and relaxin loosen the ligaments connecting the ribs and sternum. As a result, from 68.5 to 103.5 degrees, the subcostal angle of the rib cage increases (39). As a result, the functional residual capacity, expiratory reserve volume, and residual volume all fell by 20-30%. Also, tidal volume increases by 30%, and inspiratory capacity increases by 5-10%. Lung compliance and total lung capacity remain constant. Also, oxygen consumption increases by 20% during pregnancy (38). The functional residual capacity of pregnant women has decreased as a result of an increase in maternal oxygen consumption. Pregnant women are more prone to hypoxia, which makes respiratory system disorders more severe (39).

2.2.3. COVID-19 and the Coagulation Response in Pregnancy

ACE-2 interaction results in endothelial disruption in COVID-19 infection. Coagulation is initiated by the increased endothelial secretion of tissue factor and the activation of thrombin by inflammatory cytokines (40). Thrombin production is increased in healthy pregnant women, and increased intravascular inflammation is associated with thrombosis susceptibility. In a pregnant woman with COVID-19 infection, it is believed that the risk of thromboembolism increases even further (40). Pregnant women with COVID-19 infection should receive thromboprophylaxis for up to 10 days after delivery, and clinicians should evaluate the risk of thromboembolism in these patients (41).

2.2.4. COVID-19 Infection in Pregnancy: Clinical Course

2.2.4.1. Symptoms

The clinical signs of COVID-19 disease range from asymptomatic infection to severe and deadly sickness. Most pregnant women do not exhibit symptoms (42). The most frequent clinical findings in pregnant women include cough, fever, myalgia, headache, dyspnea, sore throat, diarrhea, nausea, vomiting, loss of smell and taste or other smell and taste abnormalities, rhinorrhea or nasal congestion, chills, exhaustion, disorientation, and chest discomfort. (31). Advanced maternal age, high body mass index, any pre-existing comorbidity, chronic hypertension, preeclampsia, gestational or pregestational diabetes, and any pre-existing comorbidity are maternal risk factors that determine the clinical course of the COVID-19 disease, increase the severity of the disease, and are thought to be associated with hospitalization in the intensive care unit (42).

A meta-analysis found that 17.4% of pregnant women with COVID-19 disease developed pneumonia, 17.1% received oxygen via cannula, 13% had severe disease, 4% were admitted to the intensive care unit, 1.6% required invasive ventilation, 0.11% received extracorporeal membrane oxygenation (ECMO), and 0.8% died during the disease's clinical course (42). In studies of pregnant patients with COVID-19, the death rate was higher than that of pregnant patients who died of reasons other than COVID-19, but not higher than that of non-pregnant female patients with COVID-19 (42). The risk of COVID-19 disease is not increased by pregnancy. In contrast to non-pregnant women, pregnant women have a higher risk of ICU admission, invasive ventilation, the requirement for ECMO, and mortality (6).

2.2.4.2. Lab Findings

Similar laboratory findings are observed in pregnant and non-pregnant women with COVID-19 infection. The most common laboratory abnormalities are an elevated C-reactive protein (CRP) level, lymphopenia, leukocytosis, elevated procalcitonin level, abnormal liver enzymes, thrombocytopenia, especially ferritin, fibrinogen, and D-Dimer levels. Specifically, high CRP levels are associated with severe COVID-19. In contrast, high D-Dimer levels are believed to be related to the physiological increase during pregnancy and do not aid in determining the disease's severity (43).

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2.2.4.3. Imaging Findings

Lung imaging aids in the diagnosis of COVID-19. On CT, bilateral diffuse lobulated ground-glass opacities or consolidations and peripheral air shadows on direct chest radiography are detected radiologically (44). The American College of Radiology reports that the amount of radiation transmitted to the fetus during a single direct chest radiograph is between 0.0005-0.01 mGy, which is negligible. Thorax CT delivers a 0.01-0.66 mGy dose to the fetus. Therefore, the fetal radiation dose from a thorax CT during pregnancy is within safe limits (45). Lung CT scan has high sensitivity in diagnosing COVID-19 in pregnant women and can be used to protect the fetus when necessary with informed consent.

2.2.4.4. Vertical transmission

Due to the prevalence of ACE-2 receptors in the placenta, there is a theoretical risk of vertical transmission, as with SARS. The absence of SARS-CoV-2 in cord blood has been attributed to low maternal viremia. In one study, viremia was found to be 1%; while this poses a theoretical risk for intrauterine transmission, there is insufficient evidence for vertical transmission (46).

2.3. Pregnancy Management in COVID-19

Standard infection control measures should be utilized to reduce the transmission of COVID-19 in pregnant women. All pregnant women should undergo clinical screening. In terms of follow-up, diagnosis, and treatment, a multidisciplinary approach is required to manage COVID-19 infection during pregnancy. In addition to planning the appropriate timing of delivery and postpartum care for pregnant patients with COVID-19, primary stages include early isolation, treatment for COVID-19 and other infections that may develop with it, oxygen therapy, intravenous hydration (without causing load), fetal USG and cardiotocography at regular intervals, and when necessary, mechanical ventilation (47).

It should be understood that respiratory distress can develop rapidly in asymptomatic patients, and the patient should be informed and placed in 14-day self-quarantine.

Patients who have received informed care at home should be followed. The patient should be instructed to seek medical attention in the event of worsening dyspnea, persistent fever >39°C despite the use of acetaminophen, intolerance to oral hydration and medications, persistent chest pain, or obstetric complaints

(premature contractions, vaginal bleeding, rupture of membranes, decreased fetal movement). Like with other acute viral infections, supportive treatment is advised in the home. Hydration, relaxation, and, if tolerated, regular walking exercises are all recommended (48). Depending on disease severity, comorbidities, pregnancy difficulties, and social position, symptomatic patients might be followed at home or in the hospital.

Indications for hospitalization:

• A comorbid condition (poorly controlled hypertension or diabetes, preeclampsia, prior rupture of membranes, uterine bleeding),

• Fever >39°C despite the use of acetaminophen,

• Moderate or severe respiratory symptoms,

• Critical illness (multiple organ damage such as respiratory failure) (48).

During pregnancy, maternal peripheral oxygen saturation (SpO2) should be maintained at 95%. To ensure adequate oxygen diffusion into the placenta, the mother's partial arterial oxygen pressure (PaO2) must be greater than 70 mmHg.

2.3.1. Medical Treatment in Pregnant Women with COVID-19

Hospitalized pregnant women with severe COVID-19 disease should receive routine prophylaxis against thromboembolism with low molecular weight heparin (LMWH) or unfractionated heparin, unless contraindicated.The appropriate antibiotic therapy should be added if additional bacterial pneumonia is suspected in COVID-19. In preterm pregnancy with COVID-19 infection, ACOG (American Society of Gynecology and Obstetrics) recommends the same protocol for antenatal corticosteroid therapy as in cases without COVID-19 infection. If delivery is expected within seven days, the antenatal corticosteroid is recommended for pregnant women with COVID-19 between 24 and 33 weeks and six days (early preterm). However, it is not routinely recommended for patients between 34 and 36 weeks and six days (late preterm) (49). If the pregnant patient continues to experience respiratory distress due to COVID-19 infection, standard betamethasone or dexamethasone therapy for fetal lung development can be continued with the same agents. However, hydrocortisone or methylprednisolone can be substituted to reduce fetal glucocorticoid exposure. Since the use of corticosteroids during pregnancy may increase blood sugar, care should be taken, particularly

in diabetic patients, because an increase in maternal glucose leads to hyperglycemia and hyperinsulinemia in the fetus, which increases the fetus' oxygen demand (50). Although tocolysis is controversial in COVID-19, nifedipine should be the drug of choice if it is used. Due to its interaction with ACE-2 receptors, indomethacin should not be administered because it may impair the hypoxia-protective mechanism (51). Due to its respiratory suppressing effects, the use of magnesium sulfate for maternal seizure prophylaxis and/or neonatal neuroprotection is controversial. Perinatology, Chest Diseases, and Intensive Care Specialists should all be consulted before beginning MgSO4 treatment (52). When pregnant women with COVID-19 infection need analgesics, acetaminophen should be their first choice. It has been shown that using acetaminophen during pregnancy, including the first trimester, reduces the risks of pregnancy associated with a high fever. Pain and fever may be treated with non-steroidal anti-inflammatory drugs (NSAIDs). In less than 48 hours, the lowest effective dose can be administered when fetal toxicity conditions such as oligohydramnios and premature closure of the ductus arteriosus are present. Low-dose acetaminophen is safe during pregnancy for the prophylaxis of preeclampsia (53). Using one or more combinations of Oseltamivir, Hydroxychloroquine, Azithromycin, Favipiravir, and Lopinavir/Ritonavir in various forms is advised in antiviral therapy, according to the algorithm released by the Turkish Ministry of Health (54). Considering that the concurrent use of hydroxychloroquine and azithromycin prolong the Q-T interval, there is an increased risk of death from arrhythmia (54). It is known that the lopinavir/ritonavir combination is administered during pregnancy and does not increase the risk of fetal anomaly (55). When pregnant women with influenza used oseltamivir, there was no risk to the fetus (56). As Favipravir is contraindicated in pregnant women, it was not administered to any. Molnipravir is not administered to pregnant women simultaneously (54).

2.3.2. Birth Management in COVID-19

The labor management of women who gave birth during the COVID-19 pandemic and women with confirmed or suspected COVID-19 disease remains unchanged. The timing and method of delivery should be determined based on the mother's and fetus's clinical condition. In suspected, probable, and positively diagnosed cases, delivery should occur in isolation rooms with negative pressure. Patients with asymptomatic and mild infections do not need to modify the planned delivery method. There are insufficient data to conclude that cesarean delivery reduces the risk of newborn COVID-19 infection. Those with severe and life-threatening illnesses may undergo labor induction (56). Because it may re-

duce the risk of infection transmission to the newborn and the exposure of healthcare personnel, it is recommended that the second stage of labor be shortened through induction. In cases of fetal distress, poor progression, or deterioration in the mother's general condition, induction should not be insisted uponIn situations of septic shock, severe organ failure, and fetal distress, emergency cesarean section should be done (57). Several clinics do not perform delayed cord clamping for full-term newborns since there are inadequate data on the risk of infection transmission to the baby. If the mother practices proper hand hygiene and wears a surgical mask, skin-to-skin contact is feasible. The American Academy of Pediatrics does not recommend that skin-to-skin contact be avoided (58).

2.3.2. Breastfeeding Management in COVID 19

Insufficient information exists regarding the safety of breastfeeding and mother-infant separation. If the mother's condition is clinically severe or life-threatening, mother-infant separation is the best option, and the mother should continue to express breast milk. If the mother's illness is severe, separating her from her child seems preferable. In this instance, milking is necessary to maintain milk production. The cleanliness of breast pumps should be carefully considered. If the mother is asymptomatic, the infant can be returned to her. In this situation, the mother needs to observe proper hand hygiene and wear a surgical mask. If the mother and child are to share a room, care should be taken to ensure that they are at least 2 meters apart and can be separated with a simple curtain (59).

2.3.3. Pregnancy and Neonatal Outcomes in COVID-19

It is known that COVID-19 does not increase the risk of miscarriage in pregnant women, but data on infections in the first and second trimesters are limited (60). Increasing rates of preterm birth and cesarean section were observed (42). Although the belief that delivery will alleviate maternal respiratory distress is considered a justification for cesarean delivery, there is currently insufficient evidence to support this theory. The rate of congenital fetal anomalies is not yet supported by any evidence (60). 95% of infants born to mothers with COVID-19 exhibit no symptoms. Some newborns developed a mild infections, and it was believed that most of these infections were transmitted by droplets from the mother (61). A systematic review of 12 countries found that the stillbirth rate was 0.4-0.6% and the early neonatal mortality rate was 0.2-0.3% in infants born to mothers with COVID-19 infection and that these data were relatively similar to pre-COVID-19 data (62).

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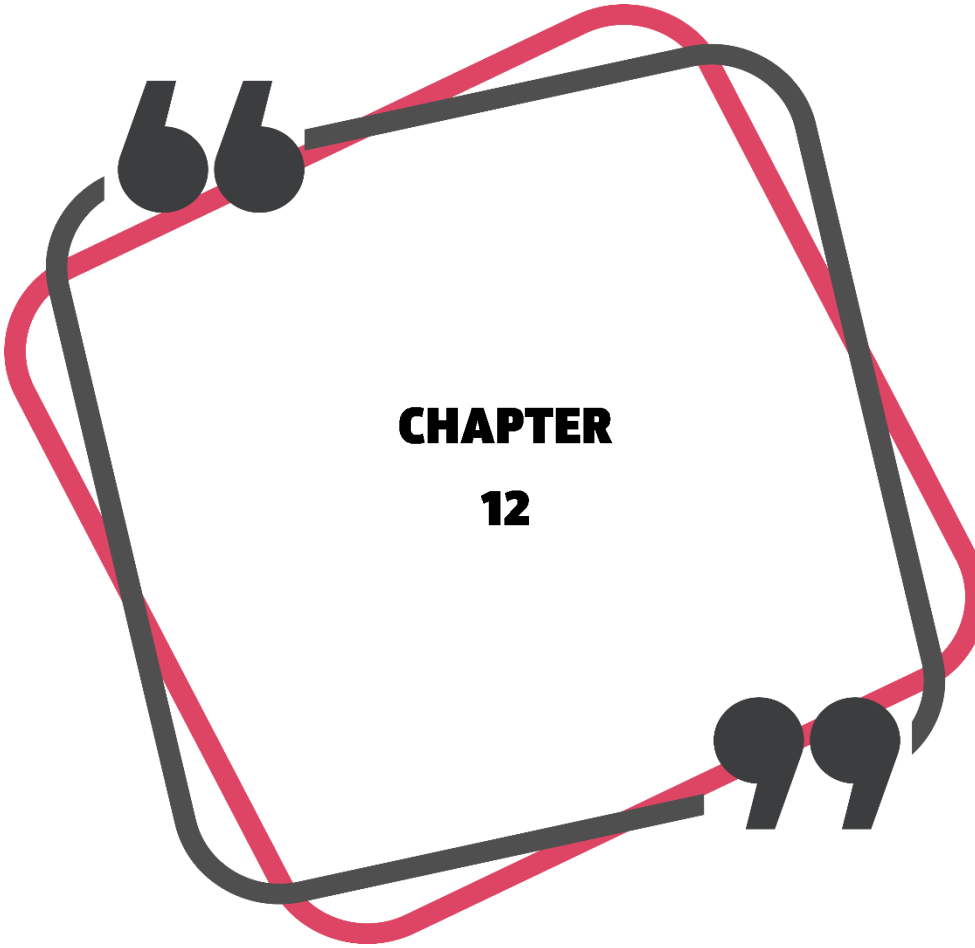
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CHAPTER
12

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PREGNANCY AND CANCER

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1. Introduction

Gynecological cancers usually occur in older women. Endometrial cancer, ovarian cancer, cervix, vulva and vagina cancers are among the main neoplasms of the female genital area. The frequency of these neoplasms, which require multiple medical approaches in their treatment, has recently increased significantly, including during pregnancy. Cancer in pregnancy is rare and information on this subject is very limited. Malignant diseases constitute 5% of maternal deaths during pregnancy (1). The most common cancer in pregnancy is breast cancer. The most common causes of death in pregnancy are central nervous system tumors, leukemia and lymphoma.

Due to scientific developments in assisted reproductive methods and women's socialization in life and work, the age of conception has moved to advanced ages due to the increase in their prominent roles. With the advancement of gestational age and the conscious spread of cancer screening tests, the number of patients diagnosed with cancer during pregnancy is increasing day by day. The incidence of cancer during pregnancy is about 1 in a thousand. Approximately 1/3 of malignant neoplasms seen in women are seen in women under the age of 45 (2). This increased the association between pregnancy and cancer.

Cancer and pregnancy coexistence can be detected during pregnancy and in the postpartum period. Even if the diagnosis and treatment of cancer during pregnancy is difficult, pregnancy does not adversely affect cancer and does not have a negative effect on treatment. In other words, pregnancy does not change the prognosis of cancer. Gynecological oncologists, with the contribution of perinatologists, take care of the well-being of the fetus and carry out the cancer treatment of the mother without delay. At the forefront of cancer treatment during pregnancy is the individualization of the patient according to the extent of the disease, the gestational week and the patient's own wishes. During pregnancy, gynecological tumors should be treated with a correct and appropriate approach. The main principles of treatment are surgery, chemotherapy and radiotherapy according to the week of pregnancy (3). When the fetal well-being comes to the fore, the loss of time in the treatment, the lack of the correct treatment and the delays in the diagnosis cause the treatment to be left to the postpartum period. It is necessary to apply the right treatment at the right time so that cancer treatment management during pregnancy does not adversely affect maternal survival and neonatal outcomes are not significantly affected.

In the opinion of oncologists, gynecological cancers can be successfully treated during pregnancy in collaboration with a multidisciplinary team, optimizing maternal care with fetal safety in mind. To maximize maternal outcome, cancer treatment should follow a standard treatment protocol as in non-pregnant patients. In order to avoid prematurity, it is necessary to individualize the treatment and to provide effective psychological support throughout pregnancy. Staging examinations such as magnetic resonance imaging and sonography and diagnostic procedures including imaging should be preferred. Open or laparoscopic pelvic surgery as part of a treatment protocol can produce beneficial results. In most standard chemotherapy regimens, it can be applied according to the gestational week. Except for cervical and vulvar cancer and significant vulvar scars, the mode of delivery is determined by the perinatologist (4).

2. Cervical Lesions in Pregnancy

Cervical cancer, which is the most common malignancy during pregnancy, is very important in terms of maternal and fetal since it is the only cancer that can be screened in prenatal follow-up. The frequency of precancerous lesions seen at younger ages has increased. In the treatment of these lesions, the expectation of treatment for the preservation of fertility has also increased. This has increased the importance of approaching cervical cancer precursor lesions in terms of follow-up, treatment and prognosis in order to protect the patient from cancer. Because of the suppression of the immune system during pregnancy and squamous

metaplasia in the cervix at the highest limits, regression is observed in precancerous lesions after pregnancy. Therefore, conservative approaches to preinvasive lesions seen during pregnancy are seen as the primary treatment (5).

2.1. Cervical Premalignant Lesions During Pregnancy

The portions of the cervix are the ectocervix, lined with squamous epithelium, and the endocervix, lined with glandular epithelium. Cervical intraepithelial neoplasia (CIN), which stands for squamous cell abnormalities, is a premalignant lesion of the cervix. Glandular cell abnormalities mean adenocarcinoma in situ and adenocarcinoma. CIN can be a low or high grade lesion. While the risk of developing cervical malignancy is minimal in patients with low-grade CIN, the probability of progression to malignancy is higher in high-grade CIN. CIN can be diagnosed by pathological examination after cervical biopsy (6). Cervical cancer screening is performed worldwide as a result of cervical cytology and research of oncogenic subtypes of human papillomavirus (HPV) (7). Cervical cancer diagnosed during pregnancy is of vital importance for both the fetus and the mother. Although cancer rates among women have dropped significantly with cytology screening, cervical cancer is still diagnosed during pregnancy. Most oncologists recommend performing a cervical cytology smear test in the early weeks of pregnancy (8). Since premalignant lesions may regress spontaneously during pregnancy and treatment may cause different complications for the patient, it is necessary to prevent progression to cancer by following aggressive treatments closely (9).

LGSIL (Low grade intraepithelial lesion) and ASCUS (Atypical squamous cells of unknown significance) are the most frequently diagnosed abnormal cervical cytology pathology results in pregnancy. HGSIL (High grade intraepithelial lesion) is less common in pregnancy. The malignancy potential in high-grade lesions has been reported to be 4-5% (10). Colposcopy should be performed on pregnant women of all ages in high-grade cervical lesions such as ASC-H (Atypical squamous cells indistinguishable from high-grade intraepithelial lesion), HGSIL, AGC (Atypical glandular cells). Colposcopy is the method that should be preferred for diagnostic purposes in cervical smear abnormalities.

Against the possibility of regression of precancerous cervical lesions seen during pregnancy, the treatment management option of the disease should be considered as a special group that should be considered in order to minimize the risk of morbidity in the mother and fetus. The rate of progression to cervical malignancy in pregnant patients is similar to that in normal non-pregnant women (11). Physiological changes in the cervix during pregnancy make colposcopy more difficult. In the visual evaluation of pregnant patients undergoing colposcopy, it is much more difficult to distinguish neoplasia from pregnancy-related changes, and

therefore, it should be known that the risk of overlooked cancer diagnosis increases. Colposcopy during pregnancy should be performed by the most experienced oncologist. Follow-up intervals should be tight. The frequency of follow-ups should be adjusted according to the experience of the oncologist performing the colposcopy, the gestational week, and the patient's preference. Although pregnancy does not change the rate and rate of progression of premalignant cervical lesions to cancer, colposcopy-guided biopsies should be performed in pregnant patients. When LGSIL is detected during pregnancy, follow-up and treatment is delayed until the postpartum 4th week, while cervical biopsy can be performed with colposcopy in the detection of HGSIL, ASCUS and ASC-H. Further treatment in malignant patients is recommended if the pathology result is interpreted as invasive. It is also another treatment method in cervical conization during pregnancy. As a result, the data on the treatment modalities of pregnancy-related cervical premalignant lesions are very limited, and a joint decision should be made by evaluating the well-being of both the mother and the fetus.

2.2. Cervical Malignant Lesions in Pregnancy

Cervical neoplasms in pregnancy; refers to cervical malignancies diagnosed during pregnancy or up to 12 months postpartum. While 50 percent of the patients are diagnosed prenatally, the remaining part is diagnosed within 12 months after birth (12). Due to the older age at conception in recent years, the increase in the incidence of pregnancy and cervical cancer has increased considerably (13). Since there are no studies illuminating the science about the treatment management of invasive cervical cancers, which are rarely seen in pregnancy, all knowledge has been formed with case reports from patients and expert opinions (14). Therefore, diagnosis and treatment in pregnant women are carried out in the same way as in non-pregnant women. The difficulties of treatment and follow-up should be considered, especially in patients who do not want to terminate their pregnancy.

According to the stage of the disease and the size of the tumor in non-pregnant patients, the symptoms of cervical cancer are the same in pregnant women. Patients are asymptomatic in the early stages. They can be diagnosed during routine screening tests. It may also present with foul-smelling purulent bloody vaginal discharges, secretions and irregular uterine bleeding. Studies have shown that; There is no significant difference in maternal survival rates between patients with malignant cervical cancer diagnosed during pregnancy and patients with malignant cervical cancer who are not pregnant (15-17). In the effect of cervical cancer on the pregnancy process; We can say that there may be a history of spontaneous abortions, preterm births, births of very low birth weight babies and it does not adversely affect pregnancy.

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Pregnancy and cervical cancer is a complex and very challenging exam that must be solved for the mother, fetus, oncologist and perinatologist. Cervical cancer diagnosed during pregnancy can be difficult to diagnose. The reason for this is of course hormonal changes associated with pregnancy. It is very important for patients who are considering pregnancy to have the necessary screenings before they become pregnant. As in all pregnancies, the priority in women with cervical cancer is always the health of the mother. During pregnancy and cervical cancer, the medical team should investigate the patient thoroughly, using all necessary time, and then plan the best follow-up and the most appropriate treatment for the patient.

If invasive cancer is suspected where dysplastic changes are observed during colposcopy, a cervical biopsy sample should be taken. If colposcopy is insufficient in the early weeks of pregnancy, it can be performed again after 6 to 12 weeks. In some cases, it can be applied as a layer with a shallow cervical conization depth (18). Colposcopy should be performed at 3-month intervals in the follow-up of lesions diagnosed during pregnancy. Thus, a possible invasive cancer diagnosis is not missed, and it should be noted that in the presence of invasive cancer, the delivery method will benefit the mother and fetus (19). Colposcopy follow-ups should be followed up and evaluated after delivery until the diagnosis is finalized (20). Wedge resection or superficial conization can be applied during pregnancy if conization cannot be distinguished only from microinvasive cervical cancer. In case of suspicion of cervical cancer or in cervical cancer stage-1a1, there is an indication for cervical conization. Conization is usually an adequate treatment for squamous cell cancers (19). The transformation zone is displaced towards the ectocervix during pregnancy, so superficial conization is usually sufficient. In the presence of endocervical neoplasms, superficial conization applied during pregnancy has no therapeutic effect. Deep conization, which includes the endocervix completely, should not be performed during pregnancy, as it may result in preterm labor and premature rupture of the membrane (19,20).

The main symptoms of cervical cancer in pregnancy are abnormal vaginal bleeding, vaginal bloody discharge and postcoital painful bleeding. The factors that determine the prognosis of cervical cancers are the same during pregnancy. These; stage, lymph node involvement, histological type, gestational week at the time of diagnosis and the patient's desire for pregnancy. In cases of cervical cancer, the most appropriate approach would be to personalize the treatment, taking into account the patient, the pathological stage of the disease, and the desire for a child. If the uterus is preserved, prognostic factors such as lymph node spread, tumor differentiation, lympho-vascular invasion must be taken into account. It should be shown that lymph node metastasis, which is the most important prog-

nostic factor in early stage cervical cancers, is absent (21). In lymph node evaluation, it should be kept in mind that decidual changes in lymph nodes caused by pregnancy may mimic metastasis. Ultrasonography and MRI are safer than intravenous pyelography to evaluate the urinary system in advanced cervical cancer cases (22-26). Even if preinvasive neoplasms of the cervix are of high grade, their treatment can be left to the postpartum period (27). Postpartum regression is usually expected in such lesions. In preinvasive lesions of the cervix, the mode of delivery depends on obstetric indications (28).

If the diagnosis of cervical squamous cell invasive cancer is made before the 24th gestational week and there is no lymph node involvement and lymphovascular region invasion, the treatment is arranged according to the stage of the cancer. Superficial conization will be sufficient in stage-1A1 women (29). Conization is insufficient in adenocancers. In women with a tumor diameter of 20 mm in stage-1A1 and 1B1 tumors, neo-adjuvant chemotherapy is preferred if pregnancy demand persists (30). If pregnancy demand continues in patients with lymph node involvement, neo-adjuvant chemotherapy or termination of pregnancy at the earliest gestational week that has a chance of survival may be considered (31,32). Chemosensitivity has been reported at rates of 80-90% in cervical cancers, therefore, neo-adjuvant chemotherapy can be applied as another treatment method in pregnancies older than 20 weeks and radiotherapy can be delayed until postpartum (33,34). Cesarean section for pregnant women diagnosed with cervical cancer other than stage-1A1 is the recommended delivery method because it reduces local recurrences. Cancer treatment in patients who do not want their pregnancy to continue is the same as for non-pregnant women (35).

3. Endometrial Cancer in Pregnancy

Endometrial cancer in pregnancy is very rare. The diagnosis of endometrial cancer during pregnancy is mostly made in the material examined after abortion or in people with increased vaginal bleeding after delivery, with pathology results after probe dilated curettage and hysterectomy (36). Apart from epithelial endometrial cancers, uterine cancers such as leiomyosarcoma, endometrial stromal sarcoma and carcinosarcoma are other uterine cancers reported (36). Implantation abnormalities and miscarriages are seen in patients diagnosed before pregnancy occurs, as pregnancy formation of the cavity will not be suitable. Endometrial cancer seen in these individuals is well differentiated and diagnosed as a focal lesion. Normally, the increase in progesterone is protective against endometrial cancer, but it has been observed that the neoplastic cells seen in endometrial cancer cases during pregnancy are unresponsive to progesterone. There are also types of endometrial cancer with a worse prognosis in which progesterone and estrogen receptors are negative and deep myometrial invasion is seen during pregnancy (37-40). Multidisciplinary treatments should be applied with medical oncology

and perinatology specialists in people who have a desire for fertility and are diagnosed.

4. Ovarian Cancer in Pregnancy

Adnexal masses during pregnancy are either functional or benign (41). 4-5% of adnexal cysts are diagnosed during pregnancy examination (42). 2-5% of these cysts during pregnancy are malignant (43). Epithelial cancers seen in pregnancy constitute about half of ovarian cancers seen in pregnancy. Stromal and germ cell neoplasms are responsible for approximately 30% of pregnancy ovarian cancers. The remaining part creates sarcomas and secondary metastases to the ovaries (44,45). The larger the adnexal mass during pregnancy, the greater the risk of malignancy. There is a 3-5% risk of malignancy in masses larger than 6 cm on average. 1/3 of ovarian neoplasms detected during pregnancy are dysgerminoma, and the other 1/3 are epithelial ovarian neoplasms. Pregnancy does not adversely affect the prognosis of cancer and does not cause delay in diagnosis. Only during pregnancy did the risk of ovarian torsion and ovarian cyst rupture increase (46-48). If there is a suspicion of ovarian cancer in the pregnant woman, a complete physical examination should be performed, evaluation of the whole body in terms of lymph involvement, breast examination, pleural fluid in the lung, intra-abdominal acid fluid, bimanual examination and rectovaginal examinations should be performed (42).

In case of diagnosis of epithelial ovarian cancer during pregnancy, treatment arrangement is made according to the patient's desire to maintain the pregnancy and the characteristics of the neoplasia lesion. In complex cysts and cases with high suspicion of malignancy, surgery can be performed before the 4th week of pregnancy, but after the 4th week of pregnancy is the safest period for surgery (49). Uterine manipulations should be avoided during surgery in order to prevent obstetric complications that may occur in patients who are considered to maintain pregnancy. Due to the increased volume of the uterus, inadequacies may occur in the evaluation of every part of the pelvis and Douglas during surgery.

During surgery, oophorectomy, taking cytology, excision of the omentum, and taking a peritoneal sample are performed. In stage 1-A cases, bilateral oophorectomy is not required, but pathological evaluation should be performed for intact ovaries. In stage 1-B cases, bilateral oophorectomy is definitely performed. Progesterone replacement must be performed after surgery performed at early pregnancy week. Chemotherapy (CT) treatment can be left after the first trimester in early stage ovarian neoplasia. 5-year survival in women with early-stage cancer who do not accept LT during pregnancy does not differ from those who receive LT (46). In the presence of advanced stage of metastatic ovarian cancer, neoad-

juvant chemotherapy can be applied in the 2nd and 3rd trimesters, and it is recommended that cytoreductive surgery be left after delivery. If advanced stage ovarian cancer is diagnosed in first trimester pregnancies, it should be recommended to start treatment as if the pregnancy did not exist (46-53). Only chemotherapy is recommended for germ cell neoplasms, except for stage I-A dysgerminomas and stage I-A grade-1 immature teratomas. In cases where treatment needs to be personalized, discontinuation of drug treatments until the end of pregnancy increases the recurrence rates, but its effect on survival does not change. There is no need for treatment in early stage sex-cord stromal neoplasia, but treatment is controversial if it is advanced stage (54-57). 1/3 of borderline ovarian neoplasms are detected during childbearing years. These borderline tumors constitute 20 percent of all ovarian malignancies (58). It is usually diagnosed incidentally during pregnancy (59).

Due to hormonal changes in pregnancy, increased epithelial atypia, increased mucin production, microinvasion and proliferation are seen more frequently in pathologists' examinations. It was observed that these pathological findings decreased in cases re-evaluated after delivery (60). In borderline ovarian tumors, unilateral oophorectomy, cytology, and exploration during surgery may be sufficient (61). Customization in the follow-up and treatment of gynecological neoplasms during pregnancy will be very beneficial for the patient.

If screening tests are used in appropriate conditions, pregnancy results will be positive, and it will also allow early diagnosis and treatment. Ultrasound is the 1st test for the diagnosis of pelvic masses (62). When malignant ovarian masses are examined in ultrasonography; We definitely see solid areas, tumoral borders are irregular, there are septations, we can see papillary projections, bilateral masses can also be seen, tumoral blood flow and intra-abdominal pelvic fluid increase in Doppler (63). Non-contrast magnetic resonance (MRI) can be used to better visualize some pelvic masses (63).

5. Premalignant Vulva Lesions in Pregnancy

During pregnancy, vulvar hyperpigmentation, vulvar edema, vulvar venous pathologies often occur depending on age and hormonal status. But these changes in the vulva usually disappear after birth. The most common vulvar lesion in pregnancy is vulvar intraepithelial neoplasia (VIN) caused by HPV. Laser and excisional vulvectomy, which are used outside of pregnancy, can be used in the treatment of these neoplasms. Because they are teratogenic, the use of podophylline and imuquimod is not recommended during pregnancy (64). The situation that should be considered in surgical interventions performed during pregnancy is that bleeding complications are more common due to pregnancy-related increase in blood supply (65,66). Radiotherapy is not recommended during pregnancy.

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If there is no lymph node involvement in vulvar neoplasia, pregnancy is continued, if lymph node involvement is present, the patients are treated as if they were not pregnant. In patients with lymph node involvement, radiotherapy is started immediately, regardless of the week of pregnancy. Only in pregnant women who are in the last trimester, delivery can be carried out immediately and treatment can be started. The mode of delivery should be cesarean section to prevent recurrence and to preserve the integrity of the existing lesion (67,68).

6. Premalignant Vaginal Lesions in Pregnancy

Vaginal intraepithelial neoplasms (VaIN) are very rare in normal women, and very rare in pregnant women. Diagnosis, follow-up, and treatment are the same as for non-pregnant women, but differ in the surgical procedure and vaginal applicability of delivery after surgical excision (69). A biopsy should be taken before VaIN treatment and if the biopsy result from the lesion is a low grade lesion, HPV test and cervical cytology should be checked. It is absolutely necessary to exclude whether there is any other accompanying anogenital lesion. If there is a high-grade lesion pathology, it is very important that the pathologist decides whether there is invasion or not. If there is no invasion, the treatment is done after the birth week is completed and when labor takes place. In postpartum surgery, it is necessary to closely monitor both the mother and the fetus during pregnancy (70). In pregnant women, imiquimod and 5-Fluorouracil are not used because of the risk of teratogenicity (71).

We can say that the probability of progression of VaIN to invasive neoplasia is 7-8 percent. VaIN lesions seen as unrelated to CIN/VIN have a higher rate of spontaneous regression when compared to VaIN associated with CIN/VIN. For this reason, since complete regression and recovery can occur with close follow-up without treatment, observation and follow-up after biopsy is much more recommended. Vulvar and vaginal intra-epithelial neoplasms are very rare in pregnant women. Cervix and anal region examinations of the patients should be done based on all studies, and the treatment of each patient should be individualized by taking into account the week of pregnancy and the course of pregnancy.

7. Gestational Trophoblastic Tumors

Gestational trophoblastic diseases (GTH) are a heterogeneous group of lesions originating from abnormal proliferation of trophoblasts. Fetal tissues constitute the pathogenesis of GTD, therefore they cannot originate from maternal tissue. Gestational Trophoblastic Neoplasia (GTN) indicates the formation of a group of malignant neoplasms as a result of abnormal proliferation of trophoblastic tissues.

Non-molar (Hidatiform mole) may also occur as a result of pregnancy. Histologically, they are divided into 4 groups; 1-Invasive mole, 2-Choriocarcinoma, 3-Placental Localized Trophoblastic Tumors, 4-Epiteloid Trophoblastic Neoplasms (72). GTN can also occur after live birth (73). The incidence of GTN is not known clearly due to lack of data. Approximately half of GTN cases may be caused by molar pregnancy, approximately 1/3 may be caused by ectopic tubal pregnancy and abortion-related pregnancies, and the remaining 1/3 by normal pregnancy (74). Molar tissue or choriocarcinoma is encountered when GTN develops after molar pregnancy, rarely placental trophoblastic neoplasms or epithelioid trophoblastic neoplasms. Local invasion and metastasis can be seen in patients after complete hydatiform. Metastasis in partial hydatiform mole is seen in 4% of patients (75). GTN developing after hydatiform mole is usually observed as choriocarcinoma, while trophoblastic tumors located in the placenta or epithelioid trophoblastic tumors can be seen rarely.

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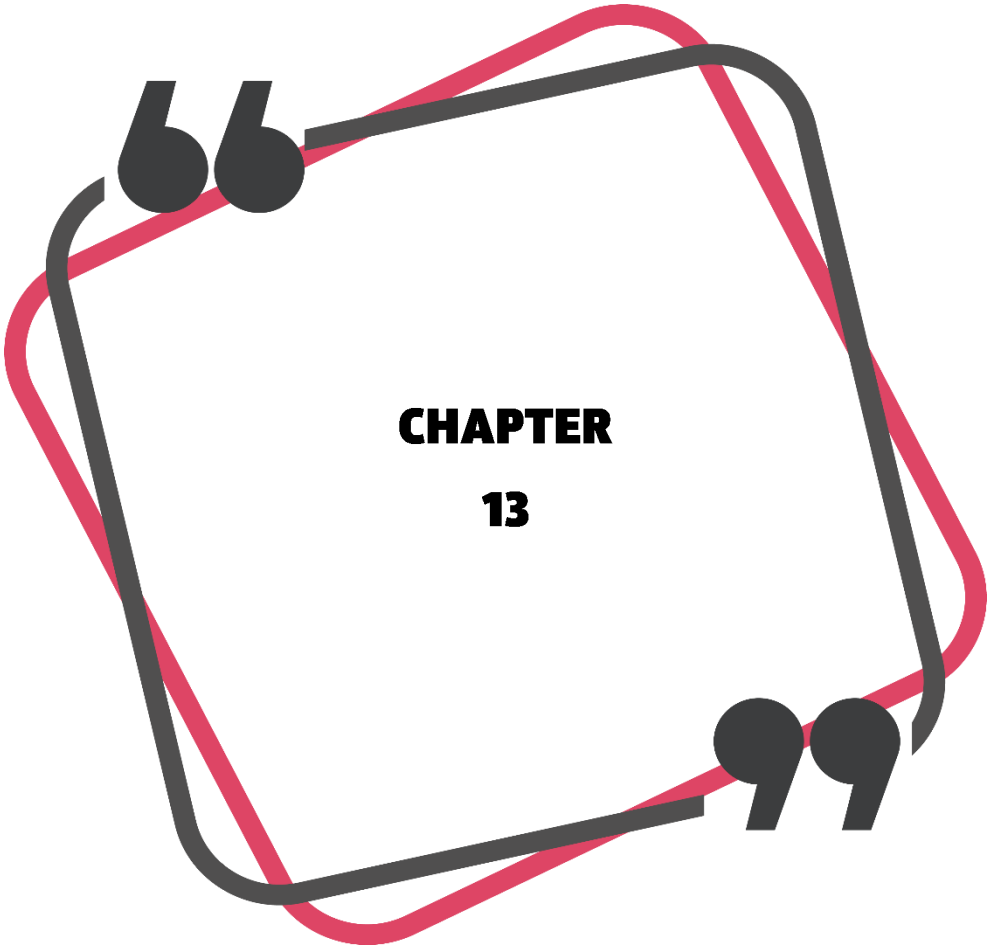
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CHAPTER
13

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THE ROLES OF CURRENT PEPTIDE AND PROTEIN-BASED HORMONES IN THE DEVELOPMENT AND FOLLOW-UP OF DIABETIC RETINOPATHY

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Introduction

Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disease accompanied by chronic hyperglycaemia [1]. Among the main causes of hyperglycaemia are genetic and environmental factors are involved. DM is caused by the absence of insulin secreted by pancreatic cells, or insulin resistance that develops against insulin despite the presence of insulin [2]. Although there are different forms of DM, such as type 1, type 2 and gestational diabetes mellitus (which occurs only during pregnancy), the main type 1 and type 2 DM cause significant health problems and many complications in all societies. 1.5-2% of the world's population is diabetic. There are an estimated more than 2 million diabetes patients in our country [3]. One of the most important complications caused by DM is diabetic retinopathy, which causes blindness [4].

Diabetic retinopathy

Diabetic retinopathy (DR) is an eye disease caused by changes in the retinal blood vessels and is one of the main causes of blindness [5]. DR is a preventable and / or treatable disease that is often seen in the 20-65 age group [3,6]. It has been reported that the incidence of DR is 23% in those with Type 2 diabetes between 11-12 years, while the incidence of DR is 60% in those with diabetes of 16 and above. 30-50% of those with diabetes mellitus have proliferative diabetic retinopathy [7]. It has been reported that those with DR have a 25 times greater risk of blindness than the general population. The cause of blindness is usually due to tractional retinal detachment or intracellular bleeding that cannot be resolved or diabetic macular edema [3]. It is estimated until 2045, the number of adults with DR, vision-threatening DR, and diabetic macular edema patients will be 161 million, 45 million and 29 million respectively [8].

Types of Diabetic Retinopathy

Currently, 19 lesions in diabetic retinopathy have been typified using standard fundus photos, which are, classified by grading separately [9].F Therefore, diabetic retinopathy is studied in two groups as Non-Proliferative Diabetic Retinopathy (NPDR) and Proliferative Diabetic Retinopathy (PDR [10,11]. While lesions in the NPDR circuit are limited only within the retina, there are signs of progression into the vitreous in addition to retinal lesions in the PDR circuit. NPDR is also studied in 4 subgroups: mild, moderate, severe and very severe [12, 13].

Mild NPDR: It is the onset of retinopathy. Microaneurysms and a small number of small retinal hemorrhages can be seen.

Moderate NPDR: The number of microaneurysms and/or retinal hemorrhage has increased and is common in at least one of the four retinal quadrants. Soft exudates, venous nodules and intraretinal microvascular anomalies (IRMA) have emerged.

Severe NPDR: Widespread intraretinal microvascular anomalies, microaneurysms, hemorrhages, venous changes have occurred. Soft exudates are also detected. Hemorrhages and microaneurysms have reached detectable levels in all retinal quadrants, venous calibration changes have reached detectable levels in at least two retinal quadrants, and IRMA has reached detectable levels in at least one retinal quadrant.

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Very Severe NPDR: In addition to the severe NPDR picture, widespread arteriolar obstructions, soft exudates, venous changes, and intense intraretinal microvascular anomalies have become dominant.

Proliferative Diabetic Retinopathy (PDR)

New vascular formation and co-proliferation of fibrous tissue have occurred on the retinal surface and/or optic disc. Neovascularizations are observed on the retinal surface, especially on the temporal quadrant and optic disc. The peculiarity of these neovascularizations is that they are surrounded by fibrous tissue with the ability to contract. In order to diagnose PDR, it is necessary to have the formation of new vessels on the retinal surface and/or optic disc and the proliferation of fibrous tissue together. PDR is grouped into two subgroups as Early PDR and High-Risk PDR. Early PDR is characterized by retinal neovascularizations and minimal fibrous tissue proliferation. High-Risk PDR: Neovascularizations have progressed to the vitreous and the accompanying fibrous tissue has become evident. They are also accompanied by preretinal and vitreous hemorrhages [3].

The Pathogenesis of Diabetic Retinopathy

Ischemic retina is formed in this disease. It leads to the formation of new vessels in the ischemic retina. These newly formed vessels stimulate the release of angiogenic substances. Thus, the neovascular tissue originating from the peripheral venules of the ischemic retinal area proceeds between the surface of the retina and the vitreous. In the initial stage, the vascular structures under the internal limiting membrane swell into the vitreous by piercing the membrane in the following periods. Thus, the vessels developing on the optic disc and the absence of an internal limiting membrane in the optic disc region progress to the vitreous in a short time [14]. These biochemical events activate a large number of circulating molecules. In particular, high circulating glucose concentrations are the primary trigger of diabetic retinopathy [15-18]. Because the results of the Diabetes Control and Complications Working Group and experimental studies show that good glucose control reduces the complications of diabetes. For example, if the values of glycolyzed haemoglobin (HbA1c) are above normal (above 6.5), the risk of developing retinopathy is 1.3 times higher [19, 20]. Glucose control is not only under the control of insulin or glucagon. In recent years, it has been reported that a large number of newly discovered peptide-structured molecules keep glucose under strict control [21, 22]. The general outlines of these peptides that provide glucose control will be discussed and their relationship with diabetic retinopathy will be revealed.

Alarin and diabetic retinopathy

Alarin (25 amino acids) is a peptide molecule and its mRNA has been detected in mouse brain, thymus and skin [17,23-26]. This peptide is a molecule with strong and dose-dependent vasoconstrictor and anti-edema activity in the cutaneous microvascular system. Alarin (1-25) has been reported to inhibit the formation of inflammatory edema in a dose-dependent manner at picomolar doses when injected locally simultaneously into the cutaneous microvascular system [26]. In a study of patients with diabetic retinopathy, plasma, aqueous alarin levels compared to the control group was significantly higher cruising and, therefore, it might play a role in the pathophysiology of diabetic retinopathy and has the capacity to be a target molecule for the treatment of diabetic retinopathy [17].

Apelin and diabetic retinopathy

Apelin is a peptide hormone. This hormone is the endogenous ligand of the G protein-coupled orphan apelin receptor (APJ) [27]. It has been isolated from the gastric juice of cattle for the first time [28]. Apelin has many physiological functions such as regulating the cardiovascular system, providing fluid homeostasis and suppressing apoptosis. In addition, apelin also has roles in the proliferation of retinal endothelial cells and the process of angiogenesis. Apelin level increases significantly in the DR group. In particular, it increases more in PDR. The increase in apelin in DR gives hope in differential diagnosis and follow-up [29, 30].

Ghrelin and diabetic retinopathy

Ghrelin is released from PD1 cells of the fundus region of the stomach [31]. It is a peptide hormone with 23 amino acids known to contain fatty acids (8-carbon or 10-carbon fatty acids). The fatty acid binds to the 3rd serine amino acid at the N-terminal end. Ghrelin attached to fatty acid is known as acylated ghrelin, if not bound to fatty acid, des-acylated ghrelin. This hormone is the hunger (appetite) hormone. It starts the eating. Their amount increases before meals and decreases after they are full [32]. The circulating amounts of ghrelin are higher in thin individuals compared to overweight individuals. Ghrelin contributes to the healing of diabetic retinal damage. Ghrelin has anti-inflammatory, antioxidant and antiapoptotic effects and is a molecule that has the potential to benefit in the treatment of many eye diseases such as cataracts, uveitis and glaucoma [33]. In a study, it was reported that ghrelin reduces the production of reactive oxygen spe-

cies (ROS), inhibits cell apoptosis and also the apoptosis of retinal cells in diabetic rats, and therefore has the potential to provide benefits in the treatment of diabetic retinopathy [33].

Glucagon-like peptide-1 and diabetic retinopathy

Glucagon-like peptide 1 (GLP-1) is a 30-amino acid and small intestine epithelial endocrine L cell-produced peptide hormone [34] that plays a role in the regulation of glycaemia and stimulates glucose-dependent insulin secretion [35], proinsulin gene expression, and β-cell proliferative and anti-apoptotic pathways, as well as inhibiting glucagon release. GLP-1R expression is synthesized in human eyes in very small amounts [36]. Glucagon-like peptide-1 receptor agonists (GLP-1 RA) have certain properties to reduce microvascular events [37]. Both observational and Mendel randomization analyses have been reported to significantly reduce the risk of DR in patients treated with GLP-1 RAs in general, although there are some conflicting reports [38]. Therefore, glucagon-like peptide-1 is an important target treatment molecule.

Neuropeptide Y and diabetic retinopathy

Neuropeptide YY (NPY) is most commonly known for its effect on satiety, food intake and reducing body weight in animals and humans [39]. However, NPY is involved in a wide variety of digestive functions, including regulating insulin secretion and glucose homeostasis. In addition, NPY is a sympathetic neurotransmitter, a potent endothelial-derived angiogenic factor, and a vascular mitogen [40]. NPY is reported to play important roles in diabetic retinopathy and retinal neovascularization [41]. For this reason, it has been reported that NPY is a valuable potential hormone in response to retinopathy medication [41].

Irisin and diabetic retinopathy

The hormone irisin was first discovered in skeletal muscle [42]. It has subsequently been reported to be synthesized in many other biological systems, including the heart muscle and adipose tissue. It is a molecule that consists of 112 amino acids. By exercising, their circulating amount increases. It is a molecule that converts white adipose tissue into brown adipose tissue, causing heat release instead of energy production, leading to weight loss in the organism [43]. Irisin

levels decrease in patients with type 2 diabetes [44]. There is a negative correlation between irisin and HbA1c and LDL levels [45]. Levels of this hormone decrease in the presence of diabetic retinopathy [46]. It has been reported that the decrease in irisin hormone in the presence of diabetic retinopathy has a role in the physiopathology of this disease and may also be an important biological marker in revealing the development of DR.

Vascular Endothelial Growth Factor and Diabetic Retinopathy

Vascular Endothelial Growth Factor (VEGF) is a signaling protein produced by cells stimulating vasculogenesis and angiogenesis [47]. The main biological task of VEGF is to form new blood vessels during embryonic development and new vessel after blood vessel damage. In addition to these biological functions, they have been reported to have roles in vascular homeostasis, cell proliferation, apoptosis and inflammation. VEGF's serum concentration has been reported to be high in diabetes [48]. Recent studies have shown that VEGF is involved in retinal exudation, hemorrhage, and retinal neovascularization, and that changes in level may be associated with the emergence and development of DR [49,50]. Therefore, it is a new promising agent for the prevention and treatment of type 2 diabetes and DR.

Conclusion

Although the main pathological changes of diabetic retinopathy are retinal hemorrhage, exudation, microvascular dilatation and neovascularization, its specific pathogenesis has not yet been fully elucidated. However, in recent years, as mentioned above, apelin, alarin, glucagon-like peptide-1, ghrelin, neuropeptide Y, irisin and VEGF molecules have been associated with diabetic retinopathy. These molecules show promise in both the diagnosis and follow-up of diabetic retinopathy.

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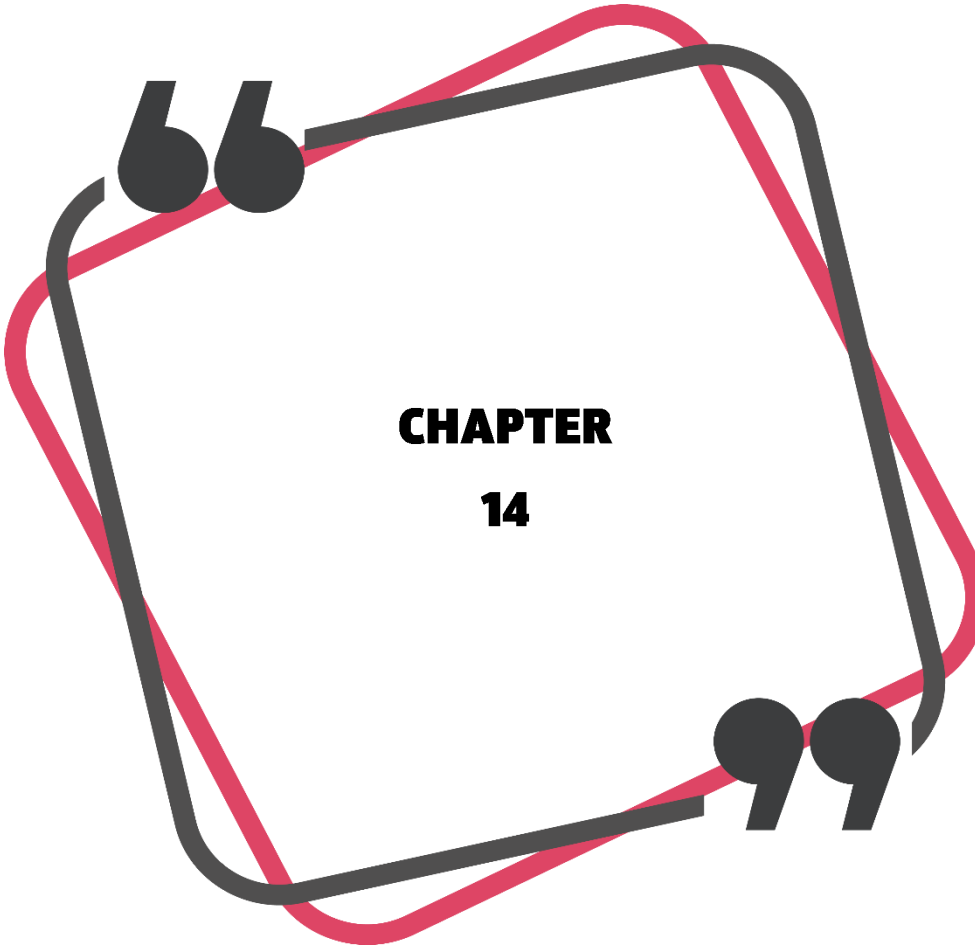
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CHAPTER
14

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OCULAR BEHÇET'S DISEASE AND CURRENT LABORATORY MARKERS OF INFLAMMATION

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Introduction

Ocular inflammation in ophthalmology is one of the most common causes of visual loss worldwide, the search for markers of inflammation for early diagnosis and prevention of loss of visual function has increased in recent years. Behçet's disease (BD) has a significant place among inflammatory diseases. Behçet's disease is a chronic, inflammatory pathological condition characterized by vascular inflammation that can affect all organs and systems, including the eye. In this disease, vision loss occurs since the retina does not receive enough oxygen as a result of blockage of the retinal vessels in the eyes [1-3].

Epidemiology

BD occurs in every race and country in the world [2,3]. However, when we compare the two hemispheres (South and North) with each other, it is most common in the northern hemisphere countries. Therefore, the countries where the disease is most prevalent are Turkiye, Iran, Japan, China, and Korea. It is rare in Italy, Portugal, Algeria, Tunisia, and Morocco and very rare in the USA and the UK. In other words, a prevalence of 20-420/100.000 in Türkiye, 20/100.000 in Saudi Arabia, 17/100.000 in Iraq, 80-100 /10.000 in Iran, 7.1/100.000 in France, 14/100.000 in China, 10/100.000 in Japan, 1. 53/100.000 in Portugal, 3.8-15.9/100.000 in Italy, 2.3-4.9/100.000 in Switzerland, 0.27-0.64/100.000 in the UK, and 5.2/100.000 in the USA are reported [2,4-7].

Etiopathogenesis

Although age, gender, infectious, psychological, genetic, and immunologic factors have been suggested to play a role in the etiology of BD, the underlying mechanisms have not yet been fully elucidated. However, in recent years, studies have focused more on markers of inflammation. These markers of inflammation have been implicated in elucidating the etiopathology and early diagnosis of the disease [1].

Diagnosis of Behçet's Disease

Although many laboratory studies on BD have been conducted in recent years, there is still no specific laboratory data that can help in the diagnosis [1]. The diagnosis of BD is based on the clinical findings recommended by the International Study Group in 1990 [8]. The diagnostic criteria of this study group are as follows: 1) First of all, oral aphthous ulcers must be present in the patient. 2) In addition to these oral aphthous ulcers, the diagnosis is made by the presence of at least 2 of the 4 major symptoms, a) Genital ulcers (anal ulcers, spots in the genital area, enlarged testicles and epididymitis in males); b) Skin lesions (papulo-pustules, folliculitis, erythema nodosum, post-pubertal acne not related to corticosteroids), c) Pathergy reaction, and d) Eye inflammations (iritis, uveitis, retinal vasculitis, vitreous), in any shape, size, and number at least 3 times in a year. In recent years, the attached scoring system for Behçet's disease has been used. Ocular lesions = 2, genital aphthae = 2, oral aphthae = 2, skin lesions = 1, neurological symptoms = 1, vascular symptoms = 1, and a positive pathergy test = 1. A score of 4 or above indicates the presence of Behçet's Disease [9].

Diagnosis of Ocular Bechet’s Disease

More than half of those affected by Behçet's disease are reported to have eye involvement. Eye involvement due to Behçet's disease is more common in the male gender and at younger ages (20s-30s) than in females. However, Behçet's Disease can occur at any age. Eye symptoms are not normally an initial symptom of Behçet's disease, but they can appear within a few years after the diagnosis of the disease. Patients complain of eye redness, discomfort, and blurred vision. A biomicroscopic eye examination reveals hypopyon. (BDCAF) is used to assess activated Behçet's disease. For the systemic activity and extraocular findings of BD, the diagnosis is made by detailed ophthalmological best corrected visual acuity, slit lamp biomicroscopy, and binocular indirect ophthalmoscopy for the last 4 weeks [2,10].

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The breakdown of the blood-retinal barrier due to inflammation leads to perifoveal capillary leakage, causing cystoid macular edema (CME) and further macular structural damage. Subsequently, vascular leakage occurs in the optic disc, peripheral retina, and macula associated with this pathologic condition. Ocular inflammation is primarily responsible for the deterioration of visual quality. Therefore, the relationship between biomolecules that play a role in ocular inflammation and diseases, and current molecules with possible roles in possible diagnosis and follow-up will be discussed in the following sections [11,12].

Ocular Behçet's disease and Interleukin 6-8

In addition to angiogenic activities, IL-6 is a molecule that induces angiogenesis and ocular inflammation in the conjunctiva, cornea, iris, retina, and orbit. The ocular effects of IL-6 differ depending on the source of secretion and the site of action. Inhibition of IL-6 activation is a target molecule that may benefit the treatment of some immune-inflammatory diseases such as Behçet's disease. This is because IL-6 is a cytokine that plays an important role in the differentiation of T cells and B cells, antibody production, and the acute phase response. IL-6 has a crucial role in ocular inflammation and angiogenesis in the conjunctiva, cornea, iris, retina, and orbit. Anti-IL-6 targeted immunotherapy has recently been used as an important treatment modality for IL-6-related ocular disorders, provided that IL-6 signaling blockade is the preferred and favorite target [13,14].

Ocular Behçet's disease and Interleukin 18.

Interleukin-18 [interferon (IFN) gamma-inducing factor (IGIF)] is a novel cytokine with a role in T cell helper type 1 (Th1) response (activation) through its ability to induce IFN-gamma production in T cells and natural killer (NK) cells. Subjects in a study with ocular active Behçet's disease and ocular inactive Behçet's disease were compared with healthy control subjects. Interleukin-18 levels were significantly increased in subjects with ocular active Behçet's disease and ocular inactive Behçet's disease; this increase was more prominent in subjects with ocular active Behçet's disease, and according to the receiving operative curve (ROC) data, it has been suggested that it may be a biomarker that has the potential to help activity and diagnosis in ocular active Behçet's disease [15,16].

Ocular Behçet's disease and other important Interleukins

Interleukin (IL)-12 (a Th1 cytokine), IL-27 (an immunomodulatory cytokine), IL-4 (a suppressor of Th1 cell growth), IL-13 (a stimulatory signal for Th2 cytokines), and IL- 33 (an epithelial cell-derived cytokine) were investigated in patients with ocular active Behçet's disease and patients with ocular inactive Behçet's disease. Researchers have reported that IL-13 and IL-33 were higher in patients with ocular active Behçet's disease. They also reported that IL-13 and IL-33 levels were positively correlated with disease activity parameters. On the other hand, they reported that IL-27 was lower in both groups. These interleukins may be involved in the pathology of ocular Behçet's disease. However, one study is not sufficient. Multicenter studies are needed [17].

Ocular Behçet's disease and neutrophil gelatinase-associated lipocalin (NGAL)

Human NGAL, also called Human Neutrophil Lipocalin (HNL), is a lipocalin-2 (lcn2) Lipocalin 24p3, Siderocalin, α1 microglobulin-related protein, which is a 25-kDa glycoprotein consisting of 178 amino acid residues. NGAL was first isolated from human neutrophil supernatants. It is involved in many biological processes, such as infection and inflammation, oxidative stress, cell necrosis, and apoptosis. Thus, NGAL is an acute-phase protein with upregulated expression in different inflammatory processes. When ocular active Behçet's disease patients were compared with healthy controls, it was significantly increased in the circulation of ocular active Behçet's disease patients, suggesting that it may be a biomarker of inflammation in ocular active Behçet's disease, as it has the potential to aid diagnosis [16,18].

Ocular Behçet's disease and Thiol/disulfide

Organic compounds containing a sulfhydryl group of sulfur and hydrogen atoms are called thiol (-SH). Disulfides (-S-S-S-) are the most important class of dynamic, redox-reactive covalent bonds formed between two thiol groups [19,20]. Thiol-disulfide homeostasis is disrupted in many diseases, including inflammatory bowel diseases. A recent study focused on patients with ocular active Behçet's disease and ocular inactive Behçet's disease and reported that disulfide levels, disulfide/ native thiol (NT) and disulfide/total thiol (TT) ratios were significantly increased in patients with OABD and OIBD compared to healthy control subjects. Therefore, it has been suggested that thiol/disulfide homeostasis may be used as a new marker of oxidative stress in ocular Behçet's disease patients [21].

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Ocular Behçet's disease and Cortistatin

Cortistatin is an endogenous anti-inflammatory cyclic neuropeptide produced by immune cells. Cortistatin shows high homology with somatostatin [22]. Cortistatin has been reported to be synthesized in various human immune cells, including lymphocytes, monocytes, macrophages, and dendritic cells. Cortistatin levels correlate with the degree of inflammatory cell differentiation and activation. This molecule also inhibits the production of proinflammatory macrophage-derived factors. In a recent study, cortistatin levels were significantly increased in patients with ocular active Behçet's disease, suggesting that it may be an important biomarker of this disease [23].

Ocular Behçet's disease and Haptoglobin,

Haptoglobin is synthesized in the liver and binds with high affinity to free hemoglobin released from erythrocytes, inhibiting its harmful oxidative activity [24]. A significant increase in haptoglobin levels has been reported between patients with active ocular disease and controls. It was also reported that there was a significant difference in haptoglobin levels between patients with inactive ocular disease and the control group. However, they reported that high serum haptoglobin levels would not be a risk factor for this disease. Possibly, although increased haptoglobin is not a risk factor, it could be introduced as a candidate diagnostic parameter in the future [25].

Ocular Behcet's disease and soluble endothelial protein C receptor

There is a link between inflammation and the coagulation mechanism. Soluble endothelial protein C receptor (PCR) is a protein that regulates protein C anticoagulant and anti-inflammatory pathways. Protein C is a natural anticoagulant [26]. This protein's anticoagulant activity inhibits inflammatory mediators and leukocyte extravasation into tissues by limiting the expression of activated protein C (APC). In a study, it was reported that plasma sEPCR concentrations were significantly higher in Behçet's disease patients compared to controls, and also that there was no statistically significant difference in serum sEPCR levels between patients with and without ocular involvement. However, researchers have suggested that polymorphism studies may be useful in elucidating the pathogenic mechanism of Behçet's disease and its ocular and systemic vascular complications [27].

Conclusion

Ocular involvement in BD is a common pathologic condition leading to blindness. The average frequency of ocular involvement in all BD is 50%, with a visual loss of up to 75%. This condition is more common and more severe in males than in females. Patients complain of eye redness, discomfort, and blurred vision. It is characterized by the presence of nongranulomatous panuveitis and retinal vasculitis with episodes and remissions. There are currently no specific laboratory parameters to diagnose ocular involvement. Furthermore, the laboratory studies mentioned above belong to some specific single-center research groups. Nevertheless, the inflation parameters discussed here are promising. Thus, these parameters may contribute to the improvement of the eye prognosis and the reduction or elimination of ocular involvement with an earlier diagnosis [11,28].

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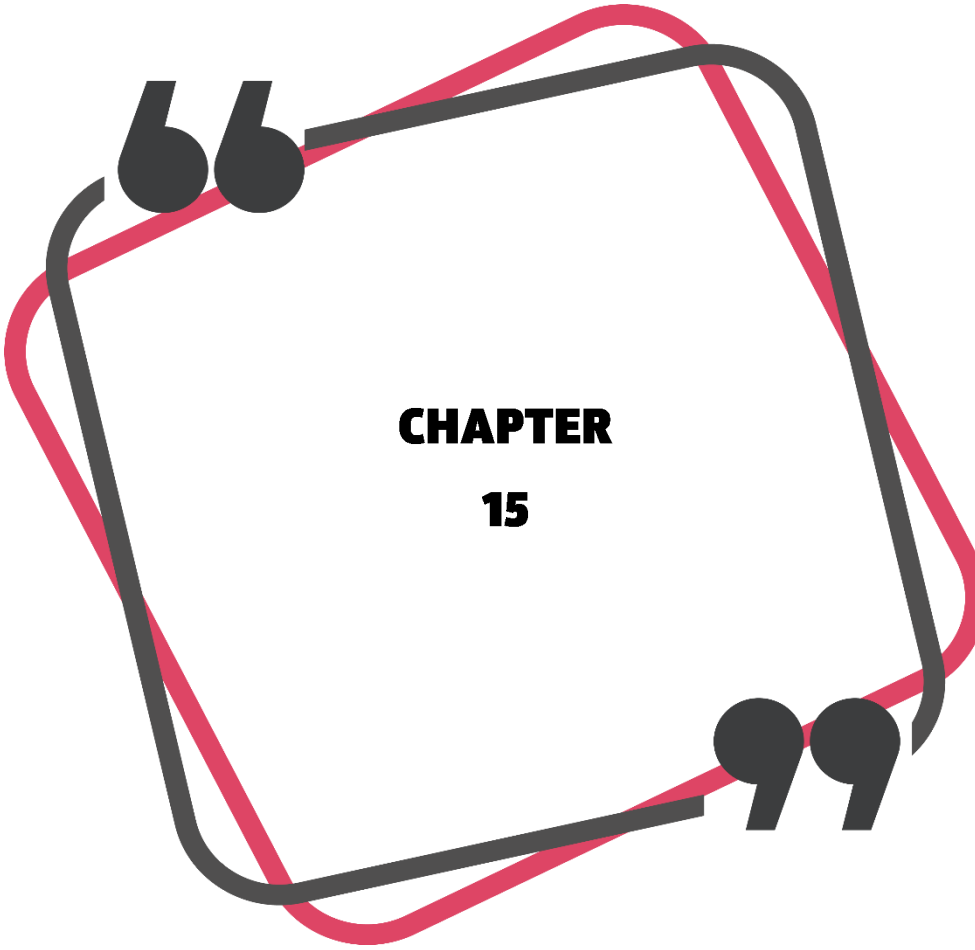
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CHAPTER
15

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COMMON DIABETIC COMPLICATIONS AND ENDOCRINAL AND PLASTIC SURGICAL APPROACHES TO THESE COMPLICATIONS

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disease and a significant public health problem that kills about 6.7 million people worldwide yearly. By 2045, the number of diabetics worldwide will be 780 million (1), and diabetes without treatment will inevitably lead to death and disability from acute and chronic complications. In this study, the complications of diabetes, such as diabetic foot, necrotizing fasciitis, cellulitis, secondary wound infections, Dupuytren's contracture, diabetic mastopathy, etc., will be discussed in light of current information, which requires a multidisciplinary approach.

Diabetic complications

Diabetic Foot

Diabetes, with its macrovascular and microvascular complications due to chronic hyperglycemia, is a disease with high morbidity and mortality. Unfortunately, no definitive treatments can reverse or completely cure organ damage (2). Microcirculation, muscle, and calcium metabolism are impaired in the diabetic foot and are among the most common complications (3). The Global Burden of Disease Study (4) reported that approximately 131 million people (34% of all people with diabetes) developed diabetes-related lower extremity complications. Of these, 105.6 million had diabetic neuropathy, 18.6 million had foot ulcers, and 6.8 million had undergone amputation. It was found that 84% of lower extremity amputations previously had foot ulcers, which are still insufficiently preventable (5). Some known risk factors for developing diabetic foot ulcers include peripheral arterial disease, peripheral neuropathy, repeated trauma, previous ulceration, and/or amputation. Other risk factors include male gender, long duration of diabetes, and low socioeconomic level (6).

Treatment

Metabolomics consists of analyzing a large number of small molecules called metabolites. Developments in metabolomics have been widely used in diagnosing and treating diabetes, and their beneficial effects have been noted. In studies, leucine, arginine, threonine, and glutamine have been used to evaluate amino acid metabolism disorders in diabetic feet. In contrast, β-hydroxy-β-methyl butyric acid has been used in fatty acid metabolism disorders. Successful results in animal studies must also be confirmed in humans. In addition, many factors influence metabolism. Changes in these factors lead to differences in the metabolites produced. Nevertheless, the future of metabolomics in the study of diabetes is promising (1).

Dracorhodin perchlorate (DP), at the research level, supports wound healing by increasing the proliferation of rat fibroblasts. Prolonged inflammation duration and inflammatory cytokines decreased when treated with DP. It was reported to promote wound healing by increasing ENOS protein response and NO content. As a result, it has been reported that DP can be developed as an adjuvant treatment for diabetic wounds (7).

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Treating diabetic wounds is a problem that cannot be solved quickly in plastic surgery, as in other branches of science. To solve this problem, the use of biocompatible and biodegradable biomaterials as scaffolds or dressings composed of various active substances or cells has become an important method to create a wound repair system integrating material cells and growth factors. In addition, stem cells and exosomes secreted into wounds regulate immune response and inflammation, stimulate angiogenesis, and regulate collagen remodeling, inhibiting scar hyperplasia while accelerating skin cell proliferation and re-epithelialization. Due to economic difficulties, it is anticipated that more materials from the research phase may be used in clinical practice and that biomaterials may be used in the future (8).

Diabetic foot ulcers are one of the most critical and dangerous complications of diabetes. In more than ¼ of these patients, lower limb amputation may occur within 6-18 months of wound occurrence (9). There are pretty complex mechanisms involved in wound healing. Infection is among the factors that negatively affect the depth and size of the wound and the healing process. Adequate and balanced nutrition is essential for wound healing. Malnutrition is common in chronic diabetic foot patients (10), and micronutrient (vitamins, minerals) deficiencies are common. This increases the risk of amputation (11). A deficiency of polyunsaturated fatty acids and a decrease in essential fatty acid derivatives are some conditions that occur in diabetic foot ulcers and negatively affect wound healing. Therefore, supportive nutritional therapy is essential. Evidence for supplementation of proteins, amino acids, omega-3, probiotics (yogurt, kefir, some unpasteurized pickles), vitamins (C, D), and trace elements (zinc, copper, chromium, selenium, magnesium, etc.) in wound healing malnutrition has been evaluated. The treatment of diabetic foot ulcers requires multidisciplinary care. In addition, although the effect of nutritional interventions on wound healing is not established, more extensive studies are needed to clarify the effects of nutritional supplements on foot ulcer healing (12).

Non-healing diabetic foot wounds are a significant public health problem affecting patients' quality of life. In general, diabetic wounds are a pathological condition associated with a lack of chemokine production, impaired immune response, inadequate epithelialization of angiogenesis, and loss of fibroblast function. Inappropriate conversion of fibroblasts to myofibroblasts decreased myofibroblast activity, and inadequate production of an extracellular matrix plays a role in non-healing wounds. From this point of view, myofibroblasts are very important for tissue repair. Studies have shown that a hyperglycemic state affects metabolic pathways and impairs wound healing. In contrast, studies on the effects

of fibroblast differentiation and myofibroblast functions on metabolic pathways continue (13).

In treating diabetic ulcers caused by peripheral sensory neuropathy and repetitive trauma, ulcer evacuation is one of the factors that have the most significant impact on healing. For this purpose, using a full-contact patch after discharge is the gold standard (14).

As one of the microvascular complications of diabetes, quality of life and life expectancy decrease in the diabetic foot. In this regard, mesenchymal stem cell exosomes (MSC-exos), one of the new treatments, are of low immunogenicity and have repair functions, as well as mesenchymal stem cells (MSCs) with efficacy. It is known that some MSCs used in diabetic wounds originate from bone marrow and are involved in the regulation of vascularization and inflammation (15). In contrast, some originate from adipose tissue and are involved in vascularization (16). Wound healing in diabetic ulcers consists of hemostasis, inflammation, proliferation, and remodeling. Healing is delayed if any of these steps are impeded (17). When wound healing is blocked by infection, peripheral neurovascular disease, hypoxia, and neuropeptides, medical costs increase, and hospital stay is prolonged. The rate of diabetic foot in patients with type 1 DM is similar to that in patients with type 2 DM, with a rate of 20%, and more than 15% of them are at risk of amputation (9). Therefore, MSC exos, with a similar mechanism of action as MSC2, support wound healing by regulating the inflammatory microenvironment in the wound, providing vascularization and antioxidant apoptosis (18). Studies have shown that M1 macrophages increase the proinflammatory cytokines interleukin-1β (IL -1β) and tumor necrosis factor-α (TNF-α), M2 macrophages have been shown to produce anti-inflammatory cytokines transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) (19). In this regard, macrophage polarization is necessary for inflammation. In diabetes, a chronic disease, the number of M1 macrophages is higher than that of M2 macrophages (20), which increases the number of proinflammatory cytokines and delays wound healing (21). MSC-Exos, on the other hand, accelerates healing by increasing the M2/M1 ratio (22). It regulates the M1/M2 ratio by inhibiting the activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway while inducing the expression of pentaerythritol tetranitrate (PETN), attenuating AKT phosphorylation, and ultimately providing a rapid transition from the inflammatory phase to the regenerative phase (15). Proangiogenic factors play a role in the continuation of vascular growth during the angiogenesis process in wound healing. However, diabetes significantly decreases the level of these factors and delays wound healing (23). Studies have reported that MSC-exos are rich

in circular RNAs, long noncoding RNAs, and microRNAs (miRNAs), which may regulate the expression of reparative genes in wound vascularization and healing. Exosomes from adipose-derived mesenchymal stem cells modified with mmu-circ-0000250 have been reported to support autophagy activation to inhibit cell apoptosis in a high glucose environment, promote wound vascularization through miR-128-3p adsorption, and accelerate the healing of diabetic wounds (24). Other studies have found that the same effects are achieved through other pathways (25). In addition, studies have found that combining MSC-Exos with hydrogel materials is more effective for vascularization and wound closure than alone (26). It is believed that MSC-Exos will be a new effective biologic agent for treating ulcers.

Charcot foot is defined as one of the chronic diabetic foot complications associated with metabolic and neuroarthrodegenerative disorders that make limb salvage difficult. Peripheral arterial disease and severe deep infections also contribute. Today, surgical interventions or corsets are still among the valid methods, and new surgical reconstruction methods are among the new treatment modalities. Apart from these, irradiation of the midfoot in Charcot foot is a new option. This load distribution device can be applied surgically using an intramedullary method. It is used in surgery to increase durability with this radiation. The study reported that irradiation makes new contributions to the surgical method (27.)

In addition, the risk of re-amputation after primary amputation is high in treating diabetic feet. In this regard, tendon compensation and intrinsic muscle contractions suitable for the residual limb are among the techniques (28). As in other surgical departments, collagen and collagen-based materials have been successfully used in plastic and reconstructive surgery for diabetic ulcers and other wounds (29).

Dupuytren's Contracture

Although the exact cause is unclear, it is defined as a fibroproliferative disorder of the hands resulting from local hypoxia and chronic ischemia. Factors responsible for the etiology include alcohol and cigarette use, advanced age, male sex, menopause, diabetes mellitus, and certain anticonvulsants (30). In Dupuytren's disease, average fascial bands, which may involve the palmar, palmodigital, or digital regions, transform into fibrotic cords (rayan GM, 2007). The prevalence is 8% worldwide, and no definitive treatment exists (Salari N, Heydari M, 2020). Dupuytren's contracture is known to be affected by multiple genes. Twenty-six

gene regions have been defined in the Genome-Wide Association Study (GWAS) (31). Heritability was reported to be 80%.

The benign fibromatous disorder of the palmar and digital fascia usually first appears as a fibrotic nodule in the 4th or 5th metacarpophalangeal joint. As the disease progresses, the nodule enlarges and forms the pathologic condition causing flexion contracture in the metacarpophalangeal and proximal interphalangeal joints (32). The epidemiology and pathophysiology of the disease are not yet known. Under the influence of stress, local fibroblasts transform into myofibroblasts by transforming growth factor β-1 (TGF-β1). These cells form strong intercellular junctions in individuals with high levels of type 3 collagen and attach to collagen fibrils in the nearest fascia (33). Myofibroblasts contract under stress, resulting in Dupuytren's contracture. In healthy individuals without risk factors, the myofibroblasts apoptosis, and the contracture resolves. However, apoptosis does not occur in at-risk groups for unknown reasons, and the contracture continues progressing (34, 35). Dupuytren's contracture has a wide range of symptoms. It is also a disease that can progress with mild to severe signs of disease. The more aggressive the progression of the disease, the higher the rate of relapse and contracture-related disability during follow-up. Recurrence can occur at any time, regardless of treatment.

Treatment

Although several surgical methods exist to treat Dupuytren's syndrome, fasciotomy is still considered the standard method. In addition, studies on TGF-β1 suppressors are currently being investigated. The ideal surgical time is still one of the controversial issues. Treatment is usually aimed at surgical correction of the narrowing. For this, there are 3 surgical methods with their advantages and risks. These are fasciectomy, percutaneous needle fasciotomy, and collagenase fasciotomy. Although not very rare in iatrogenic recurrence, complications include the treatment method chosen, inadequate fasciotomy, or hyperlaxity of the extensor mechanism.

Necrotizing fasciitis

This rare infection may spread rapidly, affecting the subcutaneous tissues and fascia and covering the entire limb within hours (36). If not diagnosed and treated promptly, the risk of death may be high. It is a disease that can be associated with muscle destruction. First and foremost, treatment requires extensive debridement

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as soon as possible and reconstructive surgical procedures if necessary. After that, it is essential to continue debridement at regular intervals.

Etiologies include surgical procedures, immunosuppressive conditions, corticosteroid use, and diabetic wounds (37). The presence of additional conditions (DM, hypertension, malignancy, immunosuppression, peripheral vascular disease) increases the risk of death. Different types of necrotizing fasciitis have been defined based on microbiological cultures (e.g., type-1, type-2) (38). Although it depends on the culture result, broad-spectrum antibiotics are usually used for gram (+), gram (-), and anaerobes. However, cases of septic shock with multiorgan failure have been reported in which arbitrarily initiated broad-spectrum antibiotics obscured the diagnosis of necrotizing fasciitis because of ulcerated wounds. Therefore, it should be remembered that the severity of the underlying pathology may be important. Early debridement of all affected tissues with large surgical incisions is the basis of treatment. Necrotizing fasciitis is an infection with high mortality in patients with diabetic feet. Therefore, early diagnosis and treatment are of great importance.

Infection involving the perineal, perianal, and genital regions is called Fournier's gangrene, and the approach is the same as for necrotizing fasciitis. Again, it should be noted that the risk of death is high here despite a serious treatment approach.

Diabetic mastopathy

The incidence of the lesion, also called lymphocytic mastopathy, fibrocystic mastopathy, or fibrocystic breast degeneration, first described in 1984, is reported to be 0.6-13% (39). It is characterized by lobular atrophy with fibrous foci and lymphocyte clusters with the periventricular and peritubular distribution. It can also occur in type 2 DM, autoimmune diseases, and healthy individuals, especially type 1 DM. The pathogenesis of the disease is still unknown, and breast cancer is the most important differential diagnosis (3). In breast lesions, infiltration of B-cell lymphocytes and autoantibodies is associated with immune responses mediated by especially HLA-DR 3, 4, and 5 antigens (4). The extracellular matrix is expanded due to hyperglycemia and increased collagen overproduction (6). Causes include exogenous insulin ingestion and anti-insulin antibodies (4). It has also been reported to occur in healthy individuals (1, 8). The lesions are approximately 0.5 cm in diameter and are usually located in the upper lateral/medial aspect of the breast (76%) in a white-gray color (9). The basement membranes of the ducts and lobules may be markedly thickened. In addition, lobules are small

and sparse. The lesions contain a keloid collagenous stroma with more stromal spindle cells than the surrounding breast tissue (41). The stroma has fibroblast and myofibroblast proliferation and abnormal collagen deposition (9). In its pathology, B-cell-rich lymphocyte infiltration mimics low-grade B-cell lymphoma (MALT lymphoma). There is an average of 20 years between the diagnosis of diabetes and mastopathy. Women are in premenopause (9). In the differential diagnosis, it is essential to note that axillary lymphadenopathy is not usually associated with mastopathy (14). Asymmetric compaction on mammography should exclude malignancy (18). In this regard, contrast-enhanced MRI partially helps to distinguish diabetic mastopathy from breast carcinoma (14), but unfortunately, it is not sufficient to distinguish malignant from benign in some lesions (42). A core biopsy is usually performed for diagnosis, and if the pathology result is inadequate, excision of the entire lesion may be required (10).

Treatment

A conservative approach and follow-up are provided in asymptomatic patients, whereas treatment is generally individualized. The surgical method should be carefully planned not to compromise the quality of life (mastectomy with reconstruction or lumpectomy) (39). Mass and breast size, patient expectations, family history, and surgical team experience are essential. The long-term prognosis is unknown and is followed up considering the benign course. Further studies are needed to determine prognostic markers in the future. The best approach for the patient is primarily a multidisciplinary approach through awareness and evaluation of clinical, imaging, and pathologic findings.

Consequently, there is currently no way to eliminate diabetes, and the most effective treatment is lifestyle modification that accompanies the patient's medical treatment. However, surgery (especially plastic surgery) is inevitable despite all this. Treatment methods are not sufficient for the complications of diabetes, and new methods are urgently needed to improve patient's quality of life and life expectancy.

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CHAPTER
16

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A BRIEF HISTORICAL JOURNEY WITH AN UP-TO-DATE PERSPECTIVE ON CATARACT PATHOPHYSIOLOGY AND SURGERY

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Introduction

Cataract, normally transparent, is the opacification of the crystalline lens of the eye (which transmits light to the retina) by losing its transparency (formation of brown and yellow discolorations) . The lens contains a capsule, lens epithelium, cortex, and nucleus. The etymology of cataract comes from the Latin word "cataracta," which means waterfall. Cataract is responsible for 33% of world blindness [1,2]. Decreased visual acuity is the most common symptom of cataract. However, in some cases, depending on the anatomical distribution of the opacities (nuclear, posterior subcapsular, cortical), other symptoms (photophobia, monocular diplopia, myopic shift, change in color vision, etc.) may occur [3]. Moreover, cataracts can occur in both eyes or in one eye, or in some cases, one eye may be more affected than the other. Cataract is a multifactorial disease associated with age, gender, genetic predisposition, tobacco use, diabetes mellitus, medication, or exposure to ultraviolet radiation. In other words, several conditions can lead to the formation of cataract. [4].

Prevalence of Cataract

Approximately 95 million people worldwide suffer from cataract [5]. Some large population studies report that the prevalence of cataract increases with age, ranging from 3.9% among 55–64 years to 92.6% among those 80 years and older [6-8]. In addition, the global prevalence of cataract in adults over 50 years of age was estimated at 47.8% [9]. In 2007, the approximate prevalence of cataract in European adults was 19.3% [10]. Cataract prevalence in adults aged 21 years and older in rural Indonesia is among the highest reported in Southeast Asia [11]. There is an explicit epidemiological study conducted in Turkey that detected the prevalence of cataract. However, the overall prevalence of Pseudoexfoliation Syndrome (PEX) was reported to be 11%. The most common cataract type in the PEX patients was mixed-type cataract, which was determined in 51.2% of patients [12]. In the United States, cataracts affect more than 24 million Americans 40 years of age and older, and the annual direct medical cost of outpatient, inpatient, and prescription services was reported to be approximately 6.8 billion dollars [13].

Historical Journey of Cataract Surgery

Couching in Cataract Surgery

The earliest known method of treating cataracts is couching, which dates back to the fifth century BC. This surgery procedure is coarse and aseptic. However, it is still used in some countries today [14,15].

Extracapsular Cataract Extraction

This type of cataract surgery was performed by Indian eye surgeons around 600 BC. This method is extremely primitive and involves extracapsular cataract extraction (ECCE) in which the lens is removed and the lens capsule is left in place. Until 1747, ECCE was the main procedure in cataract surgery until French surgeon Jacques Daviel introduced a new surgery procedure to the world [15,16].

Intracapsular Cataract Extraction

Intracapsular cataract extraction (ICCE) involves the removal of the lens and the surrounding lens capsule in one piece. Since the lens capsule acts as a barrier between the anterior and posterior chambers, its removal often causes vitreous prolapse and subsequent retinal detachments, among other complications; also,

due to the high infection rate, the use of "Intracapsular Cataract Extraction" as a surgical procedure for treating cataracts was short-lived.

Use of Intraocular Lenses (IOLs= IOLs) in Cataract Treatment

In 1949, Dr. Ridley performed the first IOL operation at St. Thomas Hospital in London. Ridley was highly criticized for putting a foreign object into the eye by the ophthalmologists at the time. Since this lens placed inside the eye led to complications such as glaucoma and frequent dislocations. This method has shed light on advances in modern cataract surgery [17]. In 1978, Kai-yi Zhou implanted the first foldable silicone IOL. The silicone intraocular lens resulted in faster recovery and less infection, thereby finding use in cataract surgery [18].

Current Cataract Surgery

Today, cataract surgeries are mostly performed with a method named phacoemulsification (phaco), in which the inner lens of the eye is emulsified with an ultrasonic hand device and aspirated from the eye. Prior to phacoemulsification, one or more small incisions are made in the eye to allow surgical instruments to enter. The Phaco probe is an ultrasonic handpiece with a titanium or steel needle, often dividing the cataract into two or four pieces. Each piece is emulsified and aspirated by absorption. Followingly, IOL is placed in the posterior chamber. This method requires very little stitching and does not require sutures. Recovery time is usually shorter. However, edema may occur due to fluctuations of macular intraocular pressure in phacoemulsification. Femtosecond Laser Assisted Cataract Surgery (FLACS) has also taken its place in eye clinics for the last 13 years. This method is used to correct corneal astigmatism or in cases where high-tech lenses such as astigmatism correctors (torics) or multifocal or trifocal lenses are worn. It is highly costly and requires more technological equipment.

Pathophysiology of Cataract

Cataract Formation and Glucose Metabolism

Diabetes has turned into an important health problem in the world, and its incidence is increasing day by day. Patients with diabetes mellitus are five times more likely to develop cataracts, which is a major cause of visual impairment. Typical diabetic cataracts contain cortical and/or posterior subcapsular opacities. Aldose reductase (ALR2) is the first and rate-limiting enzyme of the polyol pathway involved in the pathogenesis of chronic complications of diabetes mellitus. The polyol pathway may contribute to the formation of cataracts in a variety

of ways, including sorbitol accumulation, osmotic stress, and oxidative stress. ALR2 catalyzes NADPH-dependent reduction of glucose to sorbitol. Accumulation of sorbitol increases osmotic pressure in lens fiber cells, causing lens swelling, formation of water vacuoles, and eventually irreversible lens opacities. In other words, high glucose levels cause the lens to swell, reducing vision and causing cataracts [19]. In Type 2 diabetes, low-grade oxidative stress associated with chronic hyperglycemia is an important pathogenetic factor for cataract formation [20]. Recent studies report that the process involving insulin autoantibodies developed due to autoimmunity is involved in acute bilateral type 1 diabetic cataracts [21].

Cataract Formation and Galactosemia

Galactosemia is a disorder caused by the deficiency of one of three possible enzymes involved in galactose metabolism: galactokinase, transferase, or epimerase. A deficiency of any enzyme may lead to cataracts through the accumulation of galactitol in the lens. When galactosemia is detected early, starting a non-galactose diet can prevent the development of cataracts by inhibiting the accumulation of lenticular opacities [22].

Cataract Formation and Lysosomal Storage Diseases

Lysosomal storage diseases (LSDs) are a rare form of metabolic disorder that can affect almost any organ of the body. Ocular involvement is rare even compared to systemic involvement in such diseases and poses diagnostic and therapeutic challenges for ophthalmologists [23]. Lysosomal storage diseases include lipid storage disorders, neutral glycosphingolipids, gangliocytoses, leukodystrophies, neutral lipid disorders, mucopolysaccharidoses, mucolipidosis, glycoproteinoses, and lipofuscinosis. In such lysosomal storage diseases, lipid and lipoprotein metabolism disorders associated with enzyme deficiency manifest in the eye in the form of lipid accumulation in the valve, conjunctiva, and corneal arcus. For example, Tay-Sachs and Sandhoff diseases are lipidoses characterized by deposition of GM2gangliosides in neuronal lysosomes and progressive neuronal degeneration followed by β-hexoaminidase deficiency. In GM1 gangliosidosis, the Japanese flag appearance in the macula after β-galactosidase deficiency is reported at 50% in infants [24,25]. Conjunctival vasodilation, increased tortuosity, and aneurysms can be detected, as well as posterior subcapsular cataracts (37%), and increased retinal vessel tortuosity (70%) [26].

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Cataract Formation and Lipid Metabolism

Since the lens membrane is the most cholesterol-rich membrane in the body, cataracts are frequently encountered in cholesterol metabolism disorders. Apart from cataract, lens subluxation is especially common in Marfan syndrome, homocystinuria, Weil-Marchesanni syndrome, sulfide-oxidase deficiency, and hyperlysinemia. The lens subluxation in homocystinuria, especially inferior dislocation, is observed in 90% of cases. It may severely reduce vision and require surgical treatment [21].

Cataract Formation and Metabolic Syndrome

The components of metabolic syndrome include hypertension, hyperglycemia, increased waist circumference, and abnormal cholesterol and triglyceride levels, which are called the fatal quadruple today. Diabetes and hypertension are major metabolic risk factors for cataracts. Obesity and hyperlipidemia are both triggering factors for cataracts. Therefore, it is important to treat metabolic syndrome to reduce or prevent cataract formation [21].

Cataract Formation and Hypertension

A correlation is reported between cataract and hypertension [27]. Available studies report a positive correlation between blood pressure and cataract. In other words, patients with severe hypertension were at higher risk for cataracts compared to those with mild hypertension [28]. Inflammatory markers such as IL-6, TNF-alpha, and C-reactive protein are high in individuals with hypertension, so they are suggested to trigger the formation of cataracts [21].

Cataract Formation and Wilson's Disease

Wilson's disease is an autosomal recessive disease caused by impaired secretion of copper into bile. In other words, the disease causes an abnormal accumulation of copper. The most common symptom of ocular Wilson's disease is the Kayser-Fleisher ring. Sunflower cataract due to Wilson's disease was first described by Siemerling and Oloff in 1922, reporting copper accumulation in the anterior lens capsule [29].

Cataract Formation and Hepatitis

An association is reported between hepatitis B (HBV), hepatitis C (HCV), and cataracts. Associations between hepatitis B (HBV), hepatitis C, and cataracts are

reported to be mediated by high ALT and AST levels in these viral diseases. The study suggested that the association between HBV and cataracts may not be linked to liver damage and may be linked to a mechanism that has not yet been identified. HCV or HCV antigens may have a direct effect on the lens [30].

Cataract Formation and Atopic Dermatitis

Atopic dermatitis is a form of chronic eczematous dermatitis. In atopic dermatitis, the underlying mechanism behind cataract formation may be linked to oxidative stress, corticosteroid therapy, and phototherapy [31].

Cataract Formation and Systemic Lupus Erythematosus

Ocular involvement is common in one-third of patients with systemic lupus erythematosus (SLE). Cataract is the most common ocular pathology in cases with active systemic lupus erythematosus [32].

Cataract Formation and Some Syndromes

Although not very common, some syndromes are associated with cataract formation. For example, some syndromes inherited from Alport syndrome (X-linked or autosomal recessive) [33,34], Marfan syndrome (autosomal dominant) [35,36], neurofibromatosis type 2 (autosomal dominant) [37,38], Down syndrome (trisomy 21) [39] and Lowe syndrome (oculocerebrorenal, X-linked) [40,41] have been found to be associated with epiretinal membranes, retinal, hamartoma myopia, retinal detachment, glaucoma, and early cataract formation. Again, in Hurler syndrome (mucopolysaccharidosis), accumulation of GAG in the optic nerve results in compression of the lamina cribrosa and optic canal and optic nerve edema, which may lead to optic atrophy in later stages [42,43]. In all syndromes, ocular pathologies of varying degrees are frequently encountered, especially in the cornea, lens, retina, and optic nerve, which may lead to vision loss. In addition, opacities are found in the lens in disorders of cholesterol biosynthesis (Mevalonate kinase deficiency, Conradi-Hunermann syndrome [44], Smith-Lemli-Opitz syndrome).

Conclusion

In conclusiın, cataract is a multi-factorial condition associated with age and some metabolic events, in addition to hereditary diseases. Various factors such as Lysosomal diseases, oligosaccharidoses, α-mannosidosis, sialidosis, galactosiali-

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dosis, mevalonate kinase deficiency, Fabry disease [45], neuronal ceroid lipofuscinosis, glucose metabolism disorders, galactosemia, sorbitol dehydrogenase deficiency, lipid metabolism disorders, cerebrotendinous xanthomatosis, cerebrotendinous xanthomatosis, Conradi-Hünermann syndrome, Smith-Lemli-Opitz syndrome, peroxisomal diseases, Zellweger disease, neonatal adrenoleukodystrophy, infantile Refsum disease, and Copper metabolism disorders are involved in the physiopathology of cataracts [44].

There is no preventable method for cataract, which develops with age and metabolic events. However, individuals can protect themselves from cataracts by staying away from tobacco and radiation (e.g sunglasses). In addition, maintaining a healthy diet and an exercise regimen, managing and reducing the effects of existing conditions such as diabetes or hypertension, and getting an early diagnosis can also help to avoid cataracts. However, today, the best way to treat cataracts is surgery performed in experienced eye centers. In fact, modern cataract surgery is highly safe and effective. By doing so, individuals can enhance their quality of life by regaining their vision.

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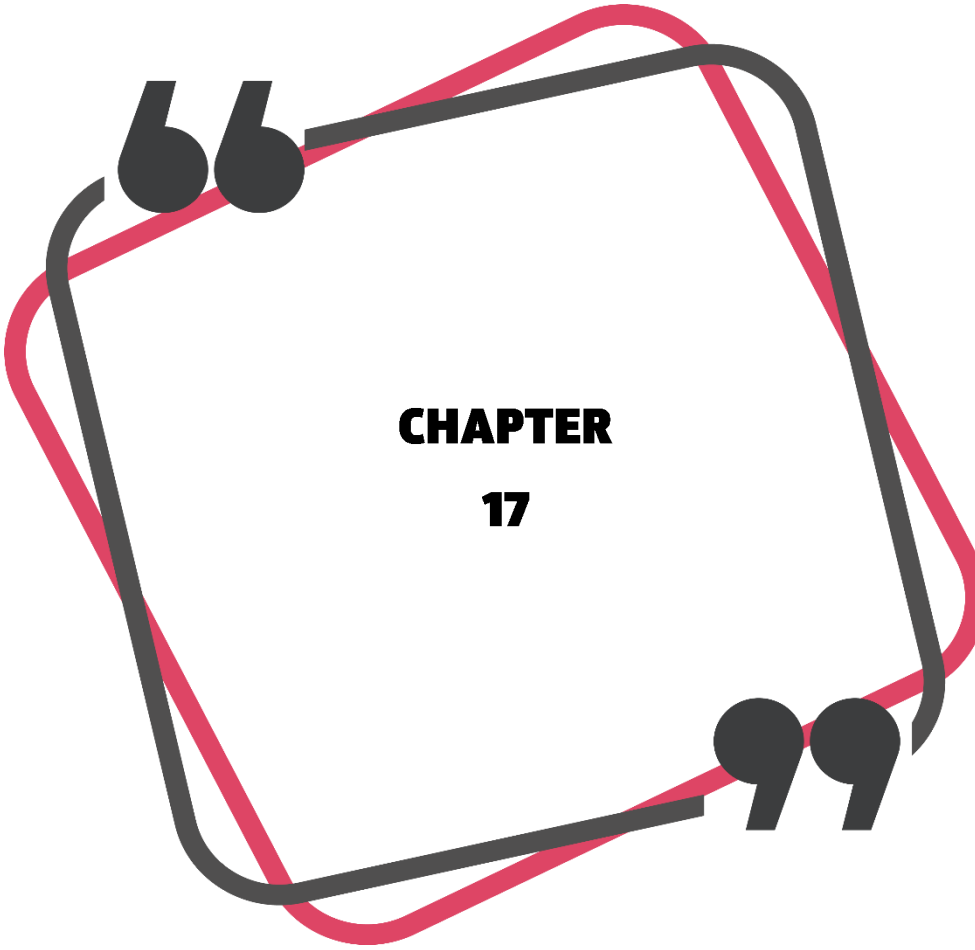
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CHAPTER
17

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THE RELATIONSHIP BETWEEN EXERCISE AND GUT MICROBIOTA IN HUMANS

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INTRODUCTION

Microbiota is an ecosystem composed of 500-1000 naturally occurring species such as bacteria, viruses, and fungi in a specific biological niche [1]. Gut microbiota (GM) can reach up to 1.5 kilograms in a 70-kg person [2]. *Firmicutes* and *Bacteroidetes*, the two bacterial phyla, constitute approximately 80-90% of the bacterial population in the gut [3, 4]. Apart from these phyla, there are also *Proteobacteria*, *Fusobacteria*, *Actinobacteria*, and *Verrucomicrobia* phyla in the intestinal flora [4]. Of the 160 species that make up the GM, only 15-20 are found in every human being. However, since these 15-20 species perform the essential functions of GM, the metabolic activities of the gut are generally steady and similar in healthy individuals. This disparity indicates that the GM varies in number and content for each person [5]. This is because the GM is a dynamic system that can be influenced by many factors [4, 6, 7].

Nutrition, antibiotics and various medicines, immune response, infections, and genetic features are all external and internal factors that influence GM [6, 7]. Exercise benefits a variety of systems, including the musculoskeletal, immunological, and cardiorespiratory systems [8]. It has been shown in recent studies that exercise may be associated with the GM [9-11]. The study by Ehrenpreis et al. [11] examining the relationship of exercise with the colonic mucosa is the first to indicate a link between exercise and GM. Exercise-induced stress may alter GM via influencing the autonomic nervous system [12-15]. The hypothalamic-pituitary-adrenal (HPA) axis, which is activated by stress, plays a critical role in the

regulation of the gut-brain axis [16]. Exercise also modulate GM through influencing the immune system [15, 17], energy metabolism [15], and lactate metabolism [10]. It has also been found that exercise activates the SIRT1 gene, which improves mitochondrial oxidative capacity and gut barrier function, potentially affecting microbiota composition [18]. Nevertheless, excessive stimulation of the HPA axis can result in the release of significant quantities of stress hormones that can cause dysbiosis in the GM [16]. Due to all these effects, exercise brings about changes in the GM diversity and bacterial composition [9, 15, 19]. In addition, it has been demonstrated by various studies that the effect of exercise on the GM may occur independently of diet, which is one of the most significant regulators of GM [20, 21].

Aside from digestion of foods, the GM carries out activities for the production, storage and consumption of energy, regulation of inflammation and hydration [22], neutralization of medicines and carcinogens, protection of the body from pathogens, and improvement of the immune system [13, 14]. It is also claimed that it may have effects on regulating cognitive function and stress response [23, 24]. Therefore, in addition to the influence of exercise on the GM, the GM may also be effective on exercise capacity and performance.

Bacteria convert carbohydrates into short-chain fatty acids (SCFAs) in the intestine. SCFAs are an important part of energy metabolism. They are also effective in strengthening the intestinal barrier and reducing the formation of inflammation [25]. Recent research has revealed that SCFAs may be a significant regulator of physical performance [26]. The GM affects neurotransmitters such as dopamine, serotonin, and GABA via the gut-brain axis, which is mostly mediated by the vagus nerve. By interacting with numerous systems, the GM can send signals to other organs in the body and influence the host [27, 28]. It has been shown that butrate, one of the SCFAs generated during carbohydrate digestion in the intestine, has been demonstrated to affect exercise performance by activating PGC-1α gene activation, which enhances respiratory capacity and fatty acid oxidation in muscle mitochondria [29]. According to the results of the review published by the 'European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases' in 2019, it has even been claimed that the GM may be a hidden risk factor for osteoarthritis [30].

The vast majority of early investigations on the association between exercise and GM were conducted on animals. In recent years, there has been a growth in the number of studies looking into the relationship between exercise and GM in humans [9, 15, 19, 20, 22, 31, 32]. Although the number of studies has increased, the relationship between exercise and GM, as well as their interactions, is still not entirely understood. The results of the conducted studies may differ due to the inclusion of healthy and unhealthy participants together in the study groups, the

participants' varying levels of physical activity and other characteristics, and the failure to appropriately control confounding factors.

DIABETES

Diabetes is one of the most prevalent health issues nowadays [33]. Sedentary lifestyles and unhealthy eating habits can cause metabolic diseases such as diabetes. As a result of this condition, the GM content decreases and dysbiosis is developed [33, 34]. These adverse changes seen in the GM cause systemic inflammation and the development of insulin sensitivity by triggering inflammatory processes caused by an increase in endotoxins in the bloodstream [35-37]. Therefore, healthy changes in the GM may be effective in the prevention and treatment of diabetes [38]. Exercise alters intestinal blood flow and temperature [39], increases intestinal motility [40], and raises the concentration of fecal bile acids [41]. In this way, it can contribute to the improvement of metabolic health and GM by reducing chronic inflammation [42] and insulin resistance [43].

There are various studies in which exercise is performed at different intensities and durations in diabetic patients. Motiani et al. [44] research that in patients with type 2 diabetes and pre-diabetes who high-intensity intermittent exercise increased the *Lachnospira* genus compared to moderate-intensity continuous exercise, and both types of exercise decreased the *Clostridium* genus and *Firmicutes/Bacteroidetes* ratio. Moreover, according to the results of this uncontrolled randomized study, although alpha diversity (the number of species detected in a particular habitat) and beta diversity (inter-habitat diversity) did not increase after exercise, exercise did lead to the lowering of systemic and the GM-specific endotoxemia markers.

Torquati et al. [45] discovered that after 8 weeks of exercise, exercise intensity had an influence on the variety of species in the GM in patients with type 2 diabetes, and this change was not related to medication, nutrition, or clinical condition. This finding supports the idea that different exercise intensities can have distinct effects on the GM There are also studies with diverse exercise durations that include diabetes patients in the literature. In a study conducted with sedentary type 2 diabetes patients, it was determined that alpha and beta diversity did not increase in the GM after 6 months of endurance, resistance, and flexibility exercises. Exercise, on the other hand, has been shown to lower systemic inflammation through modifying gut composition and gut barrier function [46]. A systematic review published in 2022 indicated that exercise can reduce markers of metabolic endotoxemia in patients with type 2 diabetes and that at least 6 weeks of moderate-to-high aerobic exercise can produce beneficial changes in the GM composition [38]. According to the findings of these studies, it is not feasible to

state definitively what influence exercise has on the GM. Nonetheless, it can be said that exercise can help control diabetes by reducing endotoxemia. Differences in research outcomes might be the consequence of inadequate control of several confounding factors or methodological biases. Furthermore, because the majority of the research is cross-sectional, it is possible to conclude that the level of evidence is low. As a consequence, high-quality studies with longitudinal and experimental research designs that control for confounding factors such as diet, age, and medication usage, which are major factors that affect GM, are required to clearly demonstrate the effect of exercise on the GM in diabetic patients.

OBESITY

Obesity is a disease with multiple etiologies, including genetic and non-genetic factors [47]. The GM is one of the determining factors in the body's energy expenditure by being effective in the digestion and storage of nutrients [48, 49]. It has been shown that disruption of intestinal homeostasis may lead to systemic inflammation and an increase in body fat [50, 51]. In a study on mice, it was found that when the microbiota of an obese mice was transferred to a germ-free mice, the mice that were transferred the microbiota had increased body adiposity, although there was no change in their diet [52]. Similarly, when microbiota from pigs with high levels of fat were transferred to a germ-free mice, it was discovered that fat production in muscle tissues increased, muscle fibers shifted toward the slow-twitch muscle fiber type, fiber size and proportion of type-2b muscle fibers decreased, and thus, adverse changes in muscle structure and function were observed in the germ-free mice [53]. In human studies, obese people were reported to have dysbiosis in the GM, and a high-fat diet was connected with dysbiosis when compared to non-obese people [50, 54]. In addition, *Firmicutes* and *Bacteroidetes* species constitute the majority of the intestinal flora, and an increase in the *Firmicutes/Bacteroidetes* ratio has been found to be linked to obesity [55]. In accordance with these findings, it is known that the GM is diminished in obese persons [56]. Due to the aforementioned characteristics of the GM, it becomes an important factor in obesity [57, 58]. Adverse changes in the GM caused by a range of factors result in the leaking of bacteria and components such as lipopolysaccharide from the bacterial mucosa as well as a reduction in the number and species of beneficial bacteria [59]. Therefore, metabolic diseases such as obesity can be prevented by protecting the health of the GM [60].

Various treatment options are available to maintain GM health, such as pre- and probiotics, dietary changes, and exercise [61]. Exercise provides important contributions in terms of protecting the intestinal flora and increasing insulin resistance [9, 43, 62]. Besides that, via modulating GM, exercise is beneficial for preventing dysbiosis and lowering systemic inflammatory responses. Thanks to

these effects, exercise may be effective in preventing obesity and reducing body fat through increasing energy expenditure [56]. One study found that 6 weeks of aerobic exercise increased the quantity of *Bacteroides* in the GM of obese people. While the fecal concentration of SCFAs increased in lean subjects, no such change was detected in obese people. However, it has been reported that these changes in the GM were restored after the exercise was terminated [63]. In a study conducted by Munukka et al. [64] with overweight women, it was found that there was no change in the *Firmicutes/Bacteroidetes* ratio after 6 weeks of endurance exercise 3 days a week, while there was an increase in the *Bifidobacteriaceae* family, which has many protective functions, and the *Akkermansia* genus, which is known to decrease in obese individuals, and a decrease in the inflammation-related *Proteobacteria* phylum. According to a study published in 2020 by Quiroga et al. [56] obese children showed changes in bacterial phylum, class, and genus levels following 12 weeks of aerobic exercise combined with strengthening exercises, and their GM profiles began to resemble those of healthy children. This study suggested that exercise could be an important non-pharmacological treatment option for obese children by modulating GM. Kern et al. [15] conducted one of the studies with the longest treatment duration assessing the effect of exercise on the GM. Cycling exercises were carried out 5 days a week for 6 months in the study, with an intensity of 50% of VO^2_{max} and 70% of VO^2_{max}. While the alpha diversity did not alter when the exercise was performed at 50% of VO^2_{max}, it was established that the exercise at 70% of VO^2_{max} increased the alpha diversity compared to the pre-exercise. In addition, it was found that exercise treatment showed minor changes in the GM compared to the control group. It was also concluded that alpha diversity was not associated with cardiorespiratory fitness or body fat mass. As can be seen from these findings, the increase in the number of bacteria or beneficial changes in the GM may not always be in conjunction with the increase in intestinal diversity. According to a systematic review dated 2022, moderate-to-high intensity and long-term exercise programs are required to improve intestinal diversity in obese individuals [38].

Apart from the effect of exercise on the GM, the GM may also emerge as a factor affecting health and performance. It has been determined that when lipopolysaccharides enter the bloodstream, they bind with toll-like receptor 4 and trigger inflammatory responses, resulting in chronic systemic inflammation. This may cause health issues, such as obesity, for the host. Furthermore, the production of lipopolysaccharides has a negative relationship with VO^2_{max}. As a result, there is a decrease in overall health [65].

Most of the studies examining the relationship between exercise and GM in obese people performed solely aerobic exercise. The effect of exercise on the GM can be demonstrated in detail by including other exercise types in study designs

and comparing exercise types. Except from that, it is seen that the duration and intensity of exercise differ in studies. Although research on obese individuals generally shows that exercise induces favorable changes in the GM, the conclusions of the studies vary. As a result, it is necessary to design new studies examining the association between exercise and GM in obese people.

ATHLETES

During exercise, GM and host benefit together in a synergistic relationship. Lactate produced by skeletal muscles during exercise leads to a selective increase in the species that use lactate as a nutrient in the gut. The increase in these species facilitates the production of beneficial metabolites, improving muscle and energy metabolism and the immune system thanks to the increase in the formation of propionate in the intestine. In this way, while the intestine is enriched with beneficial bacteria, the host who exercises as a result of the increased bacteria also benefits [26]. In the study by Petersen et al. [66], which included professional and amateur cyclists, it was discovered that the *Prevotella* genus increased with exercise duration and that *Methanobrevibacter smithii*, which plays a key role in polysaccharide digestion, was more abundant in professional cyclists. It is stated that these effects result in an increase in energy and carbohydrate metabolism in a synergistic manner.

It is known that athletes' GM have more microbial diversity than non-athletes [9]. Similarly, some studies have shown that the GM produces more dynamic responses to exercise in athletes than in sedentary people [21, 24, 66]. In a study comparing elite marathon runners and ski athletes with sedentary individuals, it was found that alpha diversity, *Firmicutes/Bacteroidetes* ratio, and *Prevotella* genus abundance increased in athletes. *Veilonella* species were found to be increased only in marathon runners [67]. In a study involving 13 university swimmers, fecal samples were collected at specific periods when the swimmers performed high-intensity exercise and when the level of the high-intensity exercise was lowered. Sample results revealed that even short-term reductions in exercise intensity were linked with decreased GM diversity [68]. In an observational study with a 4-month follow-up, Keohane et al. [69] showed that alpha diversity grew until the end of the competition and continued for 3 months in three endurance athletes, with the exception of the athlete who had antibiotic treatment before the competition.

Studies show that there may be disparities between athletes who exercise at different levels and intensities, as well as between the same athletes who change their exercise intensity. According to the results of a randomized controlled trial conducted with 24 swimmers, increasing the intensity of the exercise program

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improved intestinal alpha diversity independently of probiotics [70]. A similar result was obtained by Liang et al. [71]. In their study, which comprised 28 high- and low-level martial arts athletes, they discovered that high-level athletes had better metabolic capacity and diversity in their GM than low-level athletes. However, it is difficult to say that the change in the GM occurs only with exercise, especially in athletes, since a strict diet and other supplements are frequently used with exercise. Clarke et al. [9] included professional male rugby players and healthy non-athletes in their study. The GM of rugby players was found to be more diverse than the healthy control group. Also, the age and gender characteristics of the control group were similar to those of the athletes, while the healthy control group was separated into two groups: high BMI (>28 kg/m^2) and low BMI (<25 kg/m^2). Considering that rugby players have high BMI values, it was also discovered that the players had higher alpha diversity than the control group with high BMI. Moreover, according to the results of the analysis made considering the increased protein consumption in athletes, protein consumption was found to be associated with GM diversity. It has been stated that the microbial diversity in rugby players and the difference compared to control groups can be partially explained by protein intake. In another randomized controlled trial, additional protein intake was shown to increase the amount of *Bacteroidetes* and decrease *Roseburia*, *Blautia*, and *Bifidobacterium longum* in runners [72].

Different sports may reveal different effects on the GM of athletes due to their various dynamics and nature. The study by O'Donovan et al. [73] of Irish elite-level athletes can provide evidence for this. In the study, the researchers compared the GM of people who participated in different sports. According to the results of this research, they found differences in 5 species: *Polynucleobacter necessaries*, *Eubacterium rectale*, *Bacteroides vulgatus*, *Faecalibacterium prausnitzii*, and *Gordonibacter massiliensis*. This indicates that variations in the GM occur as a result of creatine and lactate production in response to the nature of the sport [74].

Regular exercise affects the gut-brain axis by modulating inflammatory and immune responses [75, 76]. However, prolonged or vigorous exercise may cause gastrointestinal problems by causing changes in intestinal blood flow and the sympathetic nervous system [39]. Exercise, particularly when performed at high intensity for extended periods of time, continuously activates the autonomic nervous system, lowering blood flow to the gastrointestinal tract, and causes hypoxia and oxidative stress [77]. Increased intestinal permeability, endotoxemia, inflammation, and reduced nutritional absorption are all induced by these factors. In order to prevent this, GABA and dopamine secretion increase in the gastrointestinal tract, and gastrointestinal disorders may occur [24]. According to review

studies, low- and moderate-intensity exercise can improve the gastrointestinal health, whereas high-intensity exercise lasting more than 2 hours can be detrimental to the gastrointestinal system [78, 79]. This adverse effect on the gastrointestinal tract has a major impact on the GM [31]. Pathogens and endotoxins get into the bloodstream through the intestinal barrier, causing leaky gut syndrome due to a decrease in mucosal thickness and loosening of the junctions that control the passage of substances, notably in endurance athletes. As a result of this, studies in athletes show that the frequency of gastrointestinal problems and upper respiratory tract infections is increasing [80, 81]. In the study done by Jeukendrup et al. [82] with 29 triathlon athletes, the lipopolysaccharide levels of the athletes were examined before, immediately after, 2 and 16 hours after triathlon. Digestive problems were observed in 93% of the participants immediately after the triathlon, and there was a significant increase in lipopolysaccharide levels. The fact that this effect and the presence of endotoxemia continued in 68% of the athletes 1 hour after the race also supports this result. Especially when the exercise is not prescribed correctly and the exercise is excessively intense, conditions such as vomiting, diarrhea, and even bleeding can be seen after exercise in athletes [82, 83]. Therefore, care should be taken to ensure adequate rest periods to reduce the negative effects of exercise on the gastrointestinal tract [84]. The use of pro- and prebiotics in athletes is recommended in order to prevent such problems [85].

As a result, it can be said that there is a relationship between exercise and GM in athletes, and this circumstance will be a significant component that needs to be examined in terms of rehabilitation and increasing athlete performance. It is critical to demonstrate how exercise affects dietary habits and the GM.

When the studies involving athletes are evaluated, it is seen that the studies have a low number of participants in general. In order to increase the level of evidence, studies with more participants should be designed. It has not been determined precisely to what extent the intensity and frequency of exercise affect GM in athletes. In addition, since the diet of athletes differs greatly from that of non-athletes, nutrition in athletes may emerge as an important confounding factor affecting GM. For this reason, attention should be paid to controlling factors such as protein intake and pro- and prebiotic use in studies to be carried out with athletes. Given the possible impacts of the GM on performance as well as the influence of exercise on the GM, new studies revealing the extent to which changes in the GM affect the performance of professional and competitive athletes may enable GM to emerge as a crucial determinant of performance for athletes.

ELDERS

The imbalance in anti-inflammatory and pro-inflammatory responses with aging is known to cause low-level chronic systemic inflammation [86, 87]. It is thought that this situation may be brought on by disruptions in the regulation of the immune system. As a consequence, circulating endotoxin levels rise, and systemic inflammation emerges with the decrease of beneficial bacteria that produce SCFAs and an increase in pathogenic bacterial species in the GM [87, 88]. Furthermore, several studies have linked dysbiosis to diseases such as type 2 diabetes [89], hypertension [90], atherosclerosis and stroke [91, 92], Alzheimer's disease [93], Parkinson's disease [94], colorectal cancer [95], physical fragility and sarcopenia [96], rheumatoid arthritis [97], and osteoporosis [98], the prevalence of which increases with age.

Exercise promotes beneficial bacteria by increasing SCFAs production, modulating bile acid and mucus production, decreasing lipopolysaccharides, and slowing nutrient passage through the gut. In this way, exercise is useful in preventing many chronic diseases in the elderly and is an important factor in healthy aging [99, 100]. According to the findings of a study conducted on sedentary older women, 8 weeks of exercise showed favorable improvements in the GM. Exercise has been demonstrated to reduce the number of phyla with anti-inflammatory effects, such as *Verrucomicrobia*, while enhancing the abundance of phyla with pro-inflammatory effects, such as *Proteobacteria* [101]. Since the GM has been shown to provide health benefits in recent years, researchers have begun to focus on the influence of exercise on the GM and its interaction with the GM [39, 62]. However, research on the relationship between exercise and GM in the elderly population is lacking.

In a study of 33 Japanese women aged 62 to 76 years, it was discovered that 5 weeks of cycling exercise resulted in a significant decline in *Clostridium difficile* and a substantial increase in *Oscillospira* species, despite the fact that the employed 5-week endurance exercise had a small effect on the GM. It was also concluded that cardiovascular risk factors were associated with alterations in the GM [19]. Conversely, another study with senior adults found that 24 weeks of aerobic and resistance exercise had a substantial influence on alterations in the GM and might reverse dysbiosis, which worsens with age [102]. According to the analysis results of the sample consisting of 897 elderly and 1589 adults obtained from the American Gut Project, it has been shown that the changes that occur with regular exercise in the elderly, especially in overweight people, may be effective in maintaining the diversity of the GM [103]. In the study conducted by Morita et al. [104] on 32 sedentary older women, the participants were divided into two separate groups: the aerobic exercise group and the trunk muscle strengt-

hening group. At the end of 12 weeks, it was revealed that the amount of *Bacteroides* species increased in parallel with the increase in cardiorespiratory fitness in the aerobic exercise group.

As seen in studies conducted on the elderly, the duration of exercise varies significantly between studies. For reasons such as the limited number of studies and differences in study methodologies and samples, it is not yet clear how beneficial exercise is on specific bacterial species, as well as whether certain exercise types and intensities are more effective on the GM. Secondly, it can be said that the generalizability of the research results is low due to the small number of participants in most of the studies conducted with the elderly. For this reason, more research with a larger sample size is required to improve the generalizability of the findings of studies looking into the association between exercise and GM in the elderly.

CONCLUSION

Exercise may have varied impacts on the GM in different populations and at different intensities and durations. Besides that, the type of exercise is an important determinant in terms of generating alterations in the GM. Most research evaluating the connection between exercise and GM employed endurance exercises. While it has been shown in several studies that endurance exercise changes GM in various aspects, other studies have found that resistance exercise has no significant impact. It is thought that this situation arises due to the different metabolic and physiological effects of different exercise regimens. Besides exercise, factors such as nutrition, age, and gender are other major determinants in the regulation of the GM. This may explain why outcomes differed in studies where confounding factors were not sufficiently controlled. Future research should focus on the homogeneity of groups based on participant characteristics such as age, BMI, gender, and physical activity level. Moreover, the effects on the GM of different types and durations of exercise should be studied separately.

The GM can influence health and performance in addition to how exercise affects GM. The GM may alter performance by causing changes in the production of metabolites, regulation of the immune system, absorption of nutrients, and permeability of the intestinal barrier. Clearly revealing the relationship between exercise and GM will be useful both to better understand how exercise parameters affect GM and to see the effect of changes in the GM on performance, especially in athletes. In the future, the GM may emerge as a parameter that can help predict the performance of athletes or identify issues that can impair performance. For these reasons, new research should be conducted to demonstrate the long-term

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effects of changes in the GM content and the effects of factors other than exercise on the GM.

Further study designs are required to comprehend the short- and long-term impacts of exercise and to reveal GM changes in response to exercise intensity. Given that the majority of research addressing the connection between exercise and GM in people is cross-sectional, the evidence is insufficient to establish a definitive cause-and-effect relationship between exercise and GM. As a result, longitudinal and experimental study designs evaluating the relationship between exercise and GM are required. Also, further studies should be performed to examine the association between exercise and GM in different populations, such as those with irritable bowel syndrome, children, and athletes from various sports, where differences in the GM can be seen. It is critical for human health to understand how the chance in the GM causes what kinds of physiological and performance-based differences and how these differences are modified by exercise. The GM may emerge as an important determinant of performance and health within the next few years.

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CHAPTER
18

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MEASUREMENT TOOLS USED IN DENTAL IMPLANT ASSESSMENT

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1. Introduction

Dental implants are biocompatible metal anchors surgically positioned in the jaw bone underneath the gums to support an artificial crown where natural teeth are missing (1). Periodontal indices are frequently used for the evaluation of dental implants. However, periodontal indices alone, do not define implant success or failure. Instead, these clinical indices must be related to other factors, such as an exudate or prosthesis overloading (2). The Modified versions of indices used to diagnose periodontal health and disease are also used in the evaluation of dental implants (3) (Table 1). Pain and mobility are the primary criteria for assessing implant quality and health. The presence of either one significantly compromises the implant and lead to it's removal. Also, routine probing depth is not suggested without other signs or symptoms (2). The parameters that may be used to assess the presence of peri-implant health and the severity of peri-implant disease include plaque assessment, mucosal conditions, peri-implant probing depth, the width of the peri-implant keratinized mucosa, peri-implant sulcus fluid analysis, suppuration, implant mobility and discomfort, resonance frequency analysis, and radiographic evaluation (4). Tissue thickness around the dental implant is one of the most critical parameters (5). It is suggested that 2 mm of keratinized gingiva (corresponding to 1 mm attached gingiva in this material) is adequate to maintain gingival health (6). For some patients, a lack of keratinized gingiva may be a risk factor for one or more issues: plaque accumulation, tissue soreness while brushing, increased gingival inflammation, recession, and bone loss (7).

Albrektsson's success criteria for dental implants:

- ✓ The implant should not be mobile in clinical examination.
- ✓ There should be no radiolucency around the implant in the radiographic examination.
- ✓ Vertical bone loss within the first year should be at most 1mm and this number would reduce to 0.2 mm in the following years.
- ✓ There should be no symptoms like infection, paresthesia, neuropathy, or pain during the function.

✓ At the end of 5 years, the success rate should not descend below the rate of 85% and 80% at the end of 10 years (8).

Criteria for success include:

- Absence of symptoms such as pain, infection, neuropathies, paresthesia, and detoration of vital structures.
- Implant immobility.
- No continuous peri-implant radiolucency.
- Progressive bone loss (less than 0.2 mm annually) after physiologic remodeling during the first year of function.
- Patient/dentist satisfaction with the implant-supported restoration (9).

	Modified Plaque Index	**Modified Sulcus Bleeding İndex**	**Mobility**
Score 0	No detection of plaque	No bleeding when a periodontal probe is passed along the gingival margin adjacent to the implant.	No displacement apparent upon loading of implant with 1 N in any direction ("rock solid").
Score 1	Plaque only recognized by running a probe across the smooth marginal surface of the implant. Implants covered by titanium spray in this area always score 1.	Isolated bleeding spots visible.	Loading of implant with 1 N results in minimal displacement of not more than 0.5 mm.
Score 2	Plaque can be seen by naked eye.	Blood forms a confluent red line on margin.	Implant apparently loose.
Score 3	Abundance of soft matter.	Heavy or profuse bleeding.	

Table 1. Dental implant indices (3)

This review article is aimed to explain the scoring, tools, and scales used to evaluate dental implants.

2. Pink Esthetic Score and White Esthetic Score

The white esthetic score (WES) is based on the five following parameters:

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- General tooth form, outline, and volume of the clinical crown.
- Color, which includes the assessment of the dimension's hue and value.
- Surface texture.
- Translucency and characterization (10).

All five parameters are assessed by direct comparison with the natural, contralateral reference tooth, estimating the matching degree or eventual mismatch. In the case of an optimum duplication of the esthetically relevant features inherent to the control tooth, a maximum WES of 10 is possible. Again, the threshold of clinical acceptance was set at a score of 6 (11) (Table 2).

Variables	**Major discrepancy**	**Minor discrepancy**	**No discrepancy**
Tooth Form	0	1	2
Outline/ Volume	0	1	2
Color (Hue/ value)	0	1	2
Surface Texture	0	1	2
Translucency/ Characterization	0	1	2
Maximum Score			

Table 2. White esthetic score (10)

The Pink Esthetic Score (PES) is based on seven variables. The highest possible score was 14 (12)(Table 3). The thresholds for clinical acceptance, which was currently set at values of 8/14 for the PES and 6/10 for the WES, should be more rigorous (10).

Variables		0	1	2
Mesial papilla	Shape vs reference tooth	Absent	Incomplete	Complete
Distal papilla	Shape vs reference tooth	Absent	Incomplete	Complete
Level of soft tissue margin	Level vs. reference tooth	Major discrepancy>2 mm	Minor discrepancy 1-2 mm	No discrepancy <1 mm
Soft tissue contour	Natural, matching reference tooth	Unnatural	Fairly natural	Natural
Alveolar process	Alveolar process deficiency	Obvious	Slight	None
Soft tissue color	Color vs. reference tooth	Obvious difference	Modarate difference	No difference
Soft tissue texture	Texture vs. reference tooth	Obvious difference	Modarate difference	No difference
Maximum Score				

Table 3. Variable of pink esthetic score (12)

The highest possible combined PES/WES is 20, which represents a close match of the peri-implant soft tissue conditions and the clinical single-tooth implant crown compared to the respective features present at the contralateral natural tooth site (13).

The mean total PES on the scale of 10 at the end of 4 years of follow-up for 38 subjects with dental implants in the esthetic region was respectively, 5.64 at the first visit and 7.07 at the end of a year. It revealed a significant difference between the PES scores before surgery and the follow-up examination of the single anterior implants (14).

When evaluating thirty single dental implants in various specialties, the total PES was 10.6 for prosthodontists and 9.2 for oral surgeons. The PES is a tool for reproducibly evaluating the esthetic appearance of the soft tissue around single-

tooth implant crowns. Restoring the papilla, which ranks prominently in the literature, fared the best in the assessments, while the level of the soft-tissue margin and the color of the peri-implant soft-tissue fared the worst (12).

The PES and WES were investigated in 41 single-tooth implant-supported crowns. The mean ratio of the total PES to WES was 11.19 ± 3.59. The mean PES was 5.17 ± 2.29, whereas the mean WES was 6.02 ± 1.96. The study has revealed patients' esthetic and dentists' anterior tooth perception. The PES/WES is an objective tool in rating the esthetics of implant-supported single crowns and adjacent soft tissues (13).

All 45 anterior maxillary single-tooth implants met the success criteria concerning osseointegration, including the absence of peri-implant radiolucency, implant mobility, suppuration, and pain. The mean total PES/WES ranged between 11 to 18. The mean full PES ranged: 6 to 9 documents favorable overall peri-implant soft tissue conditions. The suitability of the PES/WES index for the objective assessment of the esthetic dimension of anterior single-tooth implants was confirmed (11).

The maximum score was 14 for the PES and 10 for the WES, which illustrated a perfect match of soft and hard tissue. Therefore, considering the limitations of this study, in single-tooth implant restorations, a total PES/WES would greater than 12 which is enough to be clinically acceptable in most situations (15).

The PES/WES and the modified implant crown aesthetic index can be reliable estimates of the esthetic outcomes. The assessor's specialization would affect the esthetic evaluation with the PES/WES and the modified implant crown aesthetic index (16).

3. Implant Disease Risk Assesment

The eight vectors of the diagram include: an assessment of a history of periodontitis, the percentage of sites with bleeding on probing (BOP), the number of teeth/implants with probing depths (PD) ≥5 mm, the ratio of periodontal bone loss (evaluated from a radiograph) divided by the patient's age, periodontitis susceptibility as described from the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases (17) , the frequency/compliance with supportive periodontal therapy, the distance from the restorative margin of the implant-supported prosthesis to the marginal bone crest, and prosthesis related factors including cleanability and fit of the implant-supported prosthesis (18).

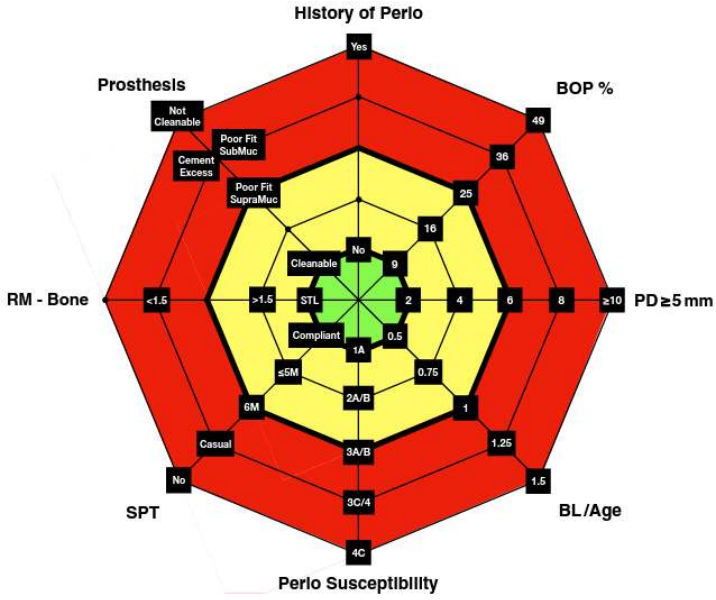


Figure 1. Implant Disease Risk Assasment (18)

When IDRA was calculated in 79 patients, 42.5% had a moderate risk, while 56.3% had a high IDRA risk. Peri-implantitis was diagnosed with moderate risk in 4 patients (12%) and high risk in 12 patients (27%). The calculated odds ratio for the development of peri-implantitis in patients at high risk of IDRA compared with patients at moderate risk of IDRA was not indicating statistically significant difference between the two groups. In conclusion, within the limitations of the present retrospective study, the IDRA algorithm might represent a promising tool to assess patients at moderate or high risk developing peri-implantitis (19).

One hundred and ten patients the dental implants' survival rate was 90% in the study. A higher risk profile was significantly associated with a decreased implant survival. At follow-up, significant differences in bleeding index, modified plaque index, and peri-implant probing depth were found among groups. Despite a statistically non-significant difference in marginal bone loss, patients with a high-risk profile of IDRA seem to be at greater risk of implant loss and biological complications (20).

The Implant Risk Assessment Questionnaire may have limited utility in its original form to identify cases of health and disease on a short-term basis. Though the clinical relevance of this study correlates with the Implant Risk Assessment Questionnaire scores in short-term implant health is limited. the Implant Risk Assessment Questionnaire remains an important tool to increase clinician awareness of risk indicators for implant therapy and conversations about implant risk with patients and other providers (21).

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Fifteen implants with low risk was peri-implant mucositis (25%), whereas the implants with moderate risk (6 implant) was peri-implantitis (75%). Based on the present study data, this risk assessment tool can be used in the early detection of peri-implant disease. Identifying the risk factor may help in the success rate of implant survival (22).

4. Implant Quality Scale

The Implant Quality of The Health Scale (IQS) allows the dentist to evaluate a health or a disease, and then set the implant accordingly. Three primary classes were established by the Consensus: success, survival, and failure. The success category describes optimum conditions, the survival category describes implants still in function but not with ideal conditions, and the loss of an implant represents an the implant that should be or already has been removed (23) (Table 4).

Implant Quality Scale Group	**Clinical Conditions**
I. Success (optimum health)	a) No pain or tenderness upon function
	b) 0 mobility
	c) <2 mm radiographic bone loss from initial surgery
	d) No exudates history
II. Satisfactory survival	a) No pain on function
	b) 0 mobility
	c) 2-4 mm radiographic bone loss
	d) No exudates history
III. Compromised survival	a) May have sensitivity on function
	b) No mobility
	c) Radiographic bone loss> 4 mm(less than ½ of implant body)
	d) Probing depth> 7 mm
	e) May have exudates history
IV. Failure (Clinical or absolute failure)	Any of following:
	a)Pain on function
	b)Mobility
	c)Radiographic bone loss>1/2 lenght of implant
	d) Uncontrolled exudate
	e)No longer in mouth

Table 4. Implant Quality Scale (2)

Implant failure is easier to describe and may consist of various factors such as: any pain, vertical mobility, uncontrolled progressive bone loss, and/or generalized peri-radiolucency warrant implant removal. IQS does not only assess the implant health-disease continuum but also relates the treatment and prognosis to the existing conditions (24).

Ninety-six patients (100 implants) showed initial early failure. Sixty-six patients with early implant failure received 67 replacement dental implants. Moreover, according to the IQS criteria, a successful implant is defined as <2 mm of radiographic bone loss from baseline. Nevertheless, in the present study, radiographs were taken after prosthetic loading. This means the initial bone remodeling (i.e., biologic width establishment) had already occurred. Thereupon, the peri-implant bone level obtained in the present study might represent an underestimation (25).

One hundred three consecutive patients (partially and edentulous) were restored, with 360 implants and 105 prostheses in function for 12 to 26 months. No early loading implant failure occurred, and all patients with implants have satisfaction for optimum health according to the Misch Implant Quality Scale. The mean early-loading bone loss was 0.29 mm (SD 0.99 mm) (26).

5. Result

Indices used to identify periodontal diseases or modified versions used for dental implants are sufficient to diagnose peri-implant health and disease states only. In addition, determining the risk and survival of the dental implant, the soft tissue around it, its prothesis, and all the patient's characteristics provides a holistic view to the dentist.

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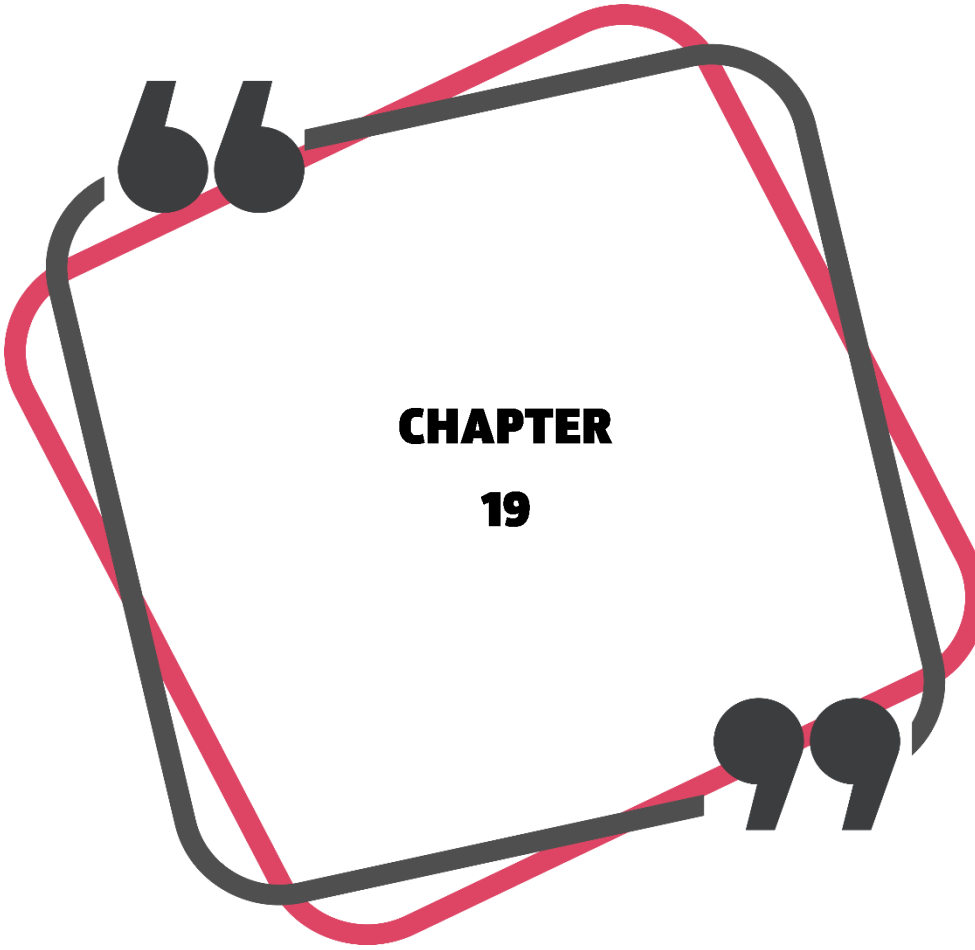
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CHAPTER
19

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DEVELOPMENT OF REVISED DYSMENORRHEA IMPACT SCALE SHORT FORM (DIS-R)

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INTRODUCTION

Dysmenorrhea is defined as painful menstrual cramps resulting from the uterus (Yasir, Kant & Dar, 2014). Dysmenorrhea is considered the most common symptom among all menstrual problems. It has been reported that it causes a greater burden of disease than any gynecological complaint, especially in developing countries (Patel, Tanksale, Sahasrabhojanee, Gupte & Nevrekar, 2006). Dysmenorrhea is causing significant economic losses due to medical care drug costs and decreased productivity (Lobo, Gershenson, Lentz & Valea, 2016). The high prevalence of dysmenorrhea in the literature, particularly in the results of studies conducted in recent years, is an indicator of how important the problem is. For example, the prevalence was found to be 64.7% in a study conducted in Ethiopia (Azagew, Kassie & Walle, 2020), 41.7% in China (Hu, Tang, Chen, Kaminga & Xu, 2020), 91.5% in Pakistan (Ullah et al., 2021), 91.5% in Ireland (Durand, Monahan & McGuire, 2021), 85% in Palestine (Helwa, Mitaeb, Al-Hamshri & Sweileh, 2018) and 74.8% in a study conducted in Spain (Fernández-Martínez, Onieva-Zafra & Parra-Fernández, 2018).

Dysmenorrhea is a problem of great importance not only for the affected individuals but also for society as a whole by negatively affecting the functional abilities of women (Chia et al., 2013). In the literature, the problem of dysmenorrhea has been associated with such problems as academic achievement and performance, social activity (Grandi et al., 2012, Karout, Hawai& Altuwaijri, 2012),

emotional health (Al-Jefout, Seham, Jameel, Randa& Luscombe, 2015), cultural and social practices, community attitudes and beliefs (Kumar, Srivastava, 2011) stress (Barcikowska et al., 2020), nutrition (Hu et al., 2020), sexual activity (Cetin, 2020), drug use (Grandi et al., 2012; Singh et al., 2008) and elimination (Agarwal & Agarwal, 2010) As a matter of fact, Gün developed a scale for measuring the effects of dysmenorrhea in 2014 based on the studies on the effects of dysmenorrhea in the literature. The study's objective is to revise the dysmenorrhea impact scale published in 2015 (Gün, Demirci & Otrar, 2015) and update it to be more functional. Research question: Is the revised form of the dysmenorrhea impact scale a valid and reliable measurement tool?

MATERIALS AND METHODS

Research Design

The research is cross-sectional and methodologically structured. It was conducted on female students at a university in Turkey. The aim of this research is to revise and update the dysmenorrhea impact scale, which measures the effects of dysmenorrhea on women and make it more useful.

Population and Sample of the Research

The research consists of female students and was conducted at a public university in Burdur. Data collection was carried out in November 2017. In the literature, Comrey and Lee (1992) stated that 100 people are weak, 200 people are moderate, 300 people are good, 500 people are very good and 1000 people are excellent (Comrey & Lee, 1992). A total of 1004 voluntary participants, aged above 18 years, experiencing dysmenorrhea, who met the sampling criteria, were reached. The scale aims to measure the effects of dysmenorrhea. The data was collected in November 2017 based on the participants' self-reports.

Data collection tools

Study data was collected with the Introductory Information Form, the revised draft of the dysmenorrhea impact scale. Introductory Information Form**:** It was prepared by the researcher in line with the literature review. The form includes questions about the characteristics of dysmenorrhea such as age, age of menarche, onset and duration of dysmenorrhea, and the status of consulting a doctor due to dysmenorrhea. Revise Draft Form of the Dysmenorrhea İmpact Scale: Based on the draft dysmenorrhea impact scale, a revised dysmenorrhea impact scale was drafted. The unrevised version of the scale is based on Gordon's eleven functional patterns. Thus, thirty nine items from the sixty three item draft scale were

collected in eleven sub-dimensions and concluded by conducting confirmatory factor analysis (Gün, 2014). The original Draft scale form is a 5-point Likert type. 5-point rating is as follows Totally agree=5, agree=4, undecided=3, disagree=2, strongly disagree=1. As the scores of the participants from the scale increase, their level of being affected by dysmenorrhea also increases (Gün, Demirci & Otrar, 2015). An exploratory factor analysis independent of Gordon's model of functional health patterns was applied in order to develop the revised form of the scale.

Data analysis

Number, average, percentage, standard deviation, minimum, and maximum values were used in the analysis of descriptive data. Kolmogorov-Smirnov and Shapiro Wilk values, in case of a large sample (n: 1004), the fact that the central stacking dimensions are close to each other to test whether the data exhibits normal distribution feature since it is less than 0.05, were tested with skewness and kurtosis values. The validity and reliability of the scale were tested by conducting content validity, face validity, exploratory factor analysis (EFA), confirmatory factor analysis (CFA), Kaiser Meyer Olkin (KMO) test, Barlett test, Cronbach Alfa, Spearman-Brown and Guttman coefficients, item total, item-rest, and item discrimination analysis, and test-retest analysis. SPSS 15.0 and AMOS software were used in the analyzes.

Ethical Considerations

Ethics committee approval was obtained before the research was conducted (Decision number: Marmara University 09.2017.503). Written and verbal information was given to the participants.

RESULTS

Descriptive Characteristics of the Participants

The average age of the participants was 19.52±1.61 (min:18, max:35), and the age of menarche was 13.27±1.03 (min:10, max:16). Dysmenorrhea in 31.4% (315) starts with menstruation, while 68.6% (689) begins a few hours before menstruation. The duration of dysmenorrhea in 67% (673) is only on the first day of menstruation, on the other hand, 33% (331) experience dysmenorrhea within the first 2-3 days of menstruation. 41.2% (414) of the participants applied to a health institution due to dysmenorrhea. Before proceeding to the analysis of the

data, the normality test of the scales used in the study was performed to determine which test techniques would be used during the analysis of the data. It is seen that the values related to kurtosis and skewness regarding normal distribution vary in the field of literature, although agglomeration measures (arithmetic mean, range, and median) are close to each other, skewness and kurtosis values between ±3.00, and as close to 0.00 as possible are considered descriptive indicators in cases where the sample is large since the Kolmogorov-Smirnov and Shapiro Wilk values used to examine the normality test are less than 0.05. Kalaycı (2014) and Tan (2008) stated that if these values are in the range of ±3, the values can be considered as a reflection of the normal distribution. It was observed that the kurtosis values of the scale items were between -1.10 and 2.98. Skewness values, on the other hand, ranged from -1.94 to 0.28.

Content and Logical Validity Study:

The expert opinion method is generally recommended and used in the literature to control the content and logical validity. For this method to give good results, it is recommended that the team consisting of people who are considered experts in the field should be at least 5 and a maximum of 40 people (Karagöz, 2021). The control of the content and logical validity of the scale was provided by taking the opinions of five experts. In the first stage of the validity and reliability studies of the scale, content validity was examined. In this regard, the appropriateness/validity levels of the scale items were determined by taking the opinions of the experts in the field. The content validation ratio takes a value between -1 and +1. If all the experts rate the scale item as "Appropriate", the content validity ratio value of that item will be 1. If the content validity ratio of the item is 0 or negative, the item has no content validity (Ayre & Scally 2014; Lawshe, 1975; Law Wilson, Pan & Schumsky, 2012). Lawshe (1975) stated that the content validity criterion for each item with a positive value should be examined at the significance level of α=0.05. Content validity criterion values are used to determine at least how many experts should approve an item before it can be decided that it is necessary or unnecessary. Its value also differs according to the number of experts. In our study, the content validity criterion values revealed by Ayre and Scally (2014) were taken as a basis. Accordingly, the content validity criterion value for 5 experts at the significance level of α=0.05 was accepted as 1,000 and the items outside this value were removed from the scale. **Following the content validity, a pilot study was conducted with 30 individuals to test the comprehensibility of the scale. Since the individuals participating in the pilot study stated that the scale items were understandable and easily answered, no additional changes were made to the scale items.**

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Construct Validity Study:

After expert opinions and preliminary trials, the scale was implemented in the real group. According to the results of this implementation, factor analyzes were made and according to the results of the analyzes, the items whose construct validity was provided were included in the final scale. Exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) were performed respectively for construct validity. Obtained results are given below in order.

Exploratory Factor Analysis (EFA) Results of the Scale:

At this stage of the study, EFA was applied to determine the construct validity of DIS-R, and "The Rotation Method" was used. In scale development studies, factor analysis is applied to reveal which structure or structures are measured by the items in the scale. These structures are defined as factors of scale. The results of the EFA applied in the study are presented in the Tables below.

Table 1. KMO and Bartlett Test Results regarding the Construct Validity of DIS-R

Kaiser Meyer Olkin (KMO) value		*.904*
Bartlett Test	**X^2 Value**	*3384.412*
	Sd	*78*
	P value	*.000*

As can be seen in Table 1, in Principal Component Analysis, the Kaiser Meyer Olkin (KMO) value was found to be 0.904. The KMO test is a measure of how suited your data is for factor analysis and the range of 0.80-0.99 is considered very good. Therefore, it can be stated that the KMO value in this study is at a very good level (Karagöz, 2021). As can be seen in Table 1, the result of the Bartlett test was found to be 3384.412 ($p<0.000$). The Bartlett test tests the hypothesis that the correlation matrix is equal to the unit matrix. Rejecting the hypothesis means that the correlation between the variables is different from 1.00 and shows that the measured variable is multivariate in the universe parameter (Karagöz, 2021). These two findings show that the sample size used in the study is sufficient for factor analysis, and the data are appropriate. In this study, no limitation was placed on the number of factors, and factors with an eigenvalue greater than 1.00

were included in the scale. Eigenvalue is a coefficient that is taken into account both in calculating the variance described by factors and in deciding on the number of significant factors. Factors with an eigenvalue of 1 or greater than 1 are considered significant factors in factor analysis (Karagöz, 2021). In this study, the eigenvalue was taken as 1.00 and two factors were determined. Figure 1 shows a scree plot of factor eigenvalues.

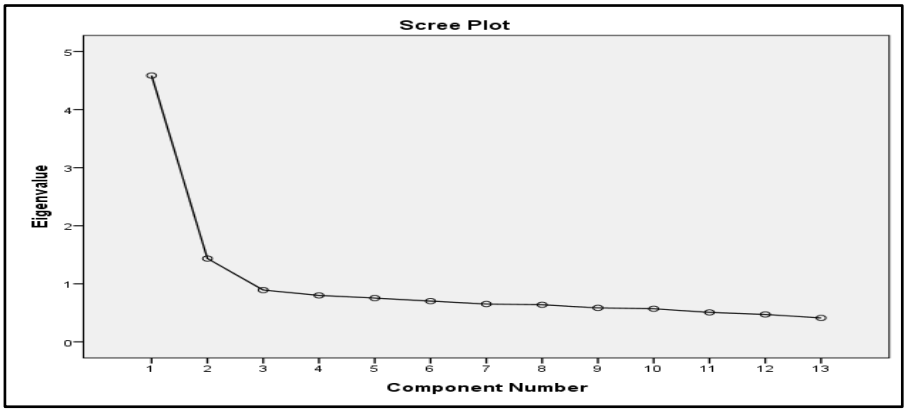


Figure 1. Scree Plot of the Sub-Dimensions of DIS-R

When the eigenvalue scree plot of the factors given in Figure 1 is examined, an elbow is seen in the two factors and after this point in the graph, a near-flat decrease is observed, albeit slowly. Therefore, it was decided that the number of factors in the scale could be limited to two. Table 2 shows the eigenvalues of the sub-dimensions and the amount of variance they explained, obtained as a result of factor analysis.

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Table 2. Ratios of Variance Explained by DIS-R Sub-Dimensions

Dimensions	Eigenvalue	Variance (%)	Stacked Variance (%)
1St-Dimension	*4.587*	*28.347*	*28.347*
2Nd-Dimension	*1.435*	*17.976*	*46.323*

As can be seen in Table 2, the variance rate explained by the first factor with an eigenvalue of 4,587 was 28.347%, and the variance rate explained by the second factor with an eigenvalue of 1.435 was 17.976%. The total explained variance rate was determined as 46,323%.

Table 3. Factor Related to the Items Constituting the Sub-Dimensions of DIS-R

Item No	Communality *(Common Factor Variance)*	Factor Loadings	
		1St-Dimension	**2Nd-Dimension**
Item 7	0.580	0.732	
Item 8	0.545	0.730	
Item 6	0.510	0.710	
Item 5	0.524	0.684	
Item 23	0.492	0.683	
Item 3	0.443	0.645	
Item 28	0.360	0.575	
Item 11	0.340	0.520	
Item 12	0.450		0.700
Item 18	0.451		0.699
Item 19	0.464		0.665
Item 15	0.400		0.602
Item 21	0.401		0.560

The common factor variances and factor loadings related to the items of the scale are presented in Table 3. Factor loading values are a coefficient that explains the relationship of items with sub-dimensions. In the literature, it is stated that factor loadings ranging from 0.30 to 0.40 can be taken as a lower cut-off point in the formation of factor pattern (Büyüköztürk, 2015). In this study, 0.50 was accepted as the lower cut-off point. Accordingly, while the 1st-factor loadings vary between 0.520 and 0.732, the 2nd-factor loadings vary between 0.560 and 0.700.

Table 4. DIS-R Sub-Dimension Names and Item Numbers that Constitute the Dimensions

Sub-Dimension No	**Sub-Dimension Name**	**Sub-dimension item numbers**
First Sub-Dimension	Cognitive/Emotional	Item 3, Item 5, Item 6, Item 7, Item 8, Item 11, Item 23, and Item 28
Second Sub-Dimension	Physiologic	Item 12, Item 15, Item 18, Item 19, and Item 21

Before proceeding to the item analysis, the sub-dimensions were named according to the sub-dimension names on the original scale. Table 4 shows the DIS-R short-form sub-dimension names and the item numbers that constitute these dimensions.

Confirmatory Factor Analysis (CFA) Results of the Scale:

In this section of the study, the results of confirmatory factor analysis (CFA), which is another analysis used to test the construct validity of the scale, are included. CFA is an analysis method that is frequently used in the development of measurement models and provides significant convenience. This method is a process for creating a latent variable based on the variables observed through a previously created model. CFA has generally been used in scale development and validity studies or for the verification of a predetermined structure (Yaşlıoğlu, 2017). Confirmatory factor analysis (DFA) reveals how well the tested model explains the presented data with fit indices. Fit indices provide data on whether

the model will be accepted or not. If the obtained data are in the appropriate reference ranges, the model is accepted, if it is not, the recommendations in the modification indices are fulfilled to improve the fit indicators of the model. If the model still does not have acceptable reference values, it is rejected (Schumacker & Lomax, 2010; Kline, 2011). The goodness of fit indices, which are most commonly used in CFA and used as a reference in this study are listed as Chi-Square test statistic (χ^2), Degrees of freedom (df), Chi-square/Degrees of Freedom (χ^2/df), The Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), and The goodness of Fit Index (GFI) (Şimşek, 2007; Çokluk, Şekercioğlu & Büyüköztürk, 2012; Koyuncu & Kılıç 2019).

CFA Multivariate Normality Test

Before starting the CFA, the structure is tested for multivariate normality. When the multivariate critical ratio (c.r.) is <10.00 in the multivariate normality test for CFA, it can be accepted that the structure has multivariate normality without looking at other values (Kline; 2011). In this regard, firstly, the multivariate normality test of the scale was applied. Kline (2011) stated that if the multivariate value is greater than 8, the data set does not conform to the multivariate normal distribution, and if it is greater than 10, there is a problem. Since the study data did not show multivariate normality and the sample size was at an acceptable level, the "Assessment of Normality" method (Running this method in AMOS, that is to do analysis, is a prerequisite to continuing CFA analysis with this method. This analysis worked because the sample size was sufficient in this study accepted the sample size in the study, and CFA was continued (Schumacher & Lomax, 2010). Although the Multivariate value of the items does not meet the normality, the kurtosis and skewness coefficients of the questions constituting the scale dimensions provide the feature of normal distribution (Kalaycı 2014).

DIS-R CFA Results:

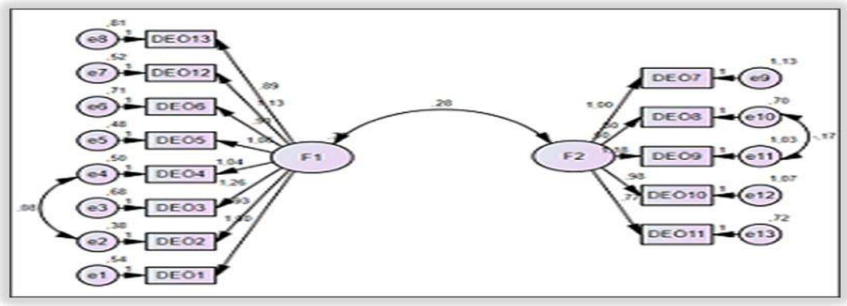
For the suitability of the model, first of all, the indicators of goodness of fit were examined. In the CFA test of the scale, it was observed that the model indicators had acceptable reference values. Table 5 shows the CFA excellent fit and good fit indicators (Çokluk, Şekercioğlu & Büyüköztürk, 2012; Schumacker & Lomax, 2010; Subaşı & Çetin, 2017; Gürbüz, 2019; Kline 2011).

Table 5. The goodness of Fit Indicators of the CFA Measurement of the Scale

Fit Indicators	Excellent Fit *	Good Fit *	Scale Values	Goodness of fit
$\chi 2$			119.16	
P value	0.05≤**p**≤1.00	0.01≤**p**≤0.05	.000	Perfect Fit
Df			62	
$\chi 2$/df	0≤***χ2/df***≤3.00	3≤***χ2/df***≤5.00	1.922	Perfect Fit
RMSEA	0≤**RMSEA**≤0.05	0.05≤**RMSEA**≤0.08	.030	Perfect Fit
CFI	0.95≤**CFI**≤1.00	0.90≤**CFI**≤0.95	.901	Good Fit
GFI	0.95≤**GFI**≤1.00	0.90≤**GFI**≤0.95	.960	Perfect Fit
AGFI	0.90≤**AGFI**≤1.00	0.85≤**AGFI**≤0.90	.930	Perfect Fit

The DIS-R goodness-of-fit values obtained as a result of the CFA are shown as scale values and goodness of fit. The results obtained are shown in table 5 respectively. As can be seen in Table 5, as a result of examining the indicators of goodness of fit as a result of CFA performed to test DIS-R, whose factor structures were created by EFA, according to the reference intervals specified in the literature, it was determined that it was a statistically appropriate model. Reference values close to 1.00 indicate a good fit between theory and collected data. This shows that there is no requirement to get exactly the same values given. Hair, Black, Babin, Anderson, (2010) emphasize that the research sample, the number of factors, structures, and variables (number of questions) evaluated in the study may affect the fit indices. The large number of structures in the model and the increase in the number of observed and latent variables are the factors that make it difficult to get fit indices. Through error terms, improvements in covariance between observed and latent variables are shown (Hair et al., 2010). To increase the goodness of the fit indices in the analysis results, a modification was required on an item. Figure 2 shows the results obtained after these operations and the PATH Diagram.

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$\chi 2$= 119,16; df= 62; $\chi 2$/df:1,922; p=,000; RMSEA= ,030; CFI=,901

Figure 2. DIS-R CFA Result Standardized PATH Diagram

Figure 2 shows the path diagrams between the measurement model used for the scale and the latent and observed variables. In addition, there is a moderately strong correlation of "0.28" in the same direction between F1 and F2.

Table 6. DIS-R Standardized Regression Coefficients

Regression* Weights			**Raw Forecast Coefficient**	**Sh**	**Critical Ratio**	**P**	**Standardized Forecast Coefficient**
DIS-R 1	**<---**	**F1**	1.00				0.643
DIS-R 2	**<---**	**F1**	0.93	0.07	14.20	***	0.681
DIS-R 3	**<---**	**F1**	1.26	0.08	16.52	***	0.687
DIS-R 4	**<---**	**F1**	1.04	0.07	15.00	***	0.672
DIS-R 5	**<---**	**F1**	1.06	0.07	15.04	***	0.687
DIS-R 6	**<---**	**F1**	0.90	0.08	11.82		0.551
DIS-R 12	**<---**	**F1**	1.13	0.07	15.16	***	0.693
DIS-R 13	**<---**	**F1**	0.89	0.07	12.53	***	0.524
DIS-R 7	**<---**	**F2**	1.00			***	0.554
DIS-R 8	**<---**	**F2**	0.80	0.07	11.10	***	0.558

Regression* Weights			Raw Forecast Coefficient	Sh	Critical Ratio	P	Standardized Forecast Coefficient
DIS-R 9	**<---**	**F2**	1.18	0.09	13.11	***	0.637
DIS-R 10	**<---**	**F2**	0.98	0.08	12.44		0.556
DIS-R 11	**<---**	**F2**	0.77	0.07	11.13	***	0.539

**** DIS-R: Dysmenorrhea Impact Scale Revised Form***

As can be seen in Table 6, standardized regression weights between the factor and the item to which it is associated are in the range of .524 to .693 and at a statistically significant level (p<.001). Standardized values are expected to be close to .70 and less than 1.00 (Brown & Moore, 2012; Jöreskog, 2004; Kline, 2011). In addition, in the CFA, each item is expected to have a correlation of .70 and above or close to .70 with the scale factors. The lowest acceptable regression load is expressed as .50 (Hair et al., 2010). The results obtained are within the scope of the minimum regression interval rule. As can be seen in Table 6, when the critical value, which is considered to be statistically 1.96, is compared with the obtained critical values, it is seen that the obtained critical values are well above the value of 1.96. The results of the analysis show that each of the parameters describing the level of effect of dysmenorrhea is significant. It was determined that the regression loads of the scale items met the criteria sought, and the lowest load was found in the 13th item with =0.524. In this regard, it can be stated that the structure validity of the scale is confirmed by the structure presented in Table 6. As a result, the standardized regression weights of the scale in Table 6 are within acceptable ranges and are significant at the p<0.001 level.

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Table 7. Results of the Variance Values of the Model Proposed in the CFA of the Scale

Variance	Parameter Estimates	sh	Critical Ratio	P*
F1	0.383	0.043	8.824	***
F2	0.503	0.057	8.813	***
e1	0.545	0.046	11.808	***
e2	0.384	0.032	12.015	***
e3	0.682	0.048	14.140	***
e4	0.502	0.036	13.844	***
e5	0.482	0.037	13.121	***
e6	0.713	0.041	17.601	***
e7	0.525	0.036	14.695	***
e8	0.806	0.049	16.591	***
e9	1.134	0.068	16.686	***
e10	0.702	0.055	12.886	***
e11	1.027	0.078	13.178	***
e12	1.072	0.069	15.436	***
e13	0.725	0.054	13.541	***

* p< . 000, F1: Cognitive/Emotional Impact Sub-Dimension, F2: Physiological Impact Sub-Dimension

As can be seen in Table 7, as a result of the CFA of the DIS-R, it was determined that the variance and covariance values of the scale were within acceptable ranges and were statistically significant at the p<.01 level. As a result of the CFA analysis performed to determine whether the factors of the DIS-R form a holistic structure, it was determined that "two factors were in the same structure and the factor structure of the scale was confirmed. When all values were examined, the

results obtained were not analyzed by second-level CFA, since the scale had two sub-dimensions (Gürbüz, 2019). Because the goodness of fit indices of the first-level and second-level CFA results of the three- and low-factor models are always the same. In such a case, it is more acceptable to rely on the always simpler, first-level CFA model (Bollen, 1989).

Reliability Study:

After the validity analysis and procedures, various reliability analyzes were performed to determine the reliability of the overall and sub-dimensions of the scale, and the results and values obtained are presented in the tables below.

Internal Consistency Coefficients of the Scale:

Reliability is an indicator of how consistently the measurement tool measures the feature or features it measures. Reliability is a concept that reveals the consistency of all questions in a measurement tool and their homogeneity in measuring the formation under consideration. It is also defined as the degree of freedom from random errors of measurement results (Tavşancıl, 2010). In a sense, reliability is part of validity. For a test to accurately measure the feature it wants to measure, that test must first measure the feature in question consistently (Tekin, 2016). In internal consistency, which is one of the methods of testing reliability, Cronbach's alpha coefficient is calculated for the whole scale and its sub-dimensions in Likert-type scales. A high Cronbach's alpha coefficient indicates that the scale items are consistent with each other and measure the same variable (Ergin, 1995; Karasar, 1994). DIS-R consists of 13 items and 2 sub-dimensions. In light of the obtained data, the internal consistency coefficients for DIS-R were calculated and the results obtained are presented in Table 8.

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Table 8. Internal Consistency Coefficients of the Scale

Coefficients	n	Number of Items	r
Cronbach α	1004	13	.836
Spearman-Brown	1004	13	.794
Guttman	1004	13	.789

As can be seen in Table 8, as a result of the analysis performed to determine the internal consistency reliability of the DIS-R (overall), Cronbach's alpha value was calculated as α=0.836. In addition to determining Cronbach's alpha coefficient, the split-half reliability coefficients obtained by dividing the items into two equivalent halves were also calculated. Accordingly, the Equal-length Spearman-Brown value was calculated as S=0.794 and the Guttman split-half value was calculated as G=0.789. In the process of calculating the Spearman and Guttman values, Cronbach's alpha coefficient was found to be α_1=0.775 for the first group from the two halves formed, and α_2=0.683 for the second group. As a result of the analysis conducted to determine the internal consistency reliability of the cognitive/emotional impact sub-dimension, Cronbach's alpha value was calculated as α=0.834. In addition to determining Cronbach's alpha coefficient, the split-half reliability coefficients obtained by dividing the items into two equivalent halves were also calculated. Accordingly, the Equal-length Spearman-Brown value was calculated as S=0.780 and the Guttman split-half value was calculated as G=0.823. In the process of calculating the Spearman and Guttman values, Cronbach's alpha coefficient was found to be α_1=0.771 for the first group from the two halves formed, and α_2=0.714 for the second group. As a result of the analysis conducted to determine the internal consistency reliability of the physiological impact sub-dimension (overall), Cronbach's alpha value was calculated as α=0.700. In addition to determining Cronbach's alpha coefficient, the split-half reliability coefficients obtained by dividing the items into two equivalent halves were also calculated. Accordingly, the Equal-length Spearman-Brown value was calculated as S=0.685 and the Guttman split-half value was calculated as G=0.687. In the process of calculating the Spearman and Guttman values, Cronbach's alpha coefficient was found to be α_1=0.603 for the first group from the two halves formed, and α_2=0.642 for the second group. According to Tavşancıl, if Cronbach's alpha internal consistency coefficient of a scale is in the range of $0.60 \leq \alpha < 0.99$, the scale is reliable (Tavşancıl, 2010). According to these results, it

can be stated that all items in the scale measure the same feature. In other words, the feature measured by the developed scale is homogeneous. According to these results, DIS-R is a reliable measurement tool.

Scale Item Analysis Results:

Another way to calculate internal consistency for scale reliability is to calculate the item total score, item-rest correlation, and the item discrimination analysis (Ergin, 1995). In this regard, item total, item-rest, and item discrimination analyses were performed to calculate the internal consistency of the scale, and the results are given below, respectively.

Item Total and Item-Rest Results of DIS-R:

Item-total correlation is based on the examination of the correlation between the score obtained from each test item (the variance of each test item) and the total score obtained from the test. In general, it is stated that items with a corrected item-total correlation coefficient of 0.30 and above are good items, items between 0.20 and 0.30 can be included in the measurement tool, and items below 0.20 should be excluded from the measurement tool (Büyüköztürk, 2015). In the item-rest correlation, the relationship between the score obtained from a certain item and the score obtained from the whole test except that item is examined. Table 9 shows the results of this analysis.

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Table 9. Item Total, Item Remaining Results of DIS-R Internal Consistency (Item Analysis) Analysis

Item No	Item Remaining		Item Total	
	r	p*	r	p*
Item 3	.822	.000	.516	.000
Item 5	.819	.000	.589	.000
Item 6	.822	.000	.515	.000
Item 7	.817	.000	.598	.000
Item 8	.820	.000	.559	.000
Item 11	.825	.000	.479	.000
Item 12	.833	.000	.388	.000
Item 15	.829	.000	.410	.000
Item 18	.829	.000	.441	.000
Item 19	.830	.000	.418	.000
Item 21	.826	.000	.463	.000
Item 23	.820	.000	.550	.000
Item 28	.826	.000	.460	.000

** $p < .000$*

As can be seen in Table 9, the item total values for all scale items vary between .388 and .598, and the item-rest correlation values vary between .817 and .833.

Discrimination Analysis Results Based on Lower and Upper Groups of DIS-R

The way to calculate the discrimination analysis is to perform the value discrimination of the scale and the t-test analysis used for unrelated groups. The larger the scale value coefficient, the more distinctive the relevant item. For the t-test, on the other hand, its distinctiveness is decided not by how large the t coefficient (critical ratio) is, but by the size of the significance level (Ergin, 1995; Baştürk, 2013). For this, the scale items are sorted according to the total score from largest

to smallest, and independent group t-test analysis is performed according to the upper and lower 27%. The results obtained are presented in Table 10.

Table 10. DIS-R Lower-Upper (27%) Group Item Analysis Results (n:544)

Item No	Groups 27%	n	A	Sd	Statistical Results		
					t	Sd	p*
Item 3	*Upper Group*	*272*	*4.86*	*.380*	*20.35*	*542*	.000
	Lower Group	*272*	*3.33*	*1.17*			
Item 5	*Upper Group*	*272*	*4.94*	*.244*	*18.43*	*542*	.000
	Lower Group	*272*	*3.62*	*1.15*			
Item 6	*Upper Group*	*272*	*4.51*	*.670*	*22.45*	*542*	.000
	Lower Group	*272*	*2.73*	*1.11*			
Item 7	*Upper Group*	*272*	*4.87*	*.392*	*20.15*	*542*	.000
	Lower Group	*272*	*3.31*	*1.21*			
Item 8	*Upper Group*	*272*	*4.59*	*.513*	*19.52*	*542*	.000
	Lower Group	*272*	*3.15*	*1.10*			
Item 11	*Upper Group*	*272*	*4.40*	*.669*	*18.86*	*542*	.000
	Lower Group	*272*	*2.92*	*1.10*			
Item 12	*Upper Group*	*272*	*4.65*	*.747*	*19.62*	*542*	.000
	Lower Group	*272*	*2.86*	*1.30*			

Item 15	*Upper Group*	*272*	*4.80*	*.567*	*15.87*	*542*	.000
	Lower Group	*272*	*3.51*	*1.21*			
Item 18	*Upper Group*	*272*	*4.73*	*.705*	*21.25*	*542*	.000
	Lower Group	*272*	*2.77*	*1.34*			
Item 19	*Upper Group*	*272*	*4.69*	*.762*	*19.45*	*542*	.000
	Lower Group	*272*	*2.95*	*1.26*			
Item 21	*Upper Group*	*272*	*4.74*	*.658*	*15.07*	*542*	.000
	Lower Group	*272*	*3.51*	*1.17*			
Item 23	*Upper Group*	*272*	*4.55*	*.560*	*20.38*	*542*	.000
	Lower Group	*272*	*3.00*	*1.12*			
Item 28	*Upper Group*	*272*	*4.43*	*.710*	*17.96*	*542*	.000
	Lower Group	*272*	*2.96*	*1.15*			

A: Arithmetic Mean, Sd: Standard Deviation, *:p<.000

As can be seen in Table 10, as a result of the independent group t-test performed to determine the discrimination of the items, the difference between the means of the lower and upper groups for all items was found to be statistically significant at the p<.001 level. As can be seen in Tables 9 and 10, the results obtained prove that each item constituting the scale is in the same structure and is distinctive in terms of the feature they measure. Based on these findings, it was determined that all the items constituting the scale were reliable.

Table 11. Pearson Product Correlation Coefficients Between Overall DIS-R and Sub-Dimensions

Variables		1	2	3
1-Overall Score	*r*	*1*		
2-Cognitive/Emotional Impact Sub-Dimension	*r*	*.916*	*1*	
3-Physiological Impact Sub-Dimension	*r*	*.777*	*.476*	*1*

p<. 001

As seen in Table 11, as a result of the Pearson Product Moment correlation analysis performed to determine whether there is a significant relationship between the scale and its sub-dimensions, the relationships between the overall score and all sub-dimensions were found to be linear and statistically significant at the p<.001 level.

Table 12. DIS-R Test-Retest Results

Scale and sub-dimensions		Posttest Overall Score	Posttest Cognitive/Emotional Impact	Posttest Physiological Impact
Pretest Overall Score	*r*	*.923*		
Pretest Cognitive/Emotional Impact Sub-Dimension	*r*		*.943*	
Pretest Physiological Impact Sub-Dimension	*r*			*.902*

As can be seen in Table 12, according to the test-retest correlation results obtained from the scale and its sub-dimensions, it was determined that the scale gave consistent measurements over time (r=0.923). In this study, due to the appropriate time between the two measurements and the fact that the compliance did not change during this period, it was determined that the scale was consistent in both applications, that is, its invariance with respect to time.

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Discussion and Conclusion

In the scale development process, it has been reported that the reliability of the measurement tool is related to its consistency. Therefore, studies that question validity, such as what is wanted to be measured with the scale and whether the scale items measure purposefully, can come to the fore. For what purpose the measurements obtained from the scale will be used, the validity of the measurement also varies depending on the purpose (Ercan & Kan, 2004; Kalaycı, 2014). Content validity is employed to understand whether the items in the draft scale represent the desired feature in a balanced way (Tavşancıl, 2010). The draft scale was evaluated by five experts in the field. Before applying to the study group, 30 participants were piloted to test the comprehensibility of the scale items. In the principal components analysis, the KMO value was found to be 0.904. The KMO test is a measure of how suited your data is for factor analysis and the range of 0.80-0.99 is considered very good. Therefore, it can be stated that the KMO value in this study is at a very good level (Karagöz, 2021). Bartlett's test result was found to be 3384.412 ($p<0.000$). Bartlett's test tests the hypothesis that the correlation matrix is equal to the unit matrix. Rejecting the hypothesis means that the correlation between the variables is different from 1.00 and shows that the measured variable is multivariate in the universe parameter (Karagöz, 2021). These two findings show that the sample size used in the study is sufficient and the data are suitable to perform factor analysis (Karagöz, 2021). In the factor analysis, the variance rate explained by the first factor with an eigenvalue of 4,587 was 28.347%, the variance rate explained by the second factor with an eigenvalue of 1.435 was determined as 17.976%, and the total explained variance amount was determined as 46,323%. In the literature, it is stated that the higher the total variance ratios of the measurement tool, the stronger the factor structure. Some sources argue that the common factor variances of items should be above 1.00 and 0.66. However, since it is difficult to meet these rates in practice, it is reported that it will be sufficient for the percentage of the factor loads to explain the total variance between 40 and 60 in the analysis (Scherer, Wiebe, Luther & Adams, 1988). Scale factor loading values are a coefficient that explains the relationship of items with sub-dimensions. In the literature, it is stated that factor loadings ranging from 0.30 to 0.40 can be taken as a lower cut-off point in the formation of factor pattern (Büyüköztürk, 2015). In this study, while the 1st-factor loads vary between 0.520 and 0.732, the 2nd-factor loads vary between 0.560 and 0.700. As a result of CFA, the $\chi 2/df$ value was found to be 1.922, RMSA 0.30, CFI 0.901, GFI 0.960, and AGFI 0.930. Chi-square calculation is affected by the sample volume, that is, it increases as the sample volume increases (Muthén,

2001). As the sample volume increases to over 200, it becomes difficult to obtain an insignificant chi-square value. The model size can also increase the chi-square value (Kline, 2011). In our study, a sample size of over 200 was used. Therefore, in accordance with the literature, chi-square was not found to be insignificant in our study. Degrees of freedom is a significant criterion in the chi-square test and its ratio to the chi-square can be used as a criterion of the goodness of fit. If this ratio is less than 5, it is accepted as a good fit indicator (Kelloway, 1998). In our finding, it was observed that this ratio was less than 5. In the literature, values below 0.05 are considered a good fit for the RMSEA value (Kline, 2011), where a value of 0 indicates a perfect fit (Hu & Bentler, 1999). In the literature, GFI statistics are valued between 0 and 1, and values close to 1 are reported to show a good fit (Bryne, 2010). Monte Carlo studies have shown that the 0.95 value is a good criterion for the model to show a good fit to the data (Hooper, Caughan & Mullen, 2008). AGFI takes a value between 0 and 1, and a value close to 1 indicates a good fit (Schermelleh-Engel, Moosbrugger & Müller, 2003). A good fit is mentioned for AGFI values above 0.90 or 0.95. In addition, for this index, 0.90 indicates a decisive good fit according to the base model, while values greater than 0.85 are acceptable fit values (Vieira, 2011). For the CFI index, which is valued between 0-1, it has been reported in the literature that high values indicate a good fit (Kline, 2011; Schermelleh-Engel, Moosbrugger & Müller, 2003). All this literature information supports the validity of the CFA indices of the scale.

As a result of the analysis performed to determine the internal consistency reliability of the scale, Cronbach's alpha value was calculated as α=0.836. The Equal-length Spearman-Brown value was calculated as S=0.794 and the Guttman split-half value was calculated as G=0.789. Reliability shows the consistency between participants' responses to the scale items and how accurately the measuring tool measured the feature it wanted to measure. In addition, reliability is known as the criterion of invariance according to time. These criteria are factors that can affect the validity of the measurement tool. The valid measurement instrument is reliable, but not all reliable measurement instrument is valid. One of the frequently used methods for measuring internal consistency in terms of reliability is Cronbach's alpha coefficient. The higher this coefficient, the higher the internal consistency of the measurement instrument. In the literature, it is reported that the alpha coefficient between 0.60-0.80 indicates the scale is reliable, while a value between 0.80-1.00 indicates that the scale has high reliability (Tavşancıl, 2010). The internal consistency values of our scale show that it is reliable. In the literature, it has been stated that the lowest correlation coefficient that can indicate the consistency of an item with the whole test should be 0.20 (Ayvaşık,

2000). It was stated that the item-total score correlation should be at least 0.30 (Özgüven, 1999). Discrimination analysis is the value discrimination of the scale and the t-test analysis is used for unrelated groups. The larger the scale value coefficient, the more distinctive the relevant item. For the t-test, on the other hand, its distinctiveness is decided not by how large the t coefficient critical ratio is, but by the size of the significance level (Ergin, 1995). The results obtained reveal that each item forming the scale is in the same structure and is distinctive in terms of the feature they measure. The test-retest method is frequently used to determine the external consistency of the scale. In this technique, the scale is applied twice with specific intervals for the same group. The correlation of points between these two applications is calculated. This calculated value is known as the reliability coefficient (r=0.923). The closer this number is to +1, the higher the reliability (Büyüköztürk, 2015). In this study, it was determined that the correlation obtained from the test-retest was quite high.

The 13-item structure of the revised short form of the dysmenorrhea impact scale developed in this study, including cognitive/emotional and physiological sub-dimensions, was found to be valid and reliable. The factor loadings of the 13 items obtained are at acceptable levels. Test-retest reliability and internal consistency values were found to be acceptable. The results of CFA and EFA also support that the scale is valid. The item analysis results also support the validity and reliability of the scale. These results showed that the scale is a valid and reliable measurement tool.

Table 13. Revised Dysmenorrhea Affective Scale (Version II), Modified Question and Dimensions (Dysmenorrhea Impact Scale-Revised Short Form) (DIS-R)

Dismenore Etki Ölçeği-Revize Kısa Form (DEÖ-R)

SAYIN KATILIMCI AŞAĞIDAKİ İFADELER ADET AĞRISI YAŞAYAN BİREYLERE YÖNELİK HAZIRLANMIŞTIR. LÜTFEN *ÖZELLİKLE ADET AĞRISI YAŞADIĞINIZ* SÜRECİ GÖZ ÖNÜNDE BULUNDURARAK CEVAPLAYINIZ. KATKILARINIZ İÇİN TEŞEKKÜR EDERİZ.

MADDELER		**Boyut Adı**
1	Adet ağrım dersime/işime odaklanmamı olumsuz etkiler.	Bilişsel/duygusal
2	Adet ağrım olduğunda keyifsiz olurum.	Bilişsel/duygusal
3	Adet ağrım başarı durumumu olumsuz etkiler.	Bilişsel/duygusal
4	Adet ağrım olduğunda canım bir şey yapmak istemez.	Bilişsel/duygusal
5	Adet ağrım olduğunda o anda yapmakta olduğum faaliyete odaklanamam.	Bilişsel/duygusal
6	Adet ağrım olduğunda ilişkilerimde duyarlı olmakta zorlanırım.	Bilişsel/duygusal
7	Adet ağrım olduğunda sorumluluklarımı yerine getirmede zorlanırım.	Bilişsel/duygusal
8	Adet ağrım varken normalde zevk aldığım aktivitelerden hoşlanmam	Bilişsel/duygusal
9	Adet ağrım olduğunda karında guruldama, ishal, kabızlık, gaz vb. şikayetlerim olur.	Fizyolojik
10	Adet ağrım olduğunda daha sık tuvalete giderim.	Fizyolojik
11	Adet ağrım olduğunda sindirim problemleri (bulantı, kusma, şişkinlik) yaşarım.	Fizyolojik
12	Adet ağrım olduğunda terlemelerim olur.	Fizyolojik
13	Adet ağrım olduğunda iştah değişiklikleri (iştahta artma/azalma) yaşarım.	Fizyolojik

Yazarların Notu: Çalışmanın kaynak olarak gösterilmesi şartıyla ölçek kullanımı için ayrıca kullanım izni alınmasına gerek yoktur. Ölçekte ters madde bulunmamaktadır. Ölçek beşli likert tipi olup, kesinlikle katılmıyorum (1), katılmıyorum (2), Kararsizim (3), Katılıyorum (4), Tamamen Katılıyorum (5) şeklinde puanlanmaktadır.

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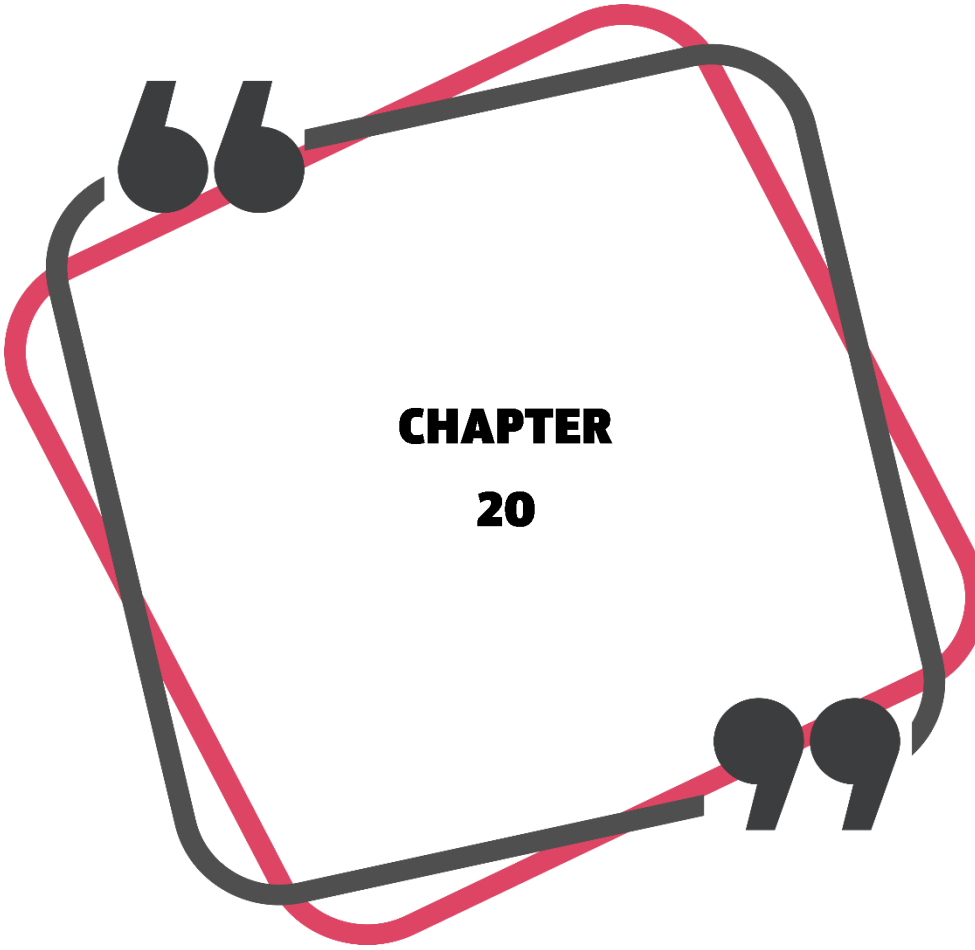
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CHAPTER
20

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THE USE OF CLINICAL PATHWAY IN NURSING EDUCATION AND PRACTICE

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1. INTRODUCTION

In nursing education and practice, students/nurses benefit from various initiatives designed to improve the quality of patient care, and to provide knowledge, skills and attitudes. The clinical pathway is used both in the efficient management of the patient care process and in the acquisition of information, attitudes and skills for nursing students and nurses

1.1. CLINICAL PATHWAY

The clinical pathway is the configuration of interventions that need to be carried out across disciplines in order to achieve outcomes during the intended patient care process within a specified timeline. Clinical pathway is a multidisciplinary clinical management tool. In short, it includes multidisciplinary initiatives, including diagnosing, planning, implementing and monitoring individuals who need clinical pathway care (Daş 1999; Özçelik et al. 2014). Multidisciplinary teams present initiatives by creating checklists for all required activities (Alşan 2009). The clinical pathway ensures effective use of quality patient care and resources by reducing application variance (Özçelik et al. 2014).

Critical pathway/paths, care path, care maps, anticipated recovery maps etc. with the same understanding of the clinical pathway in the field expressions are included. Clinical pathway is an important component of care management.

The history of clinical pathway is based on Gant semala-rina, which was developed in 1920. GANT schematics were created as schematics that visualize activities in a complex project and inspired the creation of clinical pathway. Afterwards, engineering and medicine started to be used in many fields. The use in healthcare is based upon 1980. Used for the first time at the New England Medical Center by Karen Zander and Kathleen Bower as part of the case management. Zander and Bower used the clinical pathway to see the results of resource use and health care interventions. It is currently being used in Germany, Belgium, the

Netherlands, Canada, New Zealand and Australia (Daş 1999; Alşan 2009). Furthermore, the literature also indicates that the use of the clinical pathway in Asia is leading to improvements in the care delivery process. (Cheah 2000).

Clinical pathways are intended to reduce the cost of soot, the quality of patient care and effective use of resources. However, with clinical pathway;

- Coordinate the steps in the patient care process
- Adoption of disciplinary roles in the care process
- Reaching quality standards
- Reducing hospitalizations
- Reducing patient care costs
- Improving patient satisfaction
- Increasing the quality of treatment and care
- Standardization of clinical documentation Identify and improve R&D issues related to any situation
- Carrying out interdisciplinary communication and service planning
- Collecting the data in the care process and providing the necessary control
- It is aimed to reveal the deviations, to determine the variances and to reduce them in case the patient cannot show the expected improvement. (Alşan 2009; Cheah 2000; Yu and Han 2021).

Allen et al. (2009) the clinical path according to the systematic review study; It is effective in supporting care management, receiving clinical interventions and evaluations for patients on time, improving service quality and service efficiency. In addition, it can positively support the communication process between health professionals during treatment and care for the patient. (Allen, Gillen, and Rixson 2009). The clinical pathway improves quality by standardizing care (Liu et al. 2021).

1.1.1.Clinical Pathway General Specifications

The clinical pathway is cooperation by experienced healthcare professionals and guides the treatment and care process. However, the clinical pathway differs in some aspects from other practice guidelines.

- *The clinical pathway is a multidisciplinary tool:* The clinical pathway is usually intended for a member of the healthcare team (eg: physician, nurse). This is a multidisciplinary process that requires the participation of all health professionals..

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- *Timeline:* In the clinical pathway, all interventions by the care team are shown with a timeline. The time frame applied may vary depending on the patient population or institution
- *Expected results from the patient:* During the care process, the results expected from the patient are processed on the clinical pathway. The patient's results can be displayed in aggregate, as well as as an intermediate result.
- *Key elements of care:* The main elements of care in the clinical pathway vary depending on the institution or medical diagnosis, but are usually evaluated as assessment, consultation, tests, care practices, medication, diet, activity, environmental safety, patient/caregiver education, discharge plan addressed by eight main elements. (Daş 1999; Özçelik et al. 2014).

1.1.2. Advantages of Using the Clinical Pathway

The use of the clinical pathway provides advantages for patients, healthcare professionals, and the institution.

- *Advantages for the patient:* There are undoubted advantages in using the clinical pathway in the care process. By sharing the clinical pathway with the patient or his/her relatives, the participation of the patient and their relatives in the care process can be ensured. In this case, both patients are aware of their condition and an environment of trust is created by strengthening their communication with health professionals.
- *Advantages for healthcare professionals:* All healthcare professionals can be informed about the patient care process through the clinical pathway. This situation strengthens communication and cooperation between health Professional. The clinical pathway can be used to assess the patient care process in detail. This reduces the error rate, reduces the workload of healthcare professionals and increases job saturation.
- *Advantages for the institution:* The data for assessing patient care is obtained systematically via clinical pathway. This allows improvements to be made in the care process. In this case, the quality of patient care will increase and the length of hospital stay will decrease with the clinical pathway. As resource usage in the institution will decrease, care costs will also decrease. (Daş 1999; Özçelik et al. 2014).

Chen et al. (2022), the use of clinical pathway can positively affect the effect of treatment, shorten hospital stay and increase nursing satisfaction. (Chen et al. 2022). According to Xiao et al. (2022), on the other hand, the use of clinical pathway in the care process not only reduces the hospitalization costs and length of stay of the patients, but also improves neurological function and daily living skills, decreases complications and increases patient satisfaction. (Xiao, Xiao,

and Yu 2022). The clinical pathway, however, allows the patient to understand the contents of their own care plan and encourages the patient and the patient to participate in the care (Fu et al. 2020).

1.1.3. Clinical Pathway Assessment

The clinical pathway is developed by expert healthcare professionals involved in patient care. While evaluating the clinical pathway, the functionality of the planned process, whether there are deviations and the clinical results expected from the patient are evaluated on the day or at discharge. (Özçelik 2011). In clinical pathway initiatives, deviations from possible results are called variance. Positive variance for positive results in less time than expected for the patient's condition is indicated as a negative variant for a worse outcome than expected, resulting in four key factors in variance encountered in clinical pathway.

- *Factors related to the patient or family:* Failure to perform any attempt the patient has rejected is due to the patient.
- *Factors related to health personnel:* It is caused by the people responsible for patient care.
- *Health institution or system related factors:* The operation of the institution or the functioning of systems in the institution.
- *Community-related factors:* Limited access to care services, inaccessibility to home care services, etc. states can be counted (Daş 1999).

The variances reached as a result of the evaluation of the clinical pathway should be analyzed, problems should be identified, and interventions for elimination and improvement should be planned and implemented. If necessary, improvement should be made by making necessary adjustments in the clinical pathway. (Daş 1999). The use of clinical pathways in healthcare systems is increasing and is seen as a form of quality improvement. (Jabbour et al. 2018).

1.1.4. Use of Clinical Pathways in Nursing Education and Practices

There have been many studies on the use of clinical pathways in nursing practice. In their study, Kalal et al. (2021) reported that the use of clinical pathway in the postoperative care of patients who underwent surgery for mucormycosis was effective in the positive progression of the condition of the patients. However, the researchers investigated the physiological, psychological states of the patients and the effect of drugs, etc. used clinical pathway to monitor parameters of different domains(Kalal et al., 2021). Zhao et al. (2021), on the other hand, examined the self-care capability, quality of life, and satisfaction of nurses in patients with ovarian cancer of health education using the clinical pathway. As a result of the research, they found that the patient's self-care capability and quality of life increased significantly and increased satisfaction with the nurses. As a result of the

research, they determined that the self-care capability and quality of life of the patients increased significantly and the satisfaction with the nurses increased. (Zhao et al. 2021). Chen et al. (2022), in their study examining the effect of clinical pathway use on venous thrombosis patients, routine nursing care in cardiology was performed in 85 patients in the control group, and nursing care was carried out using the clinical pathway in 85 patients in the intervention group. As a result, the incidence of deep vein thrombosis and the incidence of extremity pain were lower in the intervention group.r (Chen et al. 2022). Loeb et al. (2006) evaluated using the clinical pathway for the treatment of pneumonia and other lower respiratory tract infections in rest homes. Researchers have indicated that treating using clinical pathway reduces hospital hospitalization and health care costs (Loeb et al. 2006).

Clinical pathway is used to provide knowledge, skills and attitudes to nursing students and clinical nursing education. According to Naqib et al. (2018) in their study; pain management strategies were created in the clinical pathway for pain management and nurses were given training within the scope of the pain management training program. The researchers have improved the pain experience of patients and found an increase in overall patient satisfaction as a result of attempts with clinical pathway and nursing training (Naqip et al. 2018). The clinical pathway is one of the tools used in nursing education for the nursing student to reach clinical results accurately and effectively. The clinical pathway guides students in the management of care. In addition, new clinical pathways are designed to evaluate different clinical difficult cases and develop new strategiesThis process benefits students in developing critical thinking skills as well as professional behavior (Bradshaw 1999).

As a result, clinical pathways provide guidance for both nursing students' education and nurses' education. Since the use of clinical pathways in the patient care process contributes to the effective implementation of care management, patients' quality of life and patient satisfaction increase, hospitalizations and care costs decrease. This situation reveals the result that the clinical pathway can be used as an effective tool in nursing education and practice.

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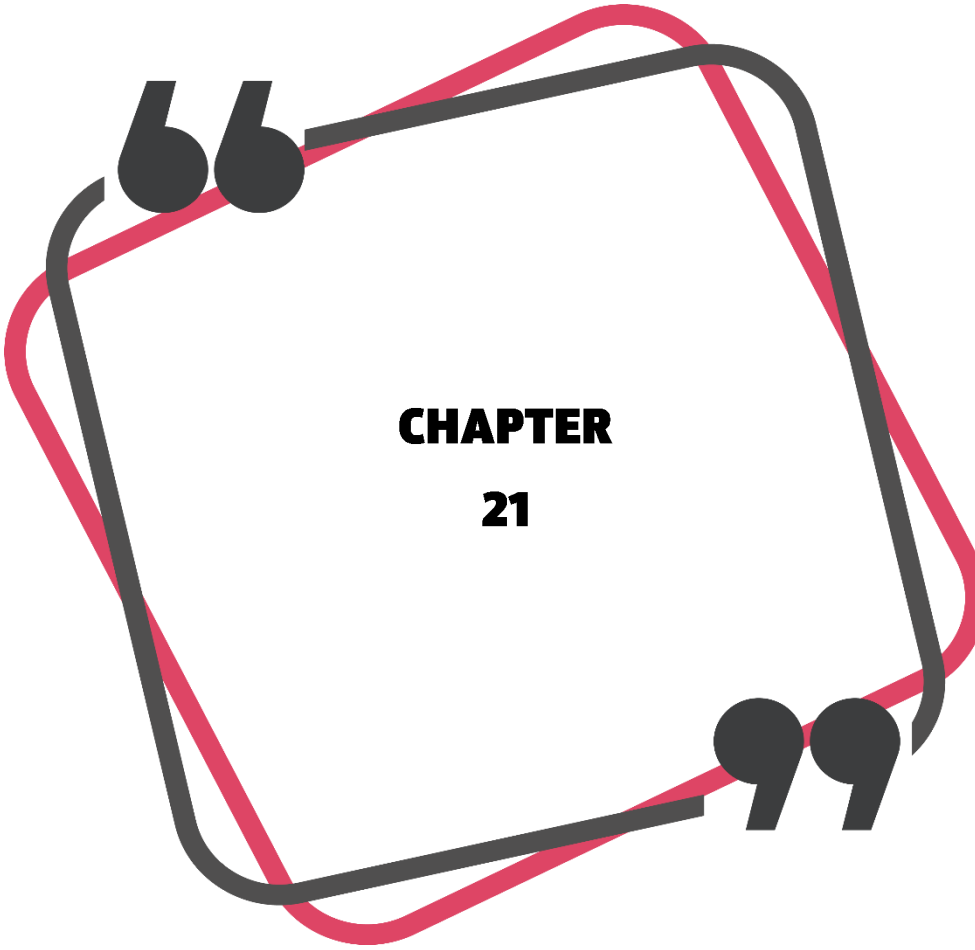
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CHAPTER
21

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SKIN TATTOOS FROM THE PERSPECTIVE OF MIDWIFERY

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Introduction

Tattoos have been regarded as a way for people to express themselves non-verbally from past to present. Before the invention of writing, with the pictures drawn on the cave walls, humanity performed the first works of art and the first written communication. The projection of this created visual language on the human body supported the individual's purpose of conveying a message and having a visual identity. Formerly, tattooing in many societies, especially in the western world was used to stigmatize certain groups such as gang members, inmates or members of the military over the years, and it has been associated with masculinity, rebellion, revolt and negative character traits. After the 1970s, with the spread of tattooing and its transformation from traditional patterns to contemporary original designs, tattooing has lost its sign indicating a status and has turned into a fashion trend among young and middle-aged individuals from all sociocultural and economic levels, regardless of sex (Çerikan et al., 2016). Tattooing, which has become increasingly widespread, has also brought about the discussion of some issues. For instance, while the themes of individual preferences and freedom have been emphasized on the one hand, social norms and health risks have come to the fore on the other hand.

Given health risks, tattooing is an invasive procedure that damages the skin since the skin acts as a barrier against external aggressions such as chemicals and microorganisms (Laux, et al., 2016). Although tattooing is considered safe by

many, one should be aware of its possible risks. Thus, people who are at risk of developing side effects should consult their healthcare professionals before they decide to have a tattoo. In the present study, the aim was to enable midwives to answer questions about tattooing asked by women, and to inform them about how to provide detailed guidance for people who want to have a tattoo but have concerns about skin diseases and/or other medical conditions.

Tattoo

"Tattoo" which has a long history is a tradition that exists in almost all cultures of the world, especially in Egypt, Mesopotamia, Anatolia, Europe, Asia and America (Karatay, 2018). Tattooing, which was used as a way of expressing fears, likes, expectations, beliefs, traditions, social and class status in primitive-communal societies, lines/signs and motifs were imprinted on naked bodies, and the messages desired to be given to another person, a society and nature are conveyed through this method. The Turkish Language Association defines tattoo as "an indelible writing or painting made by making small holes in the skin of the body with a sharp instrument such as a needle, and inserting tattoo ink into these holes" (Turkish Language Association, 2022). The word "dövme" which is the Turkish equivalent of tattoo is derived from the Turkish word "dövmek", which expresses the action of "beating the body" in Anatolian Turkish (Akalın, 2019). When the word "dövme" is used as a noun, it means "mark" or "body mark", derived from the word "dâg-tâğ". Dâg-tâğ means a mark or sign, which is marked on the human and animal body with a branding hot iron (Akalın, 2019; Karatay, 2018). The western equivalent of "dövme" is the word "tattoo". It derives from a Tahitian word "ta-tau" which is the echo of the rhythmic banging, hitting or beating sound made when tattooing is performed. (Akalın, 2019; Kanbir, 2022).

Tattoo represents sometimes a sacred object of a tribe, sometimes a secret power that protects people from evil/disease, sometimes a mark indicating that the person is a criminal, sometimes a certificate of ownership showing that a slave has been sold, sometimes a symbol of heroism of warriors, sometimes a badge of nobility of a noble person, sometimes a symbol indicating a circus clown's oddity or sometimes a tool to ornament / decorate the body (Çerikan & Alanko, 2016). In addition, it is based on a belief that a tattoo talisman protects the person from the evil eye, and that it brings plentifulness. It is also made to indicate that the person belongs to a community, or to look beautiful (Begic & Çapık, 2020; Karatay, 2018).

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History of Tattoo Art

The review of the history of the tattoo indicates that tattooing (tatuaj) has been performed for the decoration of the human body with symbols and pictures using different materials in more or less all cultures and that it dates back to ancient times in history. In 1991, Austrian archaeologist Konrad Spindler (1939–2005) stated that the primitive "Iceman" named Ötzi who is estimated to have lived 5300 years ago in the Alps on the Austrian-Italian border had a tattoo on his body. Slime tattoos in Japan are thought to date back to 5,000 B.C. (Samadelli, et al., 2015)

In the ancient times, tattoos were colored with inks composed of pigments derived from reeds or leaves. It is stated that tattoos have been found on Egyptian mummies dating from the 2000s B.C. In ancient Egypt, it was believed that tattoos had a therapeutic role, that they functioned as a permanent talisman, especially in high-risk pregnancies and difficult births, and that they protected women. These tattoos are largely done over the abdomen, thighs and chest. It was believed that as the gestational age progressed, the tattoo expanded to cover the abdomen, and thus protected the woman and the baby to be born. Examples of tattoos from Ancient Egypt shed light on the fact that tattooing was a purely female tradition there (Lineberry, 2007).

According to the results of archaeological excavations and research, it is known that noble and heroic people in ancient Eastern societies such as Huns, Kazakhs and Kyrgyz had tattoos (Çağlayandereli & Göker, 2016).

Written information about tattooing has survived until the present day thanks to the manuscripts written by ancient Greek and Roman thinkers and historians, and the Torah, the holy book of the Jews, dating from 1400 B.C. The evidence for the preliterate era is paintings and sculptures obtained from various archaeological excavations, various tools and equipment used to make tattoos, and most importantly tattooed human bodies that have been preserved by mummification (Begic & Çapık, 2020).

It is known that tattoos were used to mark criminals and slaves in Ancient Rome and in England in the following centuries. The tattoo technique, which is made by opening a wound on the skin is encountered in the natives of Australia and Central Africa. In addition, it has been determined that tattooing is an important element of indigenous folk cultures in America, the Poles and Japan. Roman physician Aetius who lived in the 6th century A.D. wrote the oldest known text on tattoo technique in his book called 'Medicae Artis Principes' (Çağlayandereli & Göker, 2016).

Religiously, tattoo marks can be found in beliefs such as Shamanism, Paganism, Animism, Sabianism, Taoism, Buddhism, Manichaeism and Zoroastrianism. The basis of tattoos is figures or images that are supposed to help protect the body and soul of the person and refers to the religious beliefs and myths of the relevant communities. In the three major religions, Judaism, Christianity and Islam, tattooing is prohibited and is not welcomed (Seval, 2017).

Information about tattoos at the beginning of the Turkish-Islamic periods in Asia and Anatolia is insufficient. It is stated that in the Oghuz Turks who moved to Anatolia, there was a symbol for each tribe as it was found in almost all of the Turkish cultures. In this sense, hawk, eagle and rabbit were the symbols of the tribes of Oghuz Turks such as Günhan, Ayhan and Yıldızhan, respectively (Çağlayandereli & Göker, 2016). In the Ottoman period, tattoos were used in meanings similar to those in Europe, especially in the 19th century, with the influence of the westernization movement. At that time, tattoo pointed to a professional group, social status or the community to which the person belonged, such as tribe and sect. For example, tattoos became widespread among Ottoman sailors through Algerian sailors. Then, as in the British army, Janissaries, a military unit in the Ottoman Empire, started to have tattoos to symbolize the division they belonged to from the 17th century until the closure of the Janissary corps (Aytuğ, 2015). After the Turks' conversion to Islam, "tattoo" has almost completely disappeared from Turkish cultural life (Begic & Çapık, 2020).

Traditional Tattoo Art

Men and women who do tattoos to express sacred wishes, good intentions about life and inexpressible emotions as artisanship are called "dekkak" and "dekkake" in Turkish, respectively. These words are derived from the Kurdish and Persian word 'deq' meaning tattoo (Akalın, 2019). As in any artisanship, dekkake and dekkak must have certain personal characteristics and certain abilities and skills. In tattooing, wishes, hopes and expectations are desired to be positive; thus, those who make tattoos are expected to have well intention and good character, and to be healthy and happy people. Dekkakes or dekkaks are also expected to know what traditional motifs should be chosen for men and women, and to be knowledgeable and experienced in materials and techniques (Yenipınar & Tunç, 2013).

While the tattoo art is executed, many different substances such as indigo, herb extracts, henna, saffron, ink, liquid, zinc-carbon, sheep milk and cow milk are used.

Especially in the Southeastern Anatolia and Eastern Anatolia regions of Turkey, breast milk, eye lashes, soot, pancreatic juice (animal bile) are used as the basic dye material (Yenipınar & Tunç, 2013; Akalın, 2019). The most striking tattoo material mentioned in the literature is breast milk taken from a mother whose baby is a girl. Such milk, which is very different from the materials used today, is called *"halip ummu'lbint"* in Arabic. Soot, obtained from the bottom of the sheet pan used to make bread, called Sirac. The pancreatic juice of the animal is called el-merare in Arabic. The mixture of these three materials is used as tattoo ink (Yenipınar & Tunç, 2013).

The motif determined for the tattoo is drawn on the skin with a pen, goose feather or a matchstick, and its borders are clarified with tattoo ink. Then, rhythmic strokes are made with needles on the motif and the ink is inserted into the skin. The number of needles varies according to the size of the motif and the area tattooed (Demir, 2015). It is believed that the tattoo ink will replace the blood that has come out and will penetrate the skin better, which makes the tattoo motif look more beautiful. Tattooing is performed between the end of March and the beginning of April, which is called *"Merbaiye"* in Arabic, because it is believed that tattoos made during this period heals faster (Begic & Çapık, 2020).

Tattoo motifs are basically a reflection of a person's personal feelings and thoughts. Motifs are stated to reflect the living geography, social environment and traces of traditions.Among the most frequently used motifs are the ones that symbolize love and plentifulness, fertility (childbearing), life and death, nature, and the "Tree of Life", which represents all life phenomena from birth to death (Yenipınar & Tunç, 2013) .

Modern Tattoo Art

Tattooing (including piercing) has been practiced worldwide for at least 5,300 years. The demand for this art has gradually increased since the 1970s and has become more common. About one-third of people living in the United States have at least one tattoo. Of these individuals, 69% have more than one tattoo. In the last 40 years, the rate of tattooing among women has quadrupled. Today, more women than men have tattoos (Rademeyer, et al., 2020).

Tattoos, which have become increasingly common in all ages and societies, have now become preferred figures that represent personal expression, individuality and creativity. Tattoos, which are made with an effort to exist in the social area, to express oneself, to look good, to feel good, to create an identity and self,

have recently become preferred by people with cosmetic concerns (vitiligo, alopecia, burns). For example, tattooing of a woman who has had a mastectomy with motifs such as flowers and clovers that are made on that nipples or give the appearance of nipples makes the woman feel good (Rademeyer, et al., 2020; Antoszewski, et al., 2010; Tsang, 2014). Today, the types of tattoos in our country, and their characteristics and purposes are summarized in Table 1.

Table 1. Types of Tattoos

Type	**Characteristics**	**Purpose**
Professional	• Deep and intense dermal pigment injections • Often multicolored	• to express oneself • to remember an important life event constantly • to state that one belongs to a group that he or she has adopted
Amateur	• More superficial and less intense injections • Usually a single color	• to express oneself • to remember an important life event constantly • to state that one belongs to a group that he or she has adopted but at a more limited cost
Cosmetic	• to support especially facial or breast reconstructive surgery • to camouflage chronic skin conditions (eg scars, burns, striae, alopecia, vitiligo)	• Popular among people with health conditions that interfere with makeup application, such as visual impairment, tremor, osteoarthritis, myopathy. • Nipple and areola tattoos contribute positively to the quality of life and body image of women undergoing breast cancer treatment and breast reconstruction.
Medical	• To create a warning sign, mark	• An alternative to medical-alert bracelet for those who have a life-threatening disease such as

	diabetes, Addison's, hypothyroidism, epilepsy, severe allergies or for those who are mentally disadvantaged (e.g. mental retardation, Alzheimer's).

Reference: Akarsu, 2022

Today, electric machines working at various speeds according to the figure to be drawn in tattoos are used. During the process, different shapes of needles with different gauges are selected in order to create a line, to color or to give a shadow effect. Ink used as a colorant in tattooing consists of pigment not fading in a liquid such as ethyl alcohol or water, and heavy metals, salts and other chemicals (Akarsu, 2022).

Although tattoo applications are performed with modern techniques and equipment, the person who has tattoo may suffer mild discomforts such as tenderness, pain, itching, swelling, edema, papule, etc. These symptoms can be managed with simple interventions. However, there are increasing concerns that new generation inks can cause cancer and mutagenicity. If absolute and relative contraindications are carefully taken into account before tattooing, the risk of tattoo-related complications decreases. Cases in which tattoo application is contraindicated are presented in Table 2.

Table 2. Situations Where Tattooing Is Contraindicated

Contraindication	Cause
Absolute contraindications	
Pregnancy and breast-feeding	• Exposure to mutagens and carcinogens in tattoo ink • Potential exposure to infectious diseases
Nickel and chromate allergy	• Most tattoo inks are contaminated with nickel, and many contain chromium.
Tattooing on or near a scar resulting from a previous malignancy, such as melanoma	• Difficulty in following-up cancer recurrence
Relative contraindications Sarcoidosis	• Black ink can trigger the development of granulomas in the lungs and other areas away from the tattoo, or cause recurrence in those in remission.
Immunosuppression	• Impaired wound healing, increased risk of infection
Chronic infection (e.g. AIDS, hepatitis B, C)	• Increased risk of systemic infection
Eczema, psoriasis	• Exacerbation of the disease at the tattoo site or distant sites
Past/current MRSA* infection or known MRSA carrier	• Increased risk for antibiotic-resistant wound infection
History of keloid scar formation	• Increased risk of abnormal scar development or deterioration of tattoo
Coagulation disorder or using anticoagulants	• Increased risk of excessive bleeding
Epilepsy	• Seizure risk due to pain and stress during or just after tattooing
Cardiac arrhythmia	• Risk of fainting during or just after tattooing
Diabetes	• Delay in wound healing • Hypoglycemia risk due to the long duration of the procedure
Latex allergy	• Anaphylaxis risk due to the use of latex gloves by the tattooist or at the tattoo studio

Heart valve disease	• Increased risk of bacterial endocarditis
Inflammatory bowel disease	• Increased risk of pyoderma gangrenosum
Systemic lupus	• Risk of developing new lesions at the tattoo site due to trauma
Melanocytic nevus where tattoo is to be done	• Increased difficulty in monitoring nevus for malignancy
Active hidradenitis suppurativa	• Increased risk of bacterial infection
Psychosocial perception (individual and social)	• Dissatisfaction, internalized stigma, susceptibility to mood changes

*MRSA: *Methicillin-resistant staphylococcus aureus*

References: Serup, 2017; Temiz & Özlü, 2021; Juhas & English, 2013; Farley, et al., 2019; De Cuyper, 2020

Healing process after tattooing

The points to be considered after the procedure are given below (Serup, et al, 2015b).

Keeping the tattooed area clean: The tattooed area is cleaned using a soft and PH-balanced soap with light touches without rubbing. While the person takes a shower, he or she should avoid direct water flow to the newly tattooed skin. He or she should make sure the water is lukewarm. The tattooed area is dried by buffering with a paper towel.

Using moisturizer: The first 24-48 hours are of great importance in tattoo care. In this process, the moisture balance of the tattooed area should be maintained. The skin is moisturized with a perfume-free cream or ointment. Generally, it is enough to moisten the tattoo 1-2 times a day, but this frequency may vary according to the needs of your skin.

Avoiding exposure to direct sunlight: The tattooed site should be kept out of the sunlight for at least a few weeks. In this process, clothing that prevents the sun's rays from reaching the skin can be used. Afterwards, creams containing sun protection factor can be used.

Avoiding swimming: Swimming in the sea or pool, or bathing in the jacuzzi may cause irritation or infection of a new tattoo, and delay in healing. Therefore, it is generally recommended not to swim four weeks after getting a new tattoo. This period may be longer if the tattoo covers a large skin surface.

Being careful in choosing clothes: Clothes that do not wrap the body, do not tighten, and allow the skin and tattoo area to breathe should be preferred. It is ideal to wear cotton clothes instead of synthetic fabrics.

Being patient: Care should be taken not to break off the crusts as the design of the tattoo may be damaged and may cause scarring. Full recovery may take about two weeks.

Women and Tattoo Art from the Midwifery Perspective:

Although tattoo is known as an art, an addiction and a passion that attracts everyone's attention in the history once it is done, it is perceived one of the leading body accessories today. People who reflect their life philosophy through a tattoo perceive tattoo as a form of identity development, and differentiation from parents (Rademeyer, et al., 2020).

Adolescence is the transitional period from childhood to adulthood during which adolescents display risk-taking behaviors such as reckless driving, drug and alcohol use, smoking, and unprotected sexual activity (Armstrong, et al., 2000). Unprotected sexual activity can lead to sexually transmitted diseases and unwanted pregnancies. Adolescents who act with emotions such as impulsive actions, invincibility, defiance, inability to predict potential negative effects, excitement seeking, and lack of attention take more risks. Armstrong and Pace-Murphy added tattooing to the list of adolescents' risk-taking behaviors, and identified the following potential health hazards of tattooing in this age group: risk of developing infection and hepatitis B, and psychosocial risks such as embarrassment, low self-esteem, and disappointment with the tattooing they have had (Armstrong & Pace-Murphy, 1997).

Tattooing has become a more common phenomenon among adolescent pregnant women. Getting tattoos, a risky behavior displayed by adolescent pregnant women, may endanger both pregnancy and the health of the fetus. Therefore, it is recommended adolescents should be monitored more closely (Armstrong, et al., 2000).

The tattoo practice, which has become widespread among young people today, is actually a traditional practice among women of Indian origin. The art of

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henna called Mehndi in Indi is traditionally associated with Indian bridal ceremonies, and it is included in the blessing ceremonies for a pregnant woman and her child. Some midwives and care providers are of the opinion that it is appropriate for a pregnant woman to get a henna tattoo on her belly. However, conditions in which tattoos are made with tattoo ink and Indian henna are not hygienic can lead to serious problems such as infection (Rademeyer, et al., 2020; Kluger, 2010).

It has been demonstrated childbearing women's having tattoos before and during pregnancy does not lead to a toxic damage such as abortion, congenital disorders, and shape malformations to reproduction. However, this issue has not been systematically studied. In the literature, it is striking that not only in the past but also today, women who want to become pregnant or who are pregnant do not hesitate to have tattoos made with tattoo ink whose content is known. However, it is also known that such tattoo materials have biokinetic and systemic effects (Serup, et al., 2015a; De Cuyper, 2020).

Since the number of studies conducted on pregnancy and tattooing is limited, it is very difficult to present fully evidence-based data. Although no teratogenic risk has been shown in women who are tattooed during pregnancy and pregnant tattoo artists who do tattoos, tattooing during pregnancy is not recommended because pharmacological agents are used in the treatment of tattoo-related textural reactions (fibrosis, granulomatosis, keratoacanthoma, etc.), skin infections and hypersensitivity reactions. This recommendation is also included in the professional standards of the Professional Tattooists Association as a rule that tattoo artists should not tattoo pregnant women (Rademeyer et al., 2020; Kluger, 2010).

Due to the increasing prevalence of tattoos, it becomes increasingly difficult to control places where tattooing is performed. This causes us to worry about many issues such as tattoo equipment, colorants, sterilization methods, and hygiene practices, as well as possible risks and problems. It should be kept in mind that tattoos made in unhealthy conditions will affect the health of people who have tattoos done. Therefore, women should be warned that they should be very careful especially if they get a tattoo during pregnancy. There are doubts about whether the materials used in tattooing affect the fetus. However, data on the teratogenic effect of tattoo inks applied in the first trimester of pregnancy are limited (Kluger, 2010; Farley & Hoover, 2019).

A young woman who was diagnosed with pregnancy got a tattoo in two sessions at the 6th and 21st gestational weeks which lasted for about 5 and 2 hours respectively. In the evening of the second session, she had no problems other than fatigue and loss of strength. In the fetal ultrasound monitoring, three lung cysts

were detected on the right lower segment of the right lung of the fetus. However, the delivery was unproblematic and the newborn developed no complications (Kluger, 2012). However, the Professional Tattooists Association has banned the implementation of this procedure on women who are pregnant. Given the exposure to many chemicals and nanoparticles in the colorant, the possibility of possible symptoms and complications should not be ignored. In addition, using various pharmacological agents in the treatment of negative results is compulsory. Considering all these, it is possible to say that this ban imposed by the Professional Tattooists Association is of great importance in protecting the health of the mother and fetus.

Among physiological changes in the breasts and abdomen during pregnancy are skin stretching and the development of striae. These changes can lead to changes in the appearance of existing tattoos. In fact, women who want to have a tattoo while they are pregnant should be warned that the appearance of the drawings might change due to the changes in their bodies and due to the return of the abdomen, which has enlarged during pregnancy, to its original state after delivery.

Epidural analgesia is often used for labor analgesia, and the back and lower back of a woman's body, where epidural analgesia is placed, are commonly used as tattoo sites in women. There is a theoretical concern among anesthesiologists that when epidural placement is performed through a tattoo, the use of a large hollow needle may carry tattoo pigment into the epidural space, resulting in an increased risk of arachnoiditis and epidermal tumors. National anesthesia standards that address this possible complication are not available. Some anesthesiologists refuse to administer epidural analgesia to women who have lumbar tattoos. Some anesthesiologists have stated that before they insert the epidural anesthesia catheter, they make a small incision from the area where the tattoo is intense to the area where the catheter will enter the epidural space. However, there is no data supporting this concern, and some experts believe that current technology eliminates this risk. More research should be conducted to eliminate this concern completely (Kluger & Sleth, 2020).

Surgical incisions or skin excision can change the appearance of the tattoo. If a woman has a tattoo on her belly, a cesarean incision should be made, and she should be counseled that the tattoo might affect her appearance. Preoperative counseling should also include investigating how important the tattoo for the individual. After surgery, some people may also want to get a new tattoo to hide the surgical scar (Rademeyer, et al., 2020; Kluger, 2010; Farley & Hoover, 2019).

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There is limited evidence on the safety of getting a new tattoo while one breastfeeds. Risks likely to arise during the breastfeeding period are similar to those observed during pregnancy.

It is recommended that getting a tattoo should be postponed until the baby is older than 8 months or until breastfeeding is completely terminated. Laser tattoo removal process should be postponed until the baby is weaned due to the possibility of fragmented pigment particles entering the systemic circulation (Rademeyer et al., 2020; Kluger, 2010). A tattoo artist who found out that the woman who wanted to get a tattoo was breastfeeding and thus was worried about it wanted to get the opinion of the Professional Tattooists Association, and the association recommended the artist to avoid tattooing during breastfeeding. No complications related to mother or baby have been reported to date after tattooing session(s) applied to a mother during pregnancy or breastfeeding (Winter, 2017).

Most tattooists probably avoid tattooing women who are to give birth or breastfeed. However, there is a possibility of tattooing some women who are pregnant but have not been diagnosed with pregnancy yet. Sometimes, there is a possibility that this procedure can be applied to women who are breastfeeding but do not tell that they are breastfeeding. Therefore, the true prevalence and potential complications of such conditions are not fully known. Potential complications pose some risks to the pregnant woman and fetus. There is no reliable data on the risk of infection and transmission of toxic chemicals through the placenta and breast milk (Kluger, 2012). However, local infection after tattooing or the transmission of blood-borne diseases such as hepatitis has rarely been reported in tattoo centers that absolutely comply with hygiene, asepsis and sterilization rules. A potential bacterial spread may be considered an exception, though always possible. It is also known that bacterial infections are rarely transmitted to the infant through breast milk. In such cases, it may be recommended to interrupt breastfeeding for some time. If antibiotics are to be used in the treatment, medication choices should be made carefully during both pregnancy and lactation (Kluger, 2012; Kluger, 2015).

Conclusion and Recommendations

The tendency to have tattoos, also called body inscriptions, has increased. With the widespread use of getting tattoos, midwives and women's health care providers are more likely to encounter pregnant and lactating women with body accessories (tattoos / piercing) that can complicate prenatal and postnatal care. It is known that these accessories do not prevent pregnancy; however, although rare, they can be the source of complications or problems during pregnancy, childbirth or breastfeeding. Obstetricians and midwives should be aware of these potential consequences and be prepared to provide comprehensive information to women who sometimes feel anxious and perhaps even guilty about getting a tattoo as a body ornament. Attitudes displayed towards women with tattoos are important because negative attitudes can affect patient care. Therefore, these patients should be approached in a non-judgmental and non-stigmatizing manner to avoid any impact on patient management.

Due to the widespread acceptance and increasing popularity of tattooing, it has become necessary to investigate the risks and complications related to tattooing and to reveal the results. Therefore, performing more and more studies on women (especially during pregnancy and lactation) and tattoos will be very valuable for this field.

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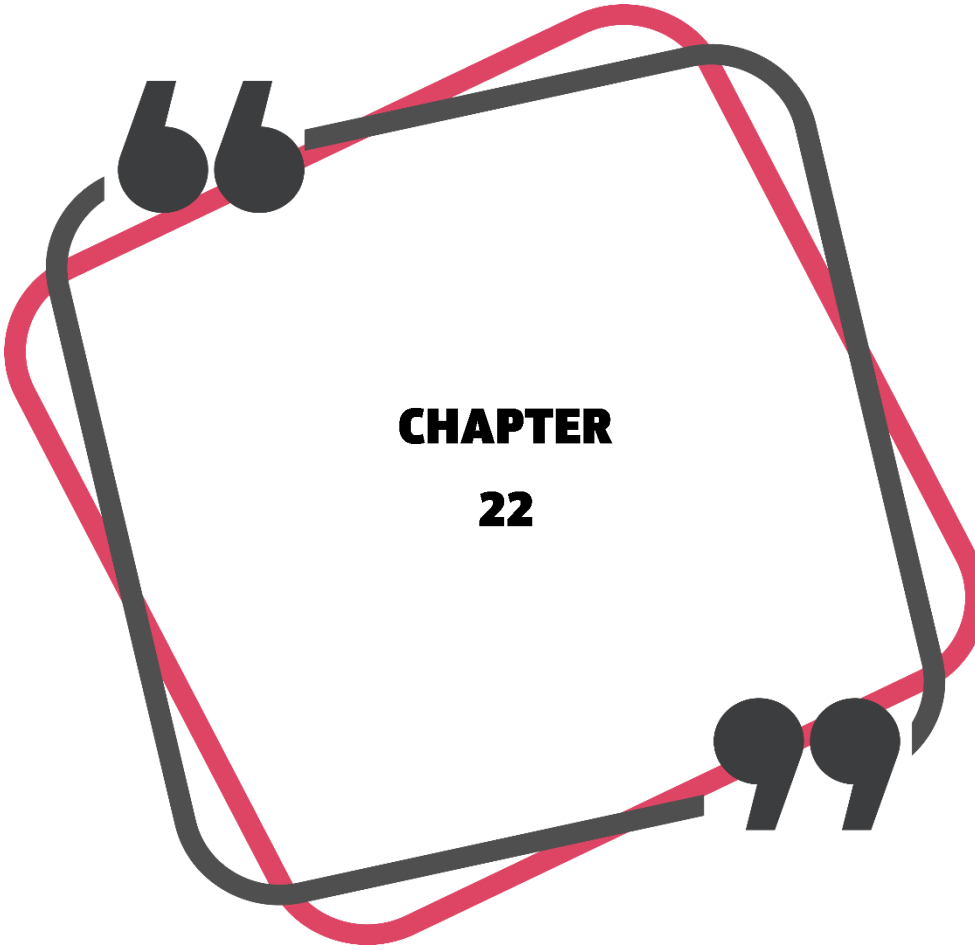
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CHAPTER
22

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MODELING THE MORTALITY PREDICTION OF PATIENTS WITH STEMI UNDERGOING PRIMARY PCI USING DATA MINING

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1 Introduction

Four major diseases -cardiovascular diseases, cancer, chronic respiratory diseases, diabetes- indicated by World Health Organization causes 71% of all deaths and continues to increase daily worldwide [1]. Fortunately, most of them can be prevented or delayed by monitoring and maintaining key health metrics [2]. It is estimated that deaths will be 22.2 million in 2030 [3]. The cardiovascular disease with the highest mortality rate throughout the world is coronary artery disease (CAD). Hence, mortality risk prediction is a necessity of promoting the quality of life and managing costs. In Turkey, 14.39% of the people who died in the year 2019 were due to CAD ischemic heart diseases [4]. PCI (Percutaneous Coronary Intervention) is a way of treating patients who have atherosclerosis. After PCI procedure, MACE (Major Adverse Cardiac Events) can occur. Death, myocardial infarction, and in-stent thrombosis occurrence during the hospitalized days of patients are some of the MACEs. Coronary stenting is a more difficult procedure than other conventional PCI procedures, but it has a high primary operational success rate [2]. Even though it is a successful procedure, some MACEs with PCI can be seen after stenting. Predictive models for determining risks of MACEs have been studied in the literature. Many risk scores for stratification MACEs after PCI have been developed and some statistical techniques have been widely used for this purpose [3]. True prediction of MACEs before the operation is an

important issue because of reliability and quality of health institution and physician. On the other hand, patient care can prevent MACE in high-risk group by using these predictions. Machine learning and data mining algorithms are used in the literature for CAD detection because of promising performance, ease of use, and low computational burden [Alizadehsani et. al. 2013, 2016); Ghiasi et al. 2020]. However, no previous studies have developed mortality prediction models by using data mining techniques to predict short and long-term mortality among patients with STEMI treated with PCI. The aim of the present study is to develop a new mortality risk prediction model with data mining for short and long-term mortality in patients with STEMI treated with PCI. Some mortality prediction algorithms for patients undergoing primary PCI were studied. General characteristics and comparative validation studies of mortality were searched and discussed. They indicated that while classic risk models use points derived from odds/hazards ratios of the predictors, contemporary models make use of complicated statistical methods [8]. However, risk models based on machine learning and data mining techniques make advanced level non-linear complex analysis because of handling multivariety and multi-dimensional data.

Tourassi and Xenopoulos [9] developed a model for predicting in-hospital mortality of patients for 5 different percutaneous interventions (stent, atherectomy, laser, rotablator, and TEC) by using feed-forward ANN and Linear Discriminant Analysis. Resnic et al. [10] modeled in-hospital mortality for 4264 PCI patients using ANN, the Simplified Risk Score they developed, and multiple logistic regression. Qureshi et al. [11] estimated in-hospital mortality risks of 9954 patients with PCI during 2 years with the risk score they developed. Chowdhary et al. [12] calculated mortality probabilities after PCI with Toronto Risk Score developed using logistic regression. The study conducted with 10694 patients was tested with 5347 patients. Romagnoli et al. [13] used the EUROScore Risk Score to predict the in-hospital mortality of 1173 patients with PCI during 1-year period in their single-center study. They measured the success of EuroScore with the help of multivariate logistic regression. Ellis et al. [14] performed logistic regression analysis to determine in-hospital mortality of patients with PCI, including neurological indicators. Peterson et al. [15] developed a logistic regression model to predict before operation, in-hospital and 30-day mortality for 588398 patients with PCI. Garg et al. [16] compared 1-year clinical findings of MI patients with ST segment who underwent PCI based on the syntax score and PAMI score. Holmes et al. [17] used a regression method in predicting in-hospital, after discharge mortality and patient's bypass status in a group of 3761 patients with stenting among PCI patients over a 4-year period. Halkin et al. [18]

modeled in-hospital and long-term mortality probabilities as 30 days after PCI and one year after PCI using Cadillac score with logistic regression and jakknife method. Today, there are accepted important risk factors of coronary heart disease. These indicate that a person's risk of developing myocardial infarction and stroke can be determined and the cardiovascular risk of the person can be reduced, the exposure to fatal and non-fatal cardiovascular events can be prevented and the risk of cardiovascular diseases can be reduced [19,20,21]. The approach to prevent cardiovascular diseases should be multidisciplinary, aiming to reduce the overall risk, not a single risk factor, and considering multiple risk factors. The purpose of preventing adverse events from cardiovascular disease is to reduce fatal and non-fatal atherosclerotic cardiovascular events, complications, and the need for percutaneous or surgical revascularization, to increase the quality of life and to prolong its duration. To reach this goal, it is essential to evaluate the total cardiovascular risk and to create a treatment strategy accordingly. In this study, we used data mining techniques for predicting mortality risk after stenting operations and comparing their prediction performance.

This study;

1. Determines the most successful prediction model for stenting operations.

2. Investigates the parameters that affect MACEs.

3. Explores the relationship of parameters and defining MACE occurrence rules.

2 Materials and methods

This study uses the data that was used in a previous study approved by the Ethics Committee and the Institutional Review Board of Erciyes University Medical School [22]. In that study, they confirmed the relationship between serum uric acid levels and post primary PCI myocardial perfusion grade using basic statistical and logistic regression analysis. The aim of this study is to predict in-hospital and after discharge mortality risk of patients with STEMI after PCI with data mining models. 289 consecutive STEMI patients who underwent PCI with stent placement at Erciyes University Heart Hospital were chosen. All patients had follow up for short and long term between 2006 and 2011. Biomarkers and clinical findings with significant impact on the course of coronary artery disease in the literature were recorded for each patient. The blood was taken from the patients with stents in the emergency service was put into tubes that provide obtaining of serum without blood coagulation, and then biomarkers requested to be

examined in central laboratory of Erciyes University were analyzed from blood serum with Elisa test. The blood taken by interns is put into a yellow capped 13x100 5 mL BD Vacutainer plastic SST gel tube and centrifuged in the machine at 1500 rpm for 10 minutes as soon as possible and taken into the freezer at -75 degrees. The gel in tubes used for centrifugation causes a physical barrier between serum and blood cells after centrifugation and accelerates serum coagulation thanks to silica particles around tube wall. Samples were never thawed before analysis and stored in the freezer until laboratory analysis. When the number of patients was completed, studies were continued at Erciyes University Central Laboratory to look biomarkers. Short-term mortality was observed in the coronary service after PCI. The independent variables regarded as modelling input were determined based on literature review and expert opinions as shown below Table 1[23].

Table 1 Independent variables for modelling

Demographic features	Clinical features	Biomarkers
Age of the patient [24], Sex [25], Body Mass Index (BMI) [26], Smoking [27]	HTN (Hypertension) [28], DM (Diabetes mellitus) [29], CAD history, Cerebrovascular diseases	GGT (Gama Glutamil Transferaz)[30], MPV (Mean Platelet Volume) [31,32], CRP (C-reactive protein) [33,34], Uric acid [22], LDL (Low Density Lypoprotein) [35], HDL (High Density Lipoprotein) [35], Triglyceride [36], Hemoglobin [37], White Blood Cell (WBC) [38], Platelet [39], RDW-cv (Red cell distribution width-coefficient variation) [40], Rdw-sd (Red cell distribution width; sd, standard deviation) [40], Serum glucose [41], Creatinine [42], CK (Creatinine Kinase), Ck-MB (Creatinine Kinase Miyocardial Band) [43], Cardiac Troponin [44], Total Cholesterol [45], Direct Bilirubin [46,47], Total bilirubin [47,48], AST (Aspartate aminotransferase) [49], ALT (Alanine aminotransferase) [48]
PCI operation features	Medication use of patients	Number of vessels intervened
Stent type (balloon predilatation, bare metal stent, drug eluting stent) [49], Stent diameter [50], Stent length,	Ace-I (Angiotensin converting enzyme inhibitors) [55], B blocker [56],	Primary percutaneous coronary intervention, single-multi vessel intervention, the number of vessels intervened

predilatation [51], stent inflation pressure, Reflow, No-reflow [52], Pain to Balloon Time [53], LAD (Left anterior descending), RCA (Right coronary artery), CX (circumflex artery) [54], IRA-RCA (Infarct-related artery- right coronary artery), IRA-CX (Infarct-related artery- circumflex artery), IRA-LAD (Infarct-related artery- right coronary artery)	Statin [57], Aspirin, Diuretic [58], Aggrastat

The detected patients were examined in terms of two different dependent variables as short and long-term mortality.

3 Work flow and analysis of inputs

Data taken from 289 patients for in-hospital mortality prediction risk modeling and 252 of 289 patients for after discharge were analyzed prior to modeling. A total of 22 in-hospital deaths with a total of 53 inputs and one output were observed and determined. All patients were admitted for STEMI and had primary PCI procedure within 6 hours of presentation by Erciyes Medicine Faculty. Work flow is given in Fig. 1.

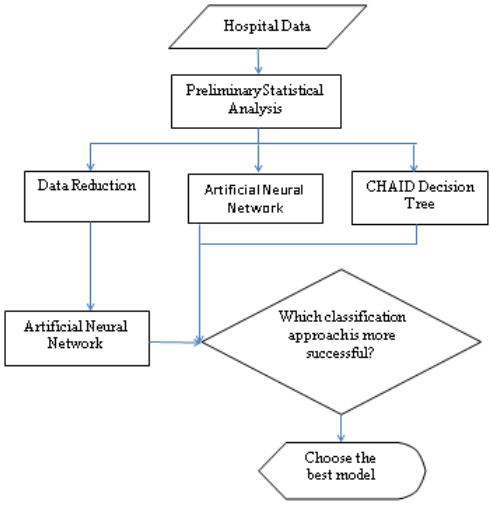


Fig. 1 Work flow of the study

Patients were excluded from the study if they had treatment of STEMI in the previous 24 hours with thrombolytic drugs, active infection, a history of gout, end stage liver and renal failure. Standard femoral approach with 7- French guiding catheter was used for performing primary PCI procedure. Direct stenting was used where appropriate; balloon dilatation was utilized for remaining cases after administration of 5000 units heparin and 300 mg clopidogrel loading dose. Bare metal stent or drug eluting stent types were selected by operating physician. 100 μg nitroglycerine injection was applied to achieve maximal dilatation in each coronary angiogram. In the emergency room, antecubital blood samples for the analyses were drawn in all patients. High sensitivity CRP was measured by BN2 model nephlometer. Citrate based anticogulated blood samples stored at +4 Co and MPV levels were measured by Sysmex K-100 auto analyzer within 30 min of sampling. In stent thrombosis, non- fatal myocardial infarction (MI) and in-hospital mortality were considered MACE. If there is a recurrent chest pain or a new ECG change with a rise of biomarkers it was classified as non-fatal MI. In-stent thrombosis was defined as total occlusion documented on angiography. Death due to the MI is defined as in-hospital mortality. In order to calculate the number of mortalities in the long term, we used Turkey's MERNIS (Central Population Management System) system. Until 2011, it was determined that 33 of the patients with stents had died. The number of cases decreased to 252 with the elimination of patients who died prior to discharge. The patients were categorized as either short-term mortality or long-term mortality. Short Term Mortality: time interval for patient between procedure and discharge: long-term Mortality: time interval after patient's discharge. Correlation is a statistic that measures the relationship between two variables and the direction of the relationship. According to Pearson correlation coefficient analysis in Fig. 2, we determined correlations between continuous variables at 0.05 significance level (2-tailed). There have been inverse (negative) correlations between such variables; Age-platelet, GGT-Tkol, GGT-LDL, CRP-Tkol, Hg-Platelet, WBC-Tkol, Ck-Stentdiameter, Tkol-ALT, stent length tostent diameter.

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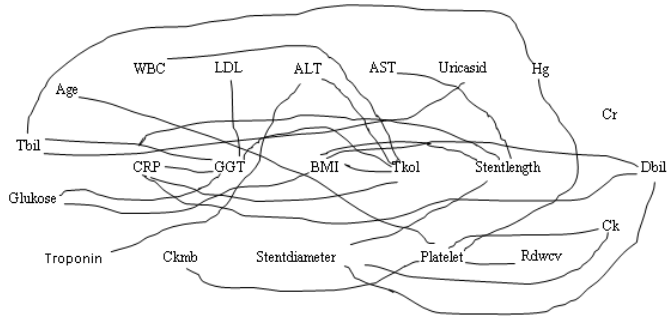


Fig. 2 Correlation map of significant variables

Considering the stent type may have an effect on long-term mortality, we decided to include in the model. In this case, long-term risk model with 49 inputs and 1 output was created.

Table 2 T test for continuous variables

	Long term			Short term		
Variables	Survivors (mean±SD)	No survivors (mean±SD)	P value	Survivors (mean±SD)	No survivors (mean±SD)	P value
Age	59±11.9	68.1±8.8	0	57.6±10.9	68.4±14.7	0
BMI	26±2.6	26.8±2.5	0.19	26±2.6	25.7±2.3	0.61
Tbil	0.9±0.9	1.3±1.2	0.04	0.9±0.5	1.2±1.9	0.38
Dbil	0.4±3.3	0.5±0.9	0.92	0.4±3.6	0.2±0.1	0.69
AST	65.7±72	203±284.3	0.03	59.3±69	105.9±94.4	0.01
ALT	38.2±35.7	124.5±230	0.09	34.8±28.9	54.3±61.6	0.08
WBC	12±4.5	15.7±5.6	0	12.1±4.7	11.5±3.4	0.46
Hg	14.5±1.8	13.7±1.6	0.07	14.6±1.7	13.3±2.2	0
Platelet	244.5±66.5	238.4±60.8	0.67	248.3±65.7	228.8±76	0.12
Rdw-cv	13.9±1.2	14.3±1.1	0.22	13.9±1.2	14.6±1.9	0.03
Rdw-sd	50.5±53.2	44.1±3.4	0.57	51.7±58.6	45.6±9.6	0.54
Serum Glicose	165.5±77	310.5±183.5	0	162.4±76.3	173±78.1	0.45
Cr	1.2±2.6	1.3±0.3	0.91	1.2±2.8	1.3±0.6	0.81
Ck	592.3±944.1	746±1007	0.46	573±944.7	693.6±918.9	0.49
Ck-mb	48.9±56	76.5±68.6	0.03	47.5±56.8	54.5±54.5	0.5
Troponin	6.9±28.4	12.5±24.6	0.36	6.7±29.7	11±26.1	0.43
TG	114.1±61.6	127.5±52	0.32	118.4±63.5	91±47.7	0.01
Tkol	181.7±44.9	171.7±42	0.31	182.6±43.3	176.1±53.2	0.43
LDL	119.04±33	109.4±29.9	0.19	119±30.7	116±44.4	0.71
HDL	39.1±23.9	37.8±13.8	0.8	39.4±26	38.1±8.7	0.78

For short term mortality, there seems to be a significant difference between living and dead patients for age, total bilirubin, AST, White Blood Cells, serum glucose, CK-MB, MPV, CRP, uric acid since p value is less than 0.05. For long-term mortality, there is a significant difference between age, AST, ALT, WBC, Rdw-sd, Tkol, MPV, CRP, Hg, uric acid with p value less than 0.05. T test results are shown in Table 2. The chi-square test was performed for categorical parameters; male as a sex, noreflow, CX, IRA-CX, Aggrastat, the number of vessels intervened for short term and noreflow in PKG, CX intervention and the number of vessels intervened for long term seems to be significant. The significance of all categorical data was examined depending on the output variable determined as "in-hospital mortality" in the chi-square test. Table 3 summarizes the effect on survivor and no survivor patients. Chi-square values in stent diameter, Pain to balloon time, and primary PCI variables could not be calculated due to the small number of observed events in each class. Modeling was performed after the data analysis.

Table 3 Pearson chi-square analysis for discrete variables

Variables	In-hospital mortality			After discharge		
	Survi-vors	No survi-vors	p value	Survi-vors	No survi-vors	p value
Male	73.36%	3.46%	0.000	69.84%	10.2%	0.832
Smoking	56.40%	4.15%	0.549	53.17%	7.14%	0.467
HTN	41.18%	3.46%	0.936	38.89%	6.75%	0.467
DM	25.26%	3.11%	0.175	24.21%	2.78%	0.423
KAH	22.15%	2.77%	0.196	18.25%	3.97%	0.231
SVH	2.42%	0%	0.591	1.98%	0.79%	*
Stent type (drug eluting)	28.64%	2.36%	0.414	9.13%	1.59%	0.456
Predilatation	69.9%	6.57%	0.255	57.54%	4.38%	0.000
No-reflow	36.68%	6.92%	0.000	29.37%	8.73%	0.000
Pain to Balloon Time	*	*	*	*	*	*
LAD	67.13%	6.23%	0.350	62.3%	9.52%	0.902
RCA	51.56%	5.54%	0.123	46.03%	9.12%	0.072
CX	40.83%	5.19%	0.030	35.32%	8.33%	0.013
IRA-RCA	2803%	2.42%	0.885	26.59%	3.57%	0.698
IRA-CX	18.34%	3.11%	0.021	16.27%	4.37%	0.053
IRA-LAD	43.25%	2.08%	0.077	41.67%	4.76%	0.214
ACE-I	44.29%	3.11%	0.526	41.67%	7.14%	0.479
Beta blocker	33.91%	4.15%	0.098	28.97%	6.35%	0.09
Statin	30.80%	2.77%	0.772	27.38%	4.76%	0.578
Aspirin	31.83%	4.15%	0.059	28.17%	5.16%	0.428
Diuretic	14.19%	0.69%	0.427	12.3%	2.78%	0.291
Aggrastat	13.15%	2.42%	0.029	11.9%	1.59%	0.805
Main coronary intervention	*	*	*	*	*	*

The number of vessels intervened (3)	21.11%	3.46%	0.022	17.86%	3.97%	0.003

*values could not be calculated.

3.1 Mortality risk modeling with decision tree

A supervised learning method used in data mining for classification and regression methods is decision tree. The widely used decision trees methods are ID3, C4.5, C5.0, CART, QUEST and CHAID algorithms. We used exhaustive CHAID (Chi-squared Automatic Interaction Detector) method for generating more classification rules and having better prediction accuracy percent compared to other methods [59,60]. CHAID is a data mining tool used to discover the relationship between one dependent and other independent variables. Chi-squared is applied to determine the splitting condition. One of the most important advantages is non-parametric statistical method of free distribution. Unlike other trees, CHAID can create nodes that are more than once branched. In order for the tree to be constructed, it does not matter whether the target and classifier variables are categorical or numeric. Exhaustive CHAID like CHAID has the same splitting and stopping steps. However, the merging step is more exhaustive than CHAID, by continuing to unite categories of the predictor variable until only two super categories are dropped. The best split for each predictor variable is Exhaustive CHAID [61].

Steps of CHAID algorithm used to construct the decision tree is given below.

1.For each explanatory variable X, find the pair of categories with the least qualifier, taking into account the Y target variable of X (with the largest p-value). The method will calculate p values based on the measurement level of Y. In this study, Y is categorical and therefore the Pearson chi-square test is performed.

$$X^2 = \sum_{j=1}^{J}\sum_{i=1}^{I}\frac{(n_{ij} - m_{ij})^2}{m_{ij}} \quad (1$$

$m_{ij} = (\sum_{j=1}^{J} n_{ij} * \sum_{i=1}^{I} n_{ij})/(\sum_{j=1}^{J}\sum_{i=1}^{I} n_{ij})$. n_{ij}: observed cell frequency, m_{ij}: estimated expected cell frequency, *J*= level number of column variable, *I*= level number of row variable. d=(J-1)*(I-1).

d: degrees of freedom.

2.For category pair of X with the largest p-value, compare p to the predetermined alpha level α_{merge}(= 0.05, default value).

i. If the value of p is larger than α_{merge}, combine this pair under a single category. Process for the new category set of X starts from Step 1.

ii. Go to Step 3 if p is less than α_{merge}.

iii. Using the appropriate Bonferroni multiplier for the category set of X and Y, calculate corrected p-value.

$$B = \sum_{i=0}^{r-1} (-1)^i \frac{(r-i)^c}{i!(r-i)!} \quad (2$$

c = number of original categories, r: number of merged categories. ($2 \leq r \leq c$).

3.Select the X estimator variable with the smallest corrected p-value (most important). Compare this p-value to the predefined alpha level α_{split}.

i.If the value of p is less than or equal to α_{split}, the node is divided based on the set of categories of X.

ii.If p is greater than α_{split}, not divide the node. This node is the end node.

Continue the tree growth process until the stopping rules are observed [60].

SPPS Clementine 10.1, a popular data mining program, was used to create the decision tree. In the exhaustive CHAID algorithm, the α value for the branching was determined as 0.05. Noreflow is specified as root node in decision tree. The result of the classification for the cases is shown in Table 4.

Table 4 In-hospital (and long term) mortality classification with exhaustive CHAID

	Survivor	No survivor	Total
Survivor	267 (219)	0 (0)	267 (219)
No survivor	0 (3)	22 (30)	22 (33)
Total	268 (222)	21 (30)	289 (252)

Short term mortality model	Long term mortality model
1. If noreflow=yes and sex=male and CRP>46.6 and Hg≤14.1 then no survivor 2. If noreflow=yes and sex=male and CRP>46.6 and Hg>14.1 and WBC>19.51 and BMI>23 then no survivor 3. If noreflow=yes and age=male and CRP≤46.6 and Uric acid>6.94 and Diüretic=0 and TG>151 and age>53 then no survivor 4. If noreflow=yes and age=male and CRP≤46.6 and 6.3<uric acid≤6.94 and HTN=yes then no survivor 5. If noreflow=yes and sex=male and CRP≤46.6 and 6.3<uric acid≤6.94 and HTN=no and TG>60 then no survivor 6. If noreflow=yes and sex=women and glucose>176 and Cr>1 then no survivor 7. If noreflow=yes and sex=women and glucose>176 and Cr≤1 and the number of vessels=3 then no survivor 8. If noreflow=no and age>75 and IRA-CX=yes and 8.8<WBC<13.5 then no survivor	1. If age≤61 and CRP>14 and sex=male and smoking=yes and Hg≤13.8 then no survivor 2. If age≤61 and CRP>14 and sex=male and smoking=yes and Tkol>178 and Agrestat=no and uric acid>6 then no survivor 3. If 61<age≤ 75 and uric acid≤6.6 and Ck-mb≤66 and Cr>1.5 and LDL>125.6 then no survivor 4. If 61<age≤75 and uric acid≤6.6 and Ck-mb>66 and LAD=yes and smoking=yes and MPV>11.5 then no survivor 5. If 61<age≤75 and uric acid≤6.6 and Ck-mb>66 and LAD=yes and smoking=yes and HTN=yes then no survivor 6. If 70<age≤75 and uric acid>6.6 and IRA-LAD =no then no survivor 7. If 61<age≤75 and uric acid>6.6 and IRA-LAD=yes and sex=male and smoking=yes and T bil>0.39 then no survivor 8. If 61<age≤75 and uric acid>6.6 and IRA-LAD=yes and sex=male and smoking=yes then no survivor 9. If age>75 and vascular intervention=1 and Cr>1.5 then no survivor 10. If age>75 and vascular intervention=2 and noreflow=no and LAD=yes and BMI>28 then no survivor 11. If age>75 and vascular intervention=2 and noreflow=no and LAD=yes

	and CRP≤5 and Cr>1.21 then no survivor 12. If age>75 and vascular intervention=2 and noreflow=no and LAD=yes and CRP>5 then no survivor 13. If age>75 and vascular intervention=2 and noreflow=yes and uric acid>4.1 then no survivor

Fig. 3 In-hospital and discharge mortality rules

3.2 Classification with ANN

A classification model was built with STATISTICA Automated Neural Network (SANN) to predict in-hospital mortality. This model is regarded as supervised learning since the output values are shown in the model so that the model can learn. It allows use of networks with different input numbers and activation functions in the software program and 500 different Multi Layer (MLP) artificial neural network models to be tested. Classification was made using ANN with 53 inputs accepted in literature [62]. STATISTICA 9.0 software package was used for the classification model. The inputs consist of 53 variables, and each level of categorical inputs was a different input. In-hospital mortality was chosen as an output. In the model, the output variable is specified as survivor/no survivor and coded as 0/1. Normalization has been performed to determine continuous input data in the range between 0 and 1. Dataset segmentation was performed as 75% training, 15% testing and 10% verification. However, since the size of the dataset is not large, the model was enabled to see at least 50% of in-hospital mortality at the training stage.

Since the mortality cases was 22, it was added to ANN model to correctly assign this class. In MLP networks, different combinations of activation functions in the input and intermediate layer cells were tried. In the output layer, only the softmax function was used to build 0-1 clusters. Error rates were chosen as cross entropy due to properties in classification. In addition, to reduce the error during back propagation process, the minimum value was set as 0.0001 for each layer. By trying 500 different networks, 5 networks with the highest success were used Broyden – Fletcher – Goldfarb – Shanno (BFGS) learning algorithm was used in all networks. This algorithm is a quasi-newton method that achieves the goal well.

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In long-term mortality prediction, an optimum network was created for ANN modeling with 49 inputs and 1 output through trial and error. The model settings made in short term measurement modeling were used without change. Training, testing, and verification segmentation were performed as 70%, 15% and 15%, respectively due to the dataset. Since each category of categorical variables was taken as input, networks with 80 inputs and 2 outputs were created. The most successful 5 networks selected are given in Table 5.

Table 5 Selected networks for in hospital and long term mortality modeling

In-hospital mortality					After discharge mortality				
Network Type	Training Perf.	Test Perf.	Valid. Perf.	Activation	Network Type	Training Perf.	Test Perf.	Valid. Perf.	Activation
MLP 89-8-2	98.62	93.02	82.14	Exponential	MLP 80-19-2	87	81	91.9	Logistic
MLP 89-8-2	*100*	*86.04*	*92.85*	*Exponential*	MLP 80-20-2	87	81	91.9	Logistic
MLP 89-17-2	93.12	90.69	89.28	Tanh	MLP 80-8-2	100	72.9	86.9	Tanh
MLP 89-17-2	93.12	90.69	89.28	Exponential	MLP 80-16-2	87	81	91.9	Exponential
MLP 89-8-2	93.12	90.69	89.28	Exponential	MLP 80-23-2	100	75.6	86.9	Identity

Since categorical groups in 53 variables are taken as separate variables, the number of inputs increased to 89 and the number of outputs was determined as survivor and no survivor. Successful modeling depends on the classification error and the rate of assignment to the correct class. For this reason, it is necessary to look at the confusion matrix of the networks before choosing the appropriate architecture as shown in Table 6. The most successful classification belongs to MLP 89-8-2 network. The activation function of hidden nodes is exponential activation function. The output function is softmax, which is developed specifically for the output units of the classification models. Optimum network has been determined in the first cycle of learning algorithm BFGS. Considering sensitivity of input parameters in the model, SVH, IRA-CX, ACE-I, HTN, Statin, B Blocker and Aspirin are the variables with the highest sensitivity. We concluded that patients who underwent PCI are under greater risk if they have additional diseases

such as Cerebrovascular and Hypertension. In addition, the use of medications such as ACE-I, Statin, B Blocker and Aspirin have a preventive effect on long-term mortality. The highest correct classification success is in 5th network with 94.5% assignment to the correct class in total. The network consisting of 80 inputs, 23 hidden nodes and 2 outputs assigned 75.8% of deaths and 97.3% of their survival status to the right class. While creating this network architecture, 23 out of 33 observed deaths were used in the training phase and all of them were assigned to the correct class. One of the 7 measurement data used in the test phase was assigned to the correct class and only 1 out of 3 deaths could be put in the correct class during the verification phase. An unsuccessful model for classification was created. Six wrong assignments were made in the correct classification.

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Table 6 Classification ability of in-hospital and discharge deaths of identified networks

Networks		in hosp m-0	in hosp m-1	in hosp m-All	Networks		long term-0	long term-1	long term-All
1.MLP 89-8-2	Total	267	22	289	1.MLP 80-19-2	Total	219	33	252
	Correct	262	16	278		Correct	219	0	219
	Incorrect	5	6	11		Incorrect	0	33	33
2.MLP 89-8-2	Total	267	22	289	2.MLP 80-20-2	Total	219	33	252
	Correct	264	17	281		Correct	219	0	219
	Incorrect	3	5	8		Incorrect	0	33	33
3.MLP 89-17-2	Total	267	22	289	3.MLP 80-8-2	Total	219	33	252
	Correct	267	0	267		Correct	214	23	237
	Incorrect	0	22	22		Incorrect	5	10	15
4.MLP 89-17-2	Total	267	22	289	4.MLP 80-16-2	Total	219	33	252
	Correct	267	0	267		Correct	219	0	219
	Incorrect	0	22	22		Incorrect	0	33	33
5.MLP 89-8-2	Total	267	22	289	*5.MLP 80-23-2*	*Total*	*219*	*33*	*252*
	Correct	267	0	267		*Correct*	*213*	*25*	*238*
	Incorrect	0	22	22		*Incorrect*	*6*	*8*	*14*

As seen in the ROC (Receiver Operating Characteristics) curves, the area under the curve of the MLP-80-23-2 network which is the most successful network is the highest with a value of 0.84. ROC analysis is a method that is generally used in diagnostic studies and provides comparison of analysis. ROC curves are given in Figure 4. The area under the ROC curve is in the 2nd network with the largest 0.90 value. The success of correctly classifying the survival status of MLP 89-8-2 network was 98.87% and the success of correctly classifying the status of death was 77.27%. In this network, 15 deaths were used in the training phase, 4 in the test phase and 3 in the verification phase. The classification success was 97.23%.

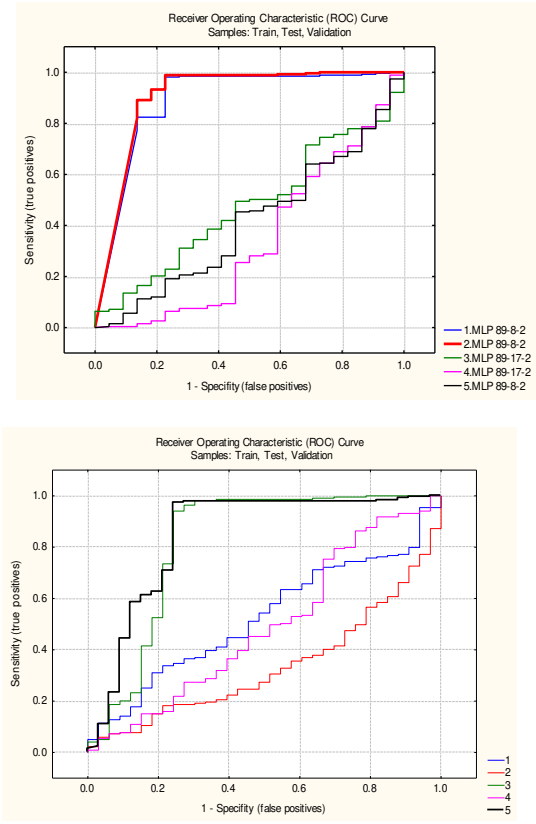


Fig. 4 ANN ROC curves for short (left) and long term mortality (right)

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All of the mortality predictions performed during the training phase were completely correct, 1 out of 4 deaths in the testing phase was correct, and 1 out of 3 deaths in the verification phase was predicted correctly. Global sensitivity analysis allows us to see the relative importance of inputs used within the network. The network is trained for each input sequentially discarded from the model, and then the errors are recorded. Inputs that give high error when discarded from the model are important inputs for the model. The results for MLP 89-8-2 network are summarized in Table 7.

Inputs of 1 and less than 1 are not important for the model. However, it is not entirely correct to remove inputs looking one model in sensitivity analysis. It is more rational to identify common important inputs by applying sensitivity analysis on multiple networks. In Table 7, variables with a sensitivity of 1 and less than 1 are not shown. If the sensitivity analysis results are interpreted, close importance level is observed in almost all variables in the ANN.

Table 7 Global sensitivity analysis for long and short-term mortality

Long Term	1	2	3	4	5	6	7	8	9
Variables	SVH	IRA CX	Ace i	HTN	Statin	Bbloc-ker	PtoB-time	Aspi-rine	Main coro-nary
Sensi-tivity	2.54	2.04	1.84	1.68	1.59	1.50	1.34	1.29	1.28
Vari-ables	IRA LAD	DM	Age	IRA RCA	TG	Diure-tic	CX	Uric Acid	Hg
Sensi-tivity	1.26	1.22	1.15	1.15	1.08	1.08	1.07	1.05	1.05
Short Term									
Vari-ables	Stent type	ACE-I	Sex	Bbloc-ker	KAH	PtoB-time	IRA RCA	Num-berof vessels	Statin
Sensi-tivity	1.2	1.17	1.11	1.10	1.09	1.08	1.08	1.08	1.07
Vari-ables	Smo-king	Stent dia-meter	CX	RCA	Main coro-nary	noref-low	ref-low	DM	HT
Sensi-tivity	1.07	1.06	1.06	1.05	1.04	1.03	1.03	1.03	1.02
Vari-ables	Gli-kose	LAD	WBC	Agres-tat	IRA-LAD				
Sensi-tivity	1.02	1.02	1.01	1.01	1.01				

3.3 PCA-ANN for short term mortality classification

Feature selection has a major impact on the model performance. Principal Component Analysis (PCA) is a modeling tool that helps to create new and fewer components by reducing the features in the data set without causing data loss. The new components obtained in this process are created by considering which feature has the stronger variance in expressing the problem. PCA was applied to eliminate the variance between variables in the input set and the effects of its integration with ANN were observed. After taking the levels of the categorical variables as a feature, a total of 89 inputs were created. Considering that there may be a correlation between the data, variables not required for the model among were removed from the 89 inputs to increase the success of the model. To express the deviation of a one-dimensional variable from the mean is Variance, but it is covariance if examined for multi-dimensional variables. PCA calculates the eigenvectors from the covariance matrix formed according to each other and tries to express the majority of the variance of the original data set with the help of fewer new components. The objectives of the PCA can be listed include simplifying data, reduce data, modeling, detect deviation, select variables, classification, and prediction.

If the variables in the original data set contain a high rate of correlation, it gives successful results. 20 or 30 original variables can be expressed as 1 or 2 principal components in highly correlated cases. The principal components are composed of original data and eigenvectors. PCA has been used frequently recently for data reduction in Artificial Neural Networks (ANN) applications. The components are used as inputs for ANN so that more successful results can be obtained. However, since PCA is a non-consultative learning model, it only looks at the correlation of inputs with each other, which sometimes creates a dilemma. In order to apply PCA to our data, advence models – NIPALS – PCA module was used in STATISTICA 9.0 package program. The high number of explanatory variables in the data set raises the possibility of multi-collinearity between variables. Non-Linear Iterative Partial Least Squares algorithm aims to obtain components that maximize covariance matrix and cannot obtain all components at the same time. The algorithm is terminated when the desired number of components is reached or when the argument matrix becomes zero. In the study, the maximum number of iterations for the NIPALS algorithm was determined as 50 and the convergence criterion was taken as 0.001. In the algorithm, components are detected by 7-cluster cross validation. In the calculation of the components, unit standard deviation values were used as the data were not in standard form. 30 components with eigenvalue greater than 1 were created with the cross validation

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technique. The reason for the high number of components is that the correlation between the data is not as high as expected. However, the number of inputs has been reduced from 89 to 30 with the PCA. 82.5% of the total variance could be explained with 30 components. The variables that best explain the first main component are Glucose, MPV, DM, CAH, reflow, no reflow, statin, Aspirin, B blocker, number of vessels intervened, single-multiple vessel intervention, Cx and uric acid. It was seen that CRP and Diuretic variables in the second main component explained most of the variance. Third main components are HTN, ACE-I and IRA CX. In the fourth fundamental component, Ck-mb and Ira LAD variables have the largest share in the variance explained by the component. All of the other components consist of partial mixing of variances of variables. With the scree plot of eigenvalues, we can check the status of the basic components we have selected. The scree plot of 30 components is given in Fig. 5. Results show the eigenvalue change has decreased considerably after the first 27 components. The first 27 components explained only 78.90% of the total variance, which was insufficient to express the original data set, so new components were added until the component eigenvalue fell below 1. The first fundamental component alone explained 11.33% of all variation in the data set. The principal component scores obtained were used as input for ANN. In this case, an MLP model with 30 inputs and 2 outputs was created, and the best network was decided by testing 500 different networks through trial and error. Previously determined ANN features were completely used to be suitable for the purpose of comparison.

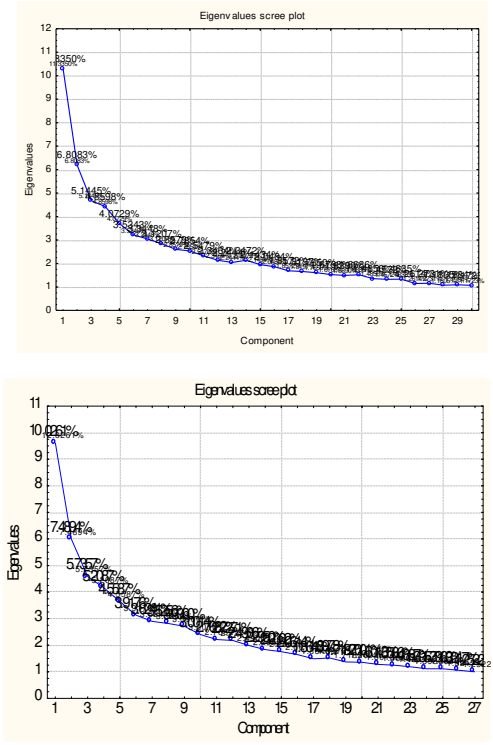


Fig. 5 Scree plot for short (left) and long term (right)

252 data divided into 70% training, 15% testing and 15% verification were used to create 27-input and 2-output MLP networks. The most successful 5 networks selected among the obtained networks are given in Table 8. Considering the interpretation of the networks in the table, the success of ANN has increased much more, and the detected models have improved in terms of their performances in the training, testing and verification stages. The success of the first three networks is 100% for each stage. To choose among these networks, the confusion matrix of the networks should be checked in Table 9. All of the first three networks accurately predicted both in-hospital deaths and short-term survival. Under these circumstances, it was decided that the simplest network architecture would be appropriate. With PCA, covariance between the inputs that have an effect on the long-term mortality were eliminated, and new independent components were created. With the help of the STATISTICA software, new components were added until the eigen value fell below 1 and the number of components was found to be 27. 12.02% of the total variance was explained with the first component. The variance with 27 components is 84.21%. 252 data divided into

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70% training, 15% testing and 15% verification were used to create 27-input and 2-output MLP networks. Thus, the effect of PCA analysis on network performance was demonstrated.

Table 8 PCA-ANN models for in hospital mortality classification

Network	Training Perf.	Test Perf.	Validation Performance	Activation	Network	Training Perf.	Test Perf.	Validation Performance	Activation
MLP 30-19-2	100	100	100	Exponential	MLP 27-9-2	97.7	72.9	83.7	Exponential
MLP 30-17-2	100	100	100	Tanh	MLP 27-13-2	87.0	81.0	91.8	Logistic
MLP 30-7-2	*100*	*100*	*100*	*Exponential*	MLP 27-7-2	94.9	75.6	83.7	Identity
MLP 30-7-2	99.54	97.67	100	Exponential	MLP 27-18-2	87.0	81.0	91.8	Exponential
MLP 30-20-2	99.54	97.67	100	Tanh	MLP 27-9-2	87.0	81.0	91.8	Identity

MLP 30-7-2 model has been determined as an active network since it has the simplest architecture. In the hidden layer nodes of this network, the activation function is exponential, and the softmax function is included in the output layer. Learning algorithm has reached the optimum network in 61st cycle of BFGS. Among these determined networks, the 2nd, 3rd and 5th models have the same performance values. However, before making this decision, it is necessary to look at the confusion matrix and ROC curves as shown in Table 9 and Figure 6, respectively. The most successful classification is in the first network with 92.1%. In addition, the area under the ROC curves of these five networks was found in MLP 27-9-2 as a value of 0.8533. The area under the ROC curves of the first three networks is equal to 1, while the other two network show values of 0.9998 and 0.9996, respectively. With the MLP 27-9-2 network, 21 out of 33 mortality cases were assigned to the correct class.

Table 9 In hospital and long term mortality classification with PCA-ANN.

Name of network		in hospm-0	in hospm-1	in hospm-All	Networks		long term-0	long term-1	long term-All
1.MLP 30-19-2	Total	267	22	289	*1.MLP 27-9-2*	*Total*	*219*	*33*	*252*
	Correct	267	22	289		*Correct*	*211*	*216*	*232*
	Incorrect	0	0	0		*Incorrect*	*83*	*123*	*207*
2.MLP 30-17-2	Total	267	22	289	2.MLP 27-13-2	Total	219	33	252
	Correct	267	22	289		Correct	219	0	219
	Incorrect	0	0	0		Incorrect	0	33	33
3.MLP 30-7-2	*Total*	*267*	*22*	*289*	3.MLP 27-7-2	Total	219	33	252
	Correct	*267*	*22*	*289*		Correct	212	16	228
	Incorrect	*0*	*0*	*0*		Incorrect	7	17	24
4.MLP 30-7-2	Total	267	22	289	4.MLP 27-18-2	Total	219	33	252
	Correct	266	21	287		Correct	219	0	219
	Incorrect	10	1	2		Incorrect	0	33	33
5.MLP 30-20-2	Total	267	22	289	5.MLP 27-9-2	Total	219	33	252
	Correct	266	21	287		Correct	219	0	219
	Incorrect	1	1	2		Incorrect	0	3	331

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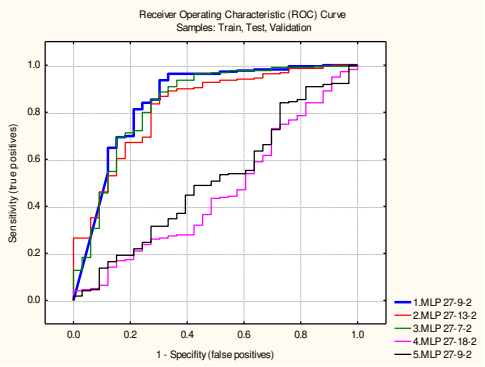


Fig. 6 ROC curves of PCA-ANN for long term

4 Conclusion

The success of classification models is directly proportional with assigning objects to the correct class. If we compare the correct classification rates made by the ANN models and the decision tree for short-term mortality classification, 100% success is achieved with the PCA-ANN model and the CHAID decision tree.

Table 10 Comparison of in-hospital mortality models

Models	Correct Classification Success
ANN	97.23%
PCA-ANN	100%
CHAID	100%

Sensitivity and sepecifity are the most important performance measures in the medical literature. ROC curve is a graphical plot which illustrate the performance of a binary clasifier system. The larger the area under ROC curve, the higher the performance of the algorithm is. Considering the sensitivity and specificity values, mortality and survivor prediction success is the same as PCA-ANN and

CHAID algorithms. In our study, sensitivity is important because 22 deaths were observed during the study period. 100% sensitivity and specificity were achieved in the models. In this case, the success of decision trees and the use of ANN for clinical problems such as diagnosis was proven. It was observed that the integrated use of PCA with ANN has a performance enhancing effect for short term measurement modeling in Table 11.

Table 11 Sensitivity and specifity values for in-hospital mortality models

Models	Sensitivity	Specifity
ANN	77.2%	98.8%
Exhaustive CHAID	100%	100%
PCA-ANN	100%	100%

We determined for decision tree and rules that no reflow during PCI has caused an 18% increase in-hospital deaths. According to the decision tree, factors that caused higher mortality included male gender, multi-vessel intervention, high CRP, elevated WBC, high TG, high glucose and uric acid values, low Hg value; age > 75 years old. The presence of hypertension triggers in-hospital mortality. Glucose, uric acid, noreflow, multi-vessel intervention, CRP and HTN variables appear as important parameters in mortality classification of PCA-ANN model, which has the same performance as the decision tree. The decision tree obtained and the rules were interpreted with expert opinion and their accuracy reviewed. When the correct classification rates of ANN and decision tree models for long-term mortality classification are compared, it is seen that the most successful classification model in Table 12 is in the exhaustive CHAID decision tree.

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Table 12 Comparison of mortality models for long-term models correct classification success

Models	Classification %
ANN	94.44%
PCA-ANN	92.06%
Exhaustive CHAID	98.8%

Also, looking at sensitivity and specificity values, survivor and no-survivor prediction success is the highest in the exhaustive CHAID model in Table 13.

Table 13 Sensitivity and specificity values

Models	Sensitivity	Specifity
ANN	75.75%	97.26%
Exhaustive CHAID	90%	100%
PCA-ANN	63.63%	96.34%

The classification success with exhaustive CHAID is high, but the decision trees was weak and showed too much branching to explain the deaths. In addition, the performance increase of PCA-ANN integration in short term death classification could not be obtained for long term death classification. Based on the rules extracted from the decision tree, it is expected that age> 75 years increases risk of death. Other factors expected to increase long-term mortality are uric acid, CRP, GGT, CK-MB, MPV, LDL, Cr, Tbil, T Arm and BMI height, Low Hg, noreflow occurrence, HTN, LAD and Ira LAD smoking, multiple vascular intervention and not using Agrastat.

With the ANN and decision trees models, variables for stented patients are estimated for the short term and long term mortality. We choose ANN for the

class determination model to process the variable and interactions affecting patient outcome after stent. Since all cases belonging to the survivor / no survivor clusters were determined, a classification method was used with supervised learning. In addition, we created decision trees and rule extraction to determine which variables had an effect on survival / no survivor status. Generally, effect on mortality was demonstrated by age, gender, smoking, presence of LAD and Ira LAD, hypertension, multiple vascular occlusions, CRP, MPV, Uric acid, BMI, T cholesterol, T Bilirubin, Cr and serum glucose, low Hg and drug use. The data reduction method "PCA" was applied to short-term mortality and 100% classification was achieved to observe the performance change of ANNs. 82.5% of the variation between PCA and 53 parameters was calculated.

Models established for short term mortality prediction are more successful than for long term mortality prediction. The fact that the obtained case values were before PCI was helpful in explaining short-term mortality. The study is successful in predicting death in the short term. However, the low number of death cases observed creates was a limitation for creating a general model. In the long-term measurement model, only 75% of the deaths could be assigned to the correct class with ANN classification models after subtracting the variables from PCI input set. The ANN model training error was almost zero, but the test phase errors were quite high. This is an indication that the network has reached its limits due to insufficient representation of data, and better models cannot be obtained.

For long-term mortality modeling, it is predicted that the input values should be taken during the recovery period of the patient and that the clinical findings obtained from the routine controls of the patient in the post-discharge period are recorded and included in the modeling can increase the success of the model. In addition, the inclusion of parameters such as BNP, Cystatin, Co peptine, S CD40 Ligand and Hyoloronic Acidis thought to affect long-term mortality after PCI. After PCI, modeling is more difficult in the long term patients due to uncontrollable factors such as diet, lifestyle and and the continued use of medications. Especially in Turkey, where patient follow-up is difficult and lack of a national database for coronary artery disease limits the ability of physicians to make predictions about their outcome. It has been observed that the use of medications such as ACE-I, statin, B Blocker and Aspirin has a mortality-reducing effect in the classification of long-term deaths with ANN.

In the decision tree, patient age is the most important factor for predicting long-term mortality. ANN based on PCA analysis could not achieve a success like the short term mortality classification due to the data. Thus, it was determined

that the integrated use of PCA and ANN has a performance enhancing effect if the data set explains the situation sufficiently.

The intended result was achieved in the study, and predictive models were created for the physicians. It is thought that modeling with much more data will give successful results. In addition, simple interfaces can detect these predictions to help physicians. In order to increase the performance of artificial neural networks, it can be integrated with models such as the genetic algorithm. By making linear models, we can determine which type of model is suitable for estimating the measurements. It is expected that working with models established for short-term mortality classification will be a good resource. Future studies are planned to use new biological descriptors for long-term mortality classification.

Declaration of Competing Interest The authors declare no conflict of interest.

Author Contribution MZ performed design of the work, some analysis and writing. GT made other analysis of the work.

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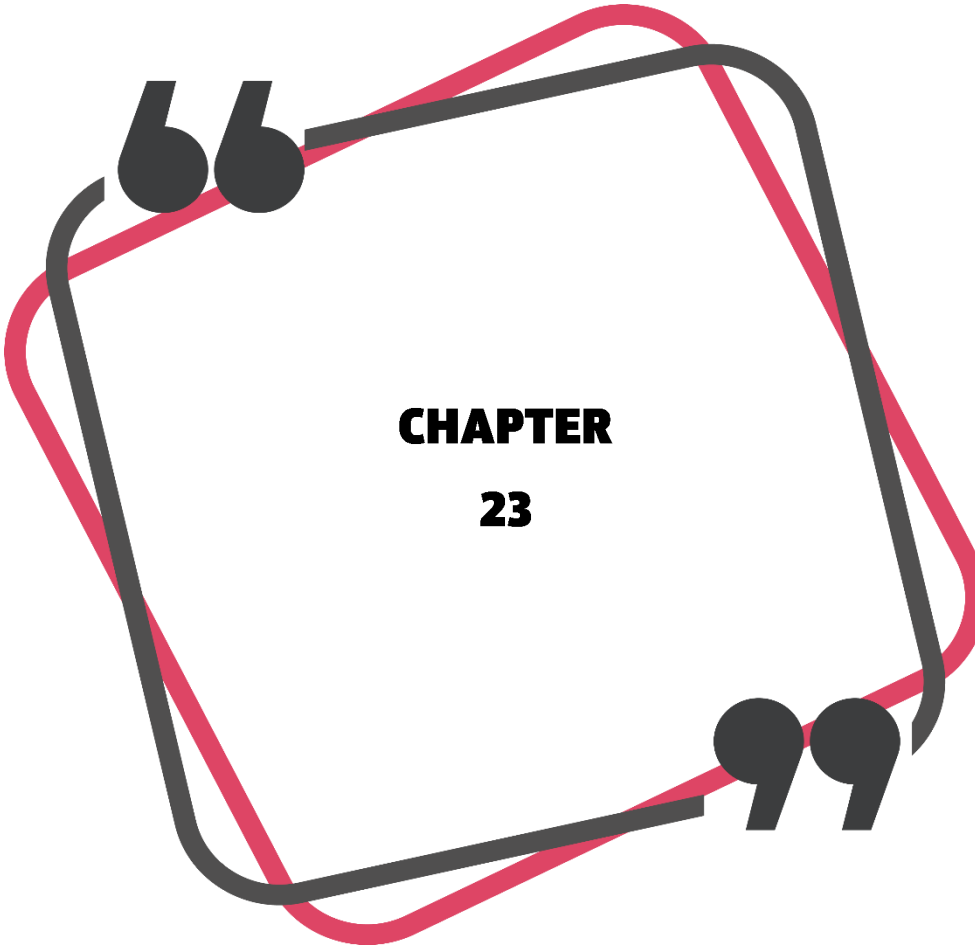
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CHAPTER
23

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LINEAR IGA DERMATOSIS AND DERMATITIS HERPETIFORMIS

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SUMMARY

Linear IgA dermatosis (LAD) is a rare autoimmune bullous disease associated with linear IgA deposition along the basement membrane zone (BMZ) characterized by subepidermal bullae formation that can be seen in both children and adults. The variant seen in children is often called childhood bullous disease or childhood linear IgA dermatosis (CLAD)[1].

In 1969, cases of linear deposition of IgA at the dermoepidermal junction were reported in patients with subepidermal bullae. In 1970, Jordan defined cases in children as benign chronic bullous disease of childhood, and in 1975 Chorzelski and Jablonska stated that LAD is a separate disease from DH. However, Lawley et al. stated that these individuals with linear IgA accumulation are pathophysiologically different from DH[2], [3]. Drug-induced LAD was first described in 1981 by Gabrielsen et al. [4].

EPIDEMIOLOGY

Linear IgA bullous dermatosis (LABD) is a rare disease among autoimmune dermatoses.

The disease peaks in two periods throughout life. In adults, LAD occurs at an average age of 60; On the other hand, the incidence of CLAD peaks at the ages of 4-5 during childhood [5]. There are rare cases reported in newborns[6].

The incidence of LAD is not known exactly, while the incidence was reported as 0.4-0.28 per 1,000,000 in studies conducted in Europe[7] while it was reported as 1.25-1.45 in 1,000,000 in studies on the incidence of CLAD in Africa[5]. The true incidence of LAD, together with the incidence in studies in different countries, is not known exactly.

In the gender distribution, it is seen that men are affected by the disease a little more than women[7].

ETIOPATHOGENESIS

The etiology or underlying pathophysiological mechanism that triggers the autoimmune response in LAD is still largely unknown. Many studies over the last 30 years have identified the presence of different antigens involved in the pathophysiology of LAD.

It has been observed that IgA autoantibodies in LAD serum react with many antigenic peptides rather than a single antigen. Studies have shown that both keratinocytes and fibroblasts are involved in the expression of LAD target antigens[8].

Two different types of LABD can be mentioned as lamina lucida and sublamina densa type, depending on the location of IgA deposition determined by immunofluorescence.

Most patients with LAD have antibodies of the IgA1 type, which target the 97 kDa antigen and the 120 kDa antigen in the basement membrane region. Both of these antigens are thought to be fragments of the extracellular portion of bullous pemphigoid antigen 2 (BP180), a transmembrane protein that plays a key role in epidermal-dermal adhesion. Other less common target antigens are IgA antibodies to the NC16a epitope on BP180, other basement membrane antigens including BP230, type VII collagen (COL7)[9], laminin-332 or laminin gamma 1[5], [8] .

The genetic background of LAD is not as well known as DH with other IgA accumulation, and there is not yet a strong enough genetic background for it.

A study of LAD HLA groups in children and adults in the UK found a very high association with the human leukocyte antigen (HLA) HLA B8. In adults, the frequency of three haplotypes HLA Cw7, HLA B8, DR3, DQ2 increased, but only HLA Cw7 was statistically significant due to the decrease in the patient sample. The presence of these three haplotypes seems to encourage greater susceptibility to early onset of the disease, although further studies are needed [10]. Unlike studies conducted in Japanese[11] and Chinese[12] populations, no direct relationship could be demonstrated with HLA groups.

LAD develops idiopathically in most patients, and in some patients it develops due to medication. Although many drug-induced LAD has been reported, the most frequently reported pharmacological agent is vancomycin[13].

The mechanisms that cause the formation of bullae are still not fully known. Neutrophil chemotaxis, which develops due to IgA-mediated and complement activity, is thought to play a role [12]. Lysosomal enzymes released from neutrophils coming to the area cause decomposition along the dermoepidermal junction and formation of subepidermal bullae.

CLINICAL FINDINGS

LAD may present with lesions on the skin, mucous membranes, or both. Although both children and adults develop LAD, there are differences in clinical manifestations between LAD and CLAD in adults.

LAD lesions are located on the extensor surfaces of the extremities, including the trunk, elbows, knees, and hips, similar to DH, which progresses with other IgA deposition.[5]

Lesions form a polymorphic picture. Lesions may be asymmetrical and scattered, or they may be observed in a DH-like herpetiform pattern and papulovesicular in nature. It can also be observed as annular plaques and tense bullous lesions around a BP-like center without a bulla. There may be severe itching accompanying the lesions. Skin findings similar to erythema multiforme (EM), erythema annular centrifugum or erythema gyratum repens are also present in reported cases[14], [15].

In CLAD, sudden onset skin lesions are often seen. less frequently, the lesions develop slowly[16]. The lesions progress with tense vesiculobullous lesions located on the background of normal skin or urticarial plaque. Involvement sites of the lesions differ from those of adult type LAD. In children, the lesions are especially localized in the lower abdomen and perineal region, and there is significant anogenital involvement[1]. Perioral region and scalp involvement can be seen on the face.

Typical lesions are described as a “string of pearls” or “jewel clusters”, with new vesicles arising from the periphery of old lesions, with vesicles or bullae with clear contents in their periphery. Sometimes, vesiculobullous lesions develop from an intact skin surface[17]

Mucosal lesions may accompany both drug-induced LAD and idiopathic type [18]. There are studies showing that the incidence of mucosal involvement is 80%[19]. In a Japanese study conducted for CLAD, mucosal involvement was observed at a much lower rate of 3%[9].

Oral, ocular, nasal, pharyngeal, esophageal, or genital mucous membranes may be involved. However, the most common involvement is seen in the oral and ocular mucous membranes. In the oral mucosa, lesions are often located on the palate, palatine arches, or buccal mucosa. [5]

Oral mucosal involvement is often in the form of painful erosive and ulcerative lesions; It can rarely be seen as chronic desquamative gingivitis. However, scarring and vision loss may develop due to eye involvement as a result of cicatricial pemphigoid-like involvement. Airway obstruction due to tracheal involvement and mucosal involvement in esophageal strictures have been reported[9], [19].

RELATED DISEASES

LAD is one of the autoimmune diseases, especially ulcerative colitis[20]; It has been reported to be associated with systemic lupus erythematosus, rheumatoid arthritis, hypothyroidism, vitiligo, Crohn's disease, ulcerative colitis, autoimmune hemolytic anemia, multiple sclerosis, glomerulonephritis, IgA nephropathy, and bone marrow aplasia[21].

It has been reported in numerous case reports that LABD co-occurs with lymphoproliferative and solid organ malignancies[1]. However, how the disease arises with malignancies is not yet understood.

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DIFFERENTIAL DIAGNOSIS

Other vesiculobullous diseases are included in the differential diagnosis with LAD.

It can be confused with LAD, as it progresses with subepidermal vesicle in DH, has grouped vesicle involvement on the extensor surfaces, and has a similar histopathology. DIF may be required to make a definite distinction[5][22].

Having polymorphous lesions similar to BP clinic, observing advanced age involvement, observing subepidermal blistering in histopathology, linear involvement of IgA and IgG in the DIF finding make it difficult to distinguish the two diseases from each other both clinically and histopathologically.

While mucosal involvement and cicatricial healing require differentiation with cicatricial pemphigoid, mucous membrane pemphigoid; It can be included in the differential diagnosis with Erythema Multiforme[23] and bullous systemic lupus erythematosus due to the presence of bullae on the urticarial background.

History of drug intake and its development may cause confusion with TEN[24]. [25]

Except for autoimmune bullous diseases; prurigo nodularis[15] can be confused with impetigo, herpes simplex, herpes zoster, scabies and sexual abuse in children.

DIAGNOSIS

Since LAD can cause polymorphic lesions and mucosal involvement, clinical signs and symptoms of the disease may be confused with other mucocutaneous-involving diseases. For this reason, the diagnosis should be confirmed by histopathology and DIF in a patient with suspected LAD in the clinical diagnosis.

Histopathological findings:

Histopathological findings for LAD are not specific. It has histopathological findings similar to other auto-immunobullous dermatoses.

Histopathologically, subepidermal bulla formation is observed. Adult, childhood and drug-induced types of LAD have similar histopathology, and in some cases, eosinophilic and lymphocytic infiltration can be detected together with neutrophilic infiltration. Neutrophilic abscesses may be localized to DH-like dermal

papilla tips. There may be BP-like eosinophilic infiltration and. Therefore, DIF examination is the gold standard for the definitive diagnosis of LAD[26].

DIF:

To perform direct immunofluorescence, it is recommended to take it from clinically normal-appearing skin around the lesion.

DIF examination is the gold standard for definitive diagnosis of LAD. It often indicates the presence of IgA deposits at the dermoepidermal junction in a linear pattern along the BMZ. Less frequently seen as a result of DIF is the presence of IgG, IgM or C3 deposition[5], [27]. [28]

IIF: Indirect immunofluorescence can be used to detect circulating IgA antibodies raised against BZM components. The proportion of circulating antibodies varies with age. Studies have shown that 30% positivity in LAD and 80% positivity in CLAD[16], [29].[28]

In the salt dissociation test, deposition occurs on the epidermal side in the lamina lucida type, while on the dermal side in the sublamina densa type [30], [31].

Other tests such as immunoelectron microscopy [32] and immunoblotting[28][8], [33] can also be used to confirm the diagnosis, but the cost and limited number of centers limit their use.

TREATMENT

LAD treatment based on case series in the literature; Although large, randomized, double-blind, controlled studies are not available, many systemic agents, especially dapsone, have been shown to be effective in both children and adults.

In drug-induced LAD, it is recommended to discontinue the responsible drug. Clinical improvement is observed in the majority of patients. However, systemic dapsone treatment can be started in patients who do not improve within 2-3 weeks.

dapsone

The most preferred agent in the systemic treatment of LAD is dapsone. Dapsone acts by inhibiting neutrophil chemotaxis and inhibiting neutrophil migration to the site. BMZ also prevents neutrophils from binding to IgA. Dapsone has been used for the treatment of LAD for a long time. Response to treatment begins in

skin lesions 48-72 hours after initiation of treatment[34]. However, if the treatment of mucosal lesions does not respond adequately, systemic corticosteroids may be added to the treatment [35].

Recommended starting point for dapsone in adults. The dose is 25 mg, and the dose can be increased up to 300 mg by increasing the dose at 1-2 week intervals until the lesions are under control [36]. In CLAD, dapsone treatment is usually started at a dose of 1 mg/kg/day. The upper limit of the dose should not exceed 3-4 mg/kg/day[5], [37].

Among the most common side effects of dapsone are; hemolytic anemia, leukopenia, methemoglobinemia, agranulocytosis, hepatitis and neuropathy. Before starting treatment, glucose-6-phosphate dehydrogenase (GPD6) enzyme level should be checked. Treatment should not be initiated in patients with G6PD deficiency.

Other sulfonamide derivatives, sulfamethoxypyridazine or sulfapyridine, can be used in patients who do not respond to dapsone therapy. In children, start with 125 mg/day and gradually increase the dose. In adults, the effective dose ranges between 500-1500 mg/day. As with dapsone, agranulocytosis, neutropenia, hepatitis, bronchiolitis, alveolitis have been reported with this drug. Sulfapyridine is another sulfonamide type drug.

The recommended dose recommendation for sulfapyridine is to start at a dose of 70 mg/kg/day in divided doses and the total daily dose should not exceed 100 mg/kg/day[38], [39].

Although there are no large, randomized, double-blind, controlled studies on the treatment of LAD in the literature, many systemic agents have been shown to be effective in both children and adults. Among these treatments, there are many different groups of drugs, from systemic antibiotics to immunosuppressants, monoclonal antibodies, IVIG therapy [5], [35].

PROGNOSIS

The prognosis for CLAD in both LAD and pediatric age groups is generally good. Spontaneous remission may occur in 30% to 60% of adult patients, and only some patients may have recurrences. In children, the disease tends to resolve spontaneously within 2 to 4 years of onset. In most pediatric patients, the disease goes into remission until adolescence [40].

Drug-induced LABD also has a good prognosis, with spontaneous resolution within 2 to 6 weeks after discontinuation of the trigger drug in most cases [41].

Although skin lesions usually heal without permanent scarring; For both LAD and CLAD, patients with mucosal involvement may experience morbidity and functional limitations due to permanent damage or dysphagia, respiratory failure, conjunctival and corneal scarring[42].

DERMATITIS HERPETIFORMIS

INTRODUCTION

Dermatitis herpetiformis, or Dühring-Brocq disease, is a rare auto-immunbullous skin disease with a chronic-recurring course that develops with gluten sensitivity.

Dermatitis herpetiformis (DH) was placed in the same category as pemphigus and pemphigoid by dermatologist Louis Duhring in 1884, forming the class of bullous diseases. In 1888, Brocq defined similar skin lesions as "polymorphic pruritic dermatitis", but after examining Duhring's report, he accepted that this was the same pathology. has done. Therefore, DH is also called Dühring-Brocq disease.[43]

Today, DH is accepted as a specific cutaneous manifestation of celiac disease (CD).[44]

There is a strong relationship between celiac disease (CD), another disease associated with gluten-sensitive enteropathy, and DH. Crypt hyperplasia and villous atrophy due to intraepithelial lymphocyte migration in the jejunum are observed in both cases due to gluten sensitivity [45]. However, in DH, unlike CD, symptoms related to enteropathy are not seen in most of the patients. A significant relationship between both diseases is HLA-DQ2, which is located on the 6th chromosome. HLA-DQ2 containing the DQA1∗0501/DQB1 ∗0201 alleles is 80-90% positive in DH and CD patients. Patients with DH and CD share many common features such as gluten sensitivity, HLA association, circulating IgA antitransglutaminase (tTG) and Anti-endomysial antibodies [46]

EPIDEMIOLOGY

DH is a rare bullous disease. However, its distribution varies geographically, and it is most commonly seen in the white race of Northern European origin in studies [47]. It is less common in people of Asian and African descent. In a 40-year prospective study conducted in Finland, the prevalence of the disease was 75.3 per 100,000, while epidemiological studies conducted in Northern Europe

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and America found incidence rates between 0.4 and 3.5 per 100,000 people and prevalence rates between 1.2 and 75.3 per 100,000 people per year. In the same study in Finland, the annual incidence of DH in the whole period was 3.5 per 100,000 and 5.2, 2.9 and 2.7 per 100,000 in three 10-year periods, respectively[48].

The disease can occur at any stage of life. However, the most common age range for patients to be diagnosed is 40-50. between the ages [49]. In Finland, where the incidence of the disease is high, the ratio of DH to other age groups in childhood was 4%[48], while in a study conducted in Italy, the ratio of the 0-10 age group to other age groups was 25% [50].

DH is more common in men, unlike CD, where female gender is more common. In general, the M/F is observed as 1.5 in studies[51]. However, as the age at diagnosis decreases, the female sex ratio increases, and it appears to be almost equally common in childhood. [48]

ETIOPATHOGENESIS

It includes many different interacting factors in which environmental, immunological and genetic predispositions play a role, in which a single factor does not play a role in the pathogenesis of DH.

GENETIC PREDISPOSITION

There are many studies showing strong associations between certain HLA genes that regulate tissue compatibility antigens and DH and CD, which are involved in the regulation of the immune response of T-cells.

More than 90% of DH and CD patients express the specific HLA class II molecule HLA-DQ2, while a proportion of patients (2-10%) serologically express the HLA-DQ8 haplotype. Monozygotic twins are highly concordant for the development of DH (70%), the prevalence among first-degree relatives is 10-15% and 40% in non-monozygotic HLA-identical siblings. Genetic differences between patients with celiac disease and DH have not yet been identified. A case of monozygotic twins with CD in one twin and DH in the other has been reported [52]. In another study, patients with DH were compared with healthy people, and it was observed that the genes encoding the HLA DQ (α1*0501, (β1*02) heterodimer were positive in 86% of the patients and 25% in the healthy control group [53] .

The presence of both the HLA DQ-2 and HLA-DQ8 alleles provides a sensitivity of close to 100% for the diagnosis of CD and DH. In individuals lacking these alleles, CD and DH are virtually excluded. Although HLA is the most well-defined genetic predisposition for CD and DH, genetic factors other than HLA have also been shown to be important in the pathogenesis of the disease. [54], [55]

GLUTEN SENSITIVITY

The common etiopathogenesis in DH and CD is gluten enteropathy, which develops as a result of gluten sensitivity. It is thought that most of the patients with DH develop gluten-induced enteropathy. However, routine bowel biopsy is not significant in the diagnosis of the disease, since enteropathy progresses in the form of segmental involvement and enteropathy symptoms develop in only 20% of DH patients [56].

Gluten responsible for the development of the disease; It consists of two peptides, gliadin and glutenin. The pathogenesis of the disease is related to gliadin. Gliadin is the alcohol-soluble portion of gluten. It is a peptide fragment that has antigenic properties and causes an immunologic response. Gliadin is divided into 4 groups according to its electrophoretic mobility: α, β, δ and λ. The intestinal disease-associated fraction is from the α-gliadin group and its immunoactivity is due to its N-terminal [54].

Gliadin, which is taken as a result of ingestion of gluten-containing foods, is absorbed from the lamina propria in the intestinal mucosa. Glutamines in gliadin are then deaminated by tissue transglutaminase (TG2) and covalent cross-links (isopeptidyl bonds) are formed between lysine residues in TG2 and glutamines in gliadin. After deamidation by TG2, Deamidegliadinpeptides are presented by binding to HLADQ2 molecules in dendritic antigen presenting cells. . With the stimulation of B cells by Thelper cells, the differentiated plasma cells initiate IgA production against many antigens such as gliadin, gliadin-dependent TG2, TG2 and epidermal transglutaminase (TG3). Stimulation of natural killer lymphocytes causes hyperplasia and villous atrophy in the crypts. [57]

The presence of circulating anti-tTGIgA is the most sensitive marker for CD and is commonly used for screening, while anti-tTG IgA antibodies are diagnostic markers for enteropathy in DH patients.[58]

In 2002, another DH-specific autoantigen, epidermal transglutaminase, eTG, was identified[59]. eTG is unique to patients with DH, is not observed in patients

with CD, and its main function in the epidermis includes cross-linking and preservation of the integrity of the corneal layer. eTG3 is not observed in all tissues, but is mainly seen in the epidermis, small intestine, brain, and testicles[60] . Anti-eTG antibodies show a sensitivity of 52% to 100% and a specificity greater than 90% for DC[61].

Although they have the same genetic background, it is not yet fully understood how antibodies against epidermal transglutaminase develop in DH and CD. One proposed model suggested that patients with CD in childhood initially had high levels of IgA anti-TG2 antibodies, but had normal levels of IgA anti-TG3 antibodies. As time passes and patients with CD become adults, some develop high levels of IgA anti-eTG antibodies unlike childhood, possibly due to "intermolecular epitope exchange". It has been assumed that these patients who develop high levels of IgA anti-eTG antibodies in adulthood have a high risk of developing DH.[62]

Circulating IgA anti-eTG antibodies form an immunocomplex with eTG and are deposited in the papillary dermis. It is thought that IgA in this storage causes chemotaxis of neutrophils, causing inflammation and forming subepidermal bullae, leading to characteristic neutrophilic dermal microabscesses for DH [63].

CLINICAL FINDINGS

DH is a rare autoimmune dermatosis with distinctive findings.

The disease, which has a unique distribution, usually involves the extensor faces such as elbows and knees, the back, hips or sometimes the scalp[64][65]. Lesions generally show a psoriasiform distribution pattern.

Lesions, which generally start with itching and burning sensation, are often in the form of grouped vesicles on an erythematous and papular base (herpetiform papulovesicular lesions).

Because of the highly pruritic lesions, excoriated papules, erythematous plaques, or erosions are often found on examination rather than intact vesicles. Therefore, the lesions exhibit polymorphic character. For this reason, the distribution pattern of the lesions has an important place in the diagnosis of the disease[66].

Although it can be seen in adults, petechiae or purpuric macules on the fingers or palmoplantar are rare clinical features of DH. These lesions are more likely to occur in children[67], [68].

Oral mucosal involvement is rarely observed in DH. Oral mucosal involvement, erythematous macules including tongue, vesicles and erosions may be observed [69], [70].

Dental disorders, especially enamel and enamal defects observed in CD, may also develop in DH. Compared with healthy control groups, dental disorders are observed in both adult and pediatric patients[71], [72].

RELATED DISEASES

Associated diseases due to malabsorption in DH

Microcytic or macrocytic anemia due to iron, folate and B12 deficiency due to malabsorption; Dental caries and hair loss due to zinc deficiency, osteoporosis starting at an early age due to calcium absorption disorder or very severe osteoporosis in advanced age may appear. Involuntary weight loss is observed in patients due to insufficient absorption.

Gastrointestinal symptoms may include diarrhea, constipation, bloating, abdominal discomfort or pain, and secondary lactose intolerance [46].

Although there is an underlying enteropathy, only 20% of DH patients have GI symptoms.

Concomitant Autoimmune diseases and malignancies

As in CD, DH can progress with many autoimmune diseases. Among them, Hashimoto's thyroiditis and diabetes mellitus type 1 are more common autoimmune diseases[73].

Pernicious anemia; More rarely, Addison's disease, alopecia areata, myasthenia gravis, autoimmune hepatitis, sarcoidosis, systemic sclerosis, vitiligo, lupus erythematosus and Sjögren's disease are other diseases reported to be associated with DH [74].

It is argued that the risk of non-Hodgkin lymphoma, especially T-cell lymphoma due to enteropathy, may be increased in DH patients as well[75]. In addition to studies reporting that the risk of developing lymphoma is increased in patients with DH and CD, there are also studies reporting no difference [76].

It is thought that early diagnosis of the disease and gluten-free diet may be protective against the development of malignancy [77].

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DIAGNOSIS

diagnosis of DH; Routine histopathological examination, direct immunofluorescence examination (DIF) and serological tests, together with lesions containing the clinical distribution of the disease itself. DIF examination reveals pathognomonic findings for DH and is considered the gold standard in diagnosis [78].

HISTOPATHOLOGY

The biopsy specimen should ideally be taken from a small, intact vesicle site. If the patient does not have an intact vesicle, biopsy should be performed from an erythematous area.

Histopathological findings in DH lesions include neutrophilic micro-abscesses or neutrophil clusters in dermal papillae with or without subepidermal bulla formation. However, these are not completely specific to DC, as similar findings can be found in other vesiculobullous diseases such as linear IgA disease and bullous lupus erythematosus[49].

DIRECT IMMUNOFLUORESCENT (DIF)

DIF examination of a biopsy sample taken perilesional from the edge of active lesions is still the gold standard test for diagnosis. It is recommended that the biopsy be taken from here, as there will be more IgA accumulation in the perilesional area at the edge of active lesions [79].

The characteristic finding in DIF is the presence of granular IgA deposits within the dermal papilla. IgA deposition may be accompanied by IgM and C3 deposition. Rarely, a fibrillar instead of granular pattern of IgA deposition is detected, or a granular IgA pattern can be observed in the dermoepidermal junction instead of the dermal papilla [78], [79].

SEROLOGY

Serological evaluation is used to aid diagnosis in patients whose clinical features are suggestive of DH but recurrent DIF and histopathological examination do not suggest DH.

In peripheral blood samples of patients, as with CD, anti-dTG, and anti-endomysial antibodies are usually highly positive. Anti-eTG, which is considered specific for DH, cannot be routinely screened [49].

Anti-dTG susceptibility (47-95%, specificity over 90%) antibodies are commonly used in the routine. Determination of anti-endomysial antibodies by indirect immunofluorescence method (sensitivity 52-100%, specificity approximately 100%) is a very specific serological method. Anti-endomysial antibodies are highly specific for DH and CD. They also demonstrate compliance with a gluten-free diet. As patients comply with the diet, a decrease in serum levels is observed[80].

TREATMENT

In the treatment of DH, removing gluten from the diet, which causes enteropathy and skin lesions, as in CD, is the first step of the treatment. Dapsone is the first choice for systemic treatment.

GLUTEN FREE DIET

Because of its association with skin lesions and enteropathy and gluten sensitivity, a gluten-free diet is the first line of treatment. After initiation of the gluten-free diet, gastrointestinal complaints regress more rapidly, while the disappearance of skin lesions occurs more slowly. This may be related to the presence of IgA antibodies, which previously appeared due to gluten, in the dermoepidermal junction for many years after the start of the gluten-free diet [78].

It has been reported that the disappearance of skin lesions may take an average of two years, and recurrence may occur within three months with the initiation of a gluten-containing diet. The benefits of a long-term gluten-free diet include; We can talk about its protective effect against the development of gastrointestinal system lymphomas and the improvement of the intestinal mucosa and the improvement of patients' symptoms due to malabsorption [49], [78], [81].

Strict adherence to a gluten-free diet, which is the basis of the treatment of the disease, is difficult for patients. Cereals with well-known gluten content belong to the Triticeae family; wheat, barley and rye. While oat intake in the diet was not recommended in the past, there are currently studies showing that oat intake is safe [82], [83]. Although patients remove foods directly related to gluten from their diets; Products that may contain gluten as food additives, preservatives and colorants may be in their diet. DH patients should choose gluten-free foods meticulously and form their diets.

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DAPSON

In the treatment of patients with DH, gluten-free diet and dapsone form the basis of treatment.

The dapsone effect, which rapidly controls disease symptoms, occurs by inhibiting the neutrophil-related inflammatory process[84]. With dapsone ingestion, a rapid regression of itching is achieved within 48-72 hours, followed by improvement in skin findings within days. However, Dapsone has no effect on the gastrointestinal tract.

The recommended starting dose is 25-50 mg/day for adults, and the starting dose for children is 0.5 mg/kg. Higher starting doses may cause side effects, especially hemolysis, in patients. Studies show that most adult patients respond fully to doses of 50 to 150 mg per day [22]. The dose is increased according to the patient's compliance, treatment response and side effects, and the maintenance dose is often 100 mg/day. Although higher doses can be increased when necessary, there is an increase in dose-related side effects rather than the effect.

Although Dapsone is generally well tolerated by patients, it can cause serious side effects. Before treatment, glucose-6-phosphate dehydrogenase (G6PD) level, complete blood count, methemoglobinemia, liver and kidney functions, as well as urinalysis should be investigated in all patients. Thereafter, patients should be reassessed weekly for the first month to monitor for symptoms of anemia, methemoglobinemia, and neuropathy. After the first months, complete blood count should be performed twice a month for the next 2 months, and then every 3 months (together with liver and kidney function tests).[84] Severe hemolysis and mortal side effects may be seen in susceptible patients who are started on high doses of dapsone without these screenings. Therefore, G6PD deficiency is an absolute contraindication to the drug.

All patients may experience varying degrees of drug-induced hemolytic anemia, but those with G6PD deficiency are at increased risk for severe hemolytic anemia. Hemolytic anemia usually occurs within the first 2 weeks [85].

One of the other side effects that can be seen with dapsone treatment is dose-related methemoglobinemia. Patients may present with symptoms such as headache and fatigue. Although methemoglobin levels are frequently below 5%, dose changes are not recommended in patients without cardiopulmonary symptoms. [22]

Another rare but important side effect is agranulocytosis, which usually occurs within 2-12 weeks. Dapsone-related hypersensitivity syndrome, which develops

in approximately 5% of patients due to dapsone ingestion, presents with flu-like symptoms, morbilliform skin rash, fever, lymphadenopathy, hepatitis, and rarely eosinophilia [86], [87].

Apart from these side effects; Gastrointestinal side effects such as nausea and vomiting, fatigue, severe drug eruptions up to toxic epidermal necrolysis, photosensitivity, peripheral neuropathy, hypothyroidism and nephropathy can be seen in patients [22].

sulphasalazine and sulphamethoxypyridazine

Sulfasalazine and sulfamethoxypyridazine may be an effective alternative to dapsone, especially when the disease is not controlled or dapsone-related therapy is contraindicated due to side effects. Recommended doses are 1–2 g/day for sulfasalazine and 0.25–1.5 g/day for sulfamethoxypyridazine. Both drugs, like dapsone, have common side effects such as hypersensitivity reactions, hemolytic anemia, proteinuria, and crystalluria[88], [89].

OTHER TREATMENTS

corticosteroids

While orally administered corticosteroids have no place in the treatment, the application of potent topical steroids may be beneficial to reduce itching and pruritus [85].

anti-histamines

Although their efficacy is not as high as corticosteroids in the treatment of DH, third-generation antihistamines with specific activity on eosinophilic granulocytes, which are classified as a third-level treatment option, can also be used in the control of pruritus and pruritus [85].

Table I. Treatment options for patients with dermatitis herpetiformis

Treatments for dermatitis herpetiformis	Level of evidence
Gluten-free Diet	IIA
Dapson †	III
Sulfapyridine †	III

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Treatments for dermatitis herpetiformis	Level of evidence
Sulfasalazine	III
Sulfamethoxypiridine	III
Systemiccorticosteroids	IV
Topical corticosteroids	IV
Antihistamines	IV

IA , Meta-analysis of randomized controlled trials (RCTs); IB, at least one RCT; IIA, at least one controlled trial without randomization; IIB, any experimental work; III, descriptive studies; IV , expert committee reports, opinions or experiences of respected authorities.

∗ The highest level of evidence is indicated.

† These treatments for dermatitis herpetiformis are approved by the US Food and Drug Administration. [84]

PROGNOSIS

It is known that the prognosis of patients with DH who comply with a gluten-free diet is good. However, studies with the lifelong course of the disease are limited. An important component of therapy to achieve or maintain remission is dapsone.

In a study, the patient group with villous atrophy in biopsy and the patient group with normal intestinal histopathology were compared and no significant difference was found between the two groups in terms of response to treatment and prognosis for the long-term course of the disease[90].

In a retrospective cohort study conducted in the USA, in the data of a cohort of 82 patients diagnosed with DH; Among the patients who did not use dapsone and other pharmacological treatment options, and who did not follow a gluten-free diet, patients who did not have skin lesions and complaints for more than two years were considered in remission. It was observed that 12% of patients (10 of 86 patients) entered remission. In the same study, age was seen as the factor affecting remission, and the earlier the disease was diagnosed, the more exacerbations would be predicted[91].

Regular follow-up of patients in terms of adherence to a gluten-free diet, accompanying autoimmune and metabolic diseases, and risk of malignancy will be beneficial in terms of monitoring the course and development of the disease.

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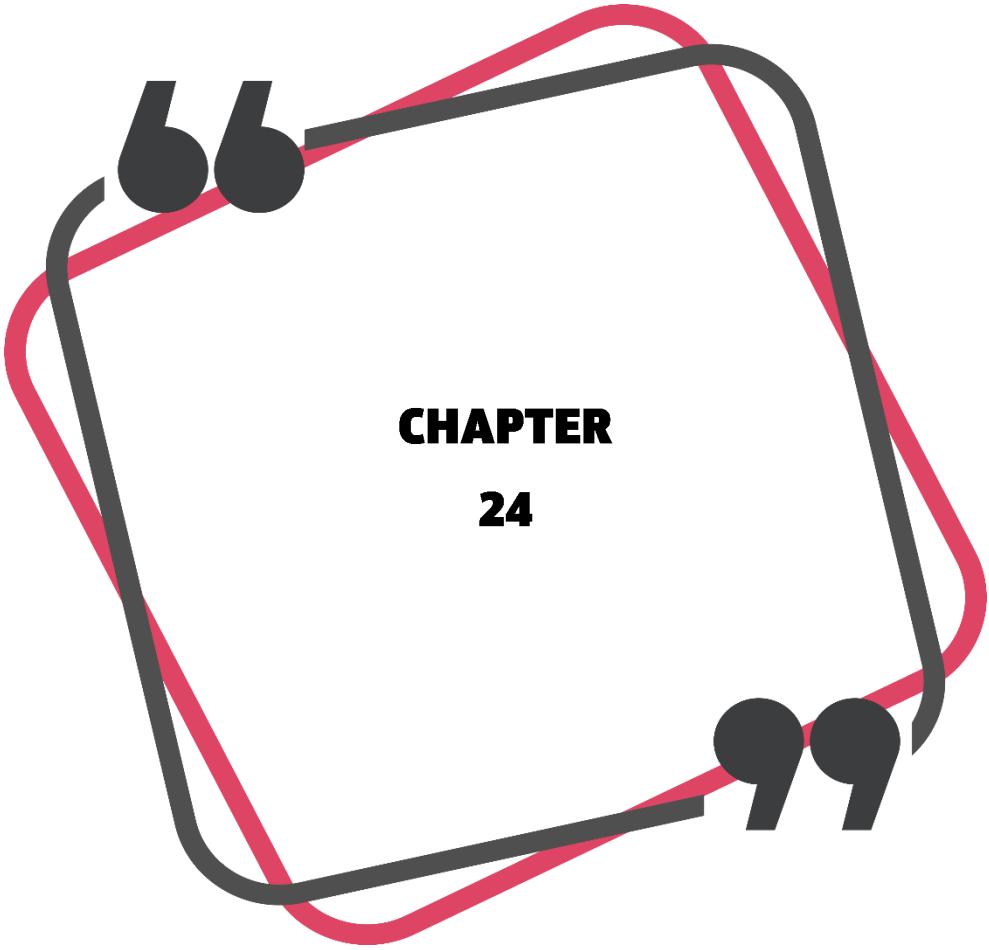
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CHAPTER
24

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MUSCULOSKELETAL DISORDERS AFTER TRAUMATIC BRAI-IN INJURY

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Traumatic brain injury is a significant social health concern because of it effecting more commonly the younger population and causing advanced functional restrictions in survivors. TBI augments neurodegenerative diseases and it causes long term cognitive, temperamental, effective disabilities (1,2). In the latest studies based on genetic researches on mouses, it was suggested that hormonal signals and neural circuits related to hypothalamus plays a crucial role in the regulation of musculoskeletal system. The dysfunctions of musculoskeletal system appearing during TBI, impacts mortality and morbidity of patients(3).

Fractures

Hormonal dysfunctions observed in TBI can activate or inhibit molecular pathways that cause changes in both osteogenesis and bone destruction. There exist certain studies which leads to the idea that dysfunctions in the endocrine and the neuropeptide system related to TBI, may affect the bone structure negatively. The damage in the brain can lead to changes in processes of skeletal reconstruction and growth by impacting hypothalami cells which control the neuroendocrine system responsible for reshaping of the bone.

Patients afflicted with TBI have a rise in their risks of osteoporosis and of osteopenia which in return results in the deformation of micro-architectural structure of bone tissue causing bone fragility and eventual fractures. The differentiations on the bone are characterized by lessened bone-mineral-density due to immobilization and other metabolic factors. Following TBI, one might observe hypoparathyroidism on the patient caused hypercalcaemia of immobilization. Also in a case of TBI, PTH activity decreases thus 1,25-dihydroxyvitamin D production and its activity decreases as a result. Hyperparathyroidism and decreased le-

vels of calcium in the serum causes an increase in the absorption of bones, a decrease in microstructural integrity and as a result of these two phenomena an increase in fracture risk of the bones(4).

It has been stated that the post-TBI osteoporosis and post-TBI fractures are related to long-term immobilization, secondly to the decrease of bone mineral density and thirdly to the hormonal shifts due to the deterioration of the hypothalamic hypophysis's axis. Deficiencies in levels of Growth Hormone and TSH may lead to increase of bone fragility and of fracture risks by changing bone metabolism. Women afflicted by TBI can observe temporary irregularities in their menstrual cycles. These afflictions may cause a decrease in oestrogen production. This decrease in its turn can cause a decrease to the bone mineral density (5).

People who have TBI also have a higher risk of epilepsy. Epileptic people, partially because of falls related to seizures, muscle weakness and loss of balance, have a 2 to 6 times higher chance of having a fracture in comparison to the general population. There are a certain number of proofs regarding the diminutive effects of antipsychotics and of anti-epileptics -which are used in treatments of neurobehavioural symptoms related to TBI- on the bone mineral density. Due to the fact that fractures are affecting the rehabilitation process, it is mandatory to take in to consideration risk factors and high risk groups related to osteoporosis, during rehabilitation. In addition to this, there is a scarcity of knowledge regarding the bone mineral densities of patients with traumatic brain injury(4).

Heterotopic ossification

Heterotopic ossification (HO) is a commonly observed complication of TBI that causes a functional disability. HO is a pathological process defined by formation of mature lamellar bones on non-osseous tissues typically on muscles or between joint capsules. HO usually appear as a result of traumatic brain injuries and spinal cord injuries(1,4). Immobility, spasticity, and fractures observed on patients with TBI present relatively a higher risk factor of resulting in a Heterotopic ossification(6).

The frequency of HO being observed in patients with TBI, ranges between 10-70% while the percentage of clinically considered symptomatically afflicted patients with HO, is in between 10-20%. The decrease in ambulation after TBI, is a major risk factor of HO development. Among other factors related to HO, we find longer coma duration, increased spasticity, pressure ulcer, infections, and decubitus ulcer. HO is most commonly observed on hip, elbow, and shoulder joints. While HO may proceed asymptomatically in certain patients, it may very well proceed with joint, fusions and total loss of functional limb usage. In other words, heterotopic ossification may proceed with varying intensities(7).

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The pathophysiology of HO isn't totally understood yet. Local factors causing apparition of HO are trauma, tissue hypoxia, venous insufficiency, and oedema. In pathological cases it has been shown that in the regions of soft tissues affected by microvascular oedematous changes, there is a formation of fibroblasts alongside osteoblasts. As a result of osteoblast accumulation transforms into osteoid and ectopic bone(8). On the affected tissues, the blood flow, inflammatory reactions and exudative cell infiltration increase. Following these phenomena, fibroblastic cell proliferation occurs. As a result of this proliferation; chondrogenesis, osteoid formation and disposition of bone matrix takes place. In order for ectopic bone formation to take place; appropriate local environment, osteoinductive factors, osteogenic precursor cells, enchondral bone formation are required(9).

Latest studies show that bone morphogenic protein (BMP) activation plays a key role as an osteoinductive agent in pathogenesis. BMP is secreted by demineralized bones, it has receptors on mesenchymal stem cells and it catalyses the transformation of primitive perivascular mesenchymal cells into osteoblasts. Specifically, BMP-2 and HO inductions cause expression of osteoblast specific transcription factors in the endoneurium. This leads to think that endoneurial progenitors are osteogenic precursors aiding HO. Moreover, osteoblasts isolated from a bone deriving from HO have shown superior osteogenic differentiation when compared to native osteoblasts derived from a normal bone. Other than BMP, PGE2(prostaglandin E2) is considered responsible for the metaplastic response of IL(interleukin-1) mesenchymal cells. Whereas; Substance P, CGRP is seen as an intermediary agent to cellular migration in the neurogenic inflammation process thus as an aiding factor in heterotopic ossification process. Studies also relate that secretion of pro-angiogenic factors are connected to the beginning of HO. Injured tissue vascularization represents an important step in the process of endochondral ossification of the joint while the passage to bone structure from hypertrophic joint(4).

The most common symptom of HO is pain. Other symptoms of HO include limitations in joint movements and functions, localized swellings, localized augmentation of corporal temperature, and erythema. Clinically, sometimes fever and increase in already existing spasticity might be observed. As a complication following HO joint ankyloses, pressure sores, difficulties related to transfer and daily activities, vascular and nerves compressions can be seen. Figure 1,2a,2b.

There aren't any laboratory findings of known related marker. Alkaline phosphatase, g-glutamyl transferase, creatinine phosphokinase and erythrocyte sedimentation rate and CRP can increase however they are not specific to HO. IL-6, IL-10, Monocyte chemoattractant protein (MCP-1), Alpha 2-glycoprotein deficiency are researched as new biomarkers(10,11).

HO can be seen on conventional radiographs, but it is not sensitive in the early stages. Figure 3,4.

Bone scintigraphy (Tc-99m) is the gold standard for early diagnosis and follow-up of maturation. However, it has low specificity in differentiating fracture, bone tumor, metastasis and osteomyelitis. Ultrasonography is helpful in differential diagnosis, calcifications can be detected earlier than radiography. CT, MRI show localization and extent of HO. They are especially used in preoperative planning. They determine the relationship of ectopic bone with surrounding tissues such as muscle, vein, and nerve(6).

Evidence for specific treatments of HO in TBI is limited. Preventive treatments include reducing spasticity, stretching exercises, maintaining joint range of motion, and avoiding local trauma such as fracture or skin injury. Once HO develops, there is no treatment to reverse the formation. The goal of treatment is to stop the growth of existing ectopic bone and to prevent the formation of new HO in other joints.

Since inflammation may be a cause of HO, NSAIDs (usually indomethacin, ibuprofen) prevent early inflammation and reduce bone formation by inhibiting PG synthesis(12). Bisphosphonates (most studies use etidronate) are also first-line treatments and are thought to disrupt ectopic bone turnover. Inhibits calcium precipitation and osteoid mineralization. While it is effective in the early period, it has no effect in the mature phase. Apart from these drugs, pamidronate, warfarin (by preventing osteocalcin carboxylation), glucocorticoid, calcitonin are used. After HO matures (6 months-1 year), radiation therapy can be used to prevent new bone growth(2). Radiotherapy prevents the transformation of pluripotent mesenchymal cells into osteoblasts, and has an anti-inflammatory effect at low doses. It can be used effectively in the early period of HO and to prevent recurrence after surgical resection.

Complications such as ankylosis, functional limitation, pressure sores, and nerve compression require surgery. It has been shown that the incidence of recurrence after surgery is high in immature heterotopic bone. Recurrence after surgery can be prevented with indomethacin, etidronate, radiotherapy, exercise or combination therapy(6). Apart from the basic treatment options in HO, research on new treatment options such as KMP type1 receptor inhibition, KMP antagonist (such as Noggin), nuclear retinoic acid receptor gamma agonist and platelet-rich plasma injections are ongoing(13).

Pain

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The primary focus for the pain of patients with TBI is not only to understand the problem and help with symptom relief, but also to improve their physical and cognitive function. Headache is the most common somatic complaint in TBI patients. The prevalence of chronic headache after TBI is approximately 58%. Traumatic brain injury (TBI) is often associated with chronic neuropathic pain. In addition to long-term physical pain after TBI, pain mediated by stress as a result of traumatic stress disorder (PTSD), hopelessness, sleep disturbance or depression is also important(14).Central pain is observed in TBI. Most central pain is a mixture of neuropathic and nociceptive types, with allodynia and hyperalgesia strongly suggestive of neuropathic components. In addition to hyperesthesia, dysesthesia, and allodynia, patients may experience muscle pain, circulatory pain, or visceral pain. It is also important to recognize that other factors, including spasticity and psychosocial factors, may contribute to pain(15).

While the rate of peripheral neuropathy in adult TBI patients ranges from 10% to 34%, it can reach up to 8% in children with TBI. Common compression neuropathies are ulnar (2.5-10% incidence), brachial plexus (10% incidence), or diffuse peroneal neuropathies. However, cases of sciatic neuropathy due to HO and retroperitoneal hematoma have also been reported(16).

Pain management in TBI should include a multidisciplinary approach. Aggressive physical therapy programs may be required, including desensitization, hydrotherapy, pacing techniques, biofeedback, transcutaneous electrical nerve stimulation, and contrast baths. Analgesics and NSAIDs are often used in pain control due to the involvement of pharmacologically inflammatory processes. Capsaicin and clonidine may be helpful in reducing pain. The use of gabapentin or pregabalin should be considered as first-line therapy because of its neuropathic pain relief effects. Antidepressants are auxiliary drugs and tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitors or SSRIs are used. Opiates do not provide much benefit in central neuropathic pain and are therefore not recommended. Medications can also be used to regulate sleep and mood disorders that often occur as a result of the chronic and severe nature of pain(16).

Pressure Sores (Decubite Ulcers)

Pressure-induced skin and soft tissue injuries are areas of localized damage from unconstrained pressure that causes damage to the underlying tissue. It usually occurs as a result of soft tissue compression between a bony prominence and the outer surface for a long time. Superficial skin is less susceptible to pressure-induced damage than deeper tissues, and therefore the external appearance may not show the extent of the damage. The consequences of pressure-induced skin

and soft tissue damage range from persistent erythema of intact skin to deep ulcers extending to the bone(17). According to the NPUAP Pressure Ulcer Staging System, pressure ulcers are diagnosed by clinical observation and staged according to the extent of the lesion.

Stage 1 Pressure Sores. The area looks red and feels warm to the touch. They are characterized by red, firm skin that is usually overlying bony tissue. Figure 4.

Stage 2 Pressure Sores. The area looks more damaged and may have an open sore, scrape, or blister. There is a disruption of the integrity of the dermis. Figure 5.

Stage 3 Pressure Sores. The area has a crater-like appearance due to damage below the skin's surface. There are lesions with partial thickness and full thickness dermis loss. Figure 6.

Stage 4 Pressure Sores. The area is severely damaged and a large wound is present. Bone, tendon or muscle tissue is damaged. Figure 7.

Deep tissue lesions, on the other hand, are lesions limited to deep tissues but characterized by intact skin. They arise in the deep muscle layers covering bony prominences as a result of high tissue stresses and are exacerbated by muscle spasticity caused by prolonged compression(18). Traditionally known, pressure sores are caused by a combination of pressure, frictional forces, and moisture. Pressure applied to the skin above the arteriolar pressure (32 mmHg) prevents the entry of oxygen and nutrients to the tissues, causing tissue hypoxia, accumulation of metabolic waste products and the formation of free radicals. Ulcer formation may occur more rapidly with higher pressures(19,20). Although hypoxia has traditionally been viewed as a major factor in the development of pressure-related skin and soft tissue injury, it is increasingly recognized that decompression and reperfusion injury also play an important role. Superficial lesions are primarily the result of moisture and friction. Exposure to moisture in the form of sweat, feces or urine can lead to skin maceration and superficial ulceration. There is little evidence to indicate the magnitude of moisture's contribution to skin damage. More than 100 risk factors for the development of pressure-related skin and soft tissue injuries have been described in the literature.

Immobilization is the most important factor contributing to the development of pressure-induced skin and soft tissue damage. Malnutrition is another risk factor for pressure ulcer development. Cross-sectional studies have shown that patients with pressure sores are more likely to have hypoalbuminemia. The role of inadequate skin perfusion in pressure ulcers is increasingly recognized. At low perfusion, pressure applied to the skin for less than two hours may be sufficient to cause serious damage. When vital organs such as the kidneys and gastrointestinal tract are not adequately perfused, blood flow to the skin is also reduced,

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increasing the risk of developing pressure-related injuries. Neurological diseases such as sensory loss, dementia, delirium, spinal cord injury and neuropathy are important risk factors for the development of pressure-related skin and soft tissue injuries. It is thought that these patients may not perceive pain or discomfort caused by prolonged pressure. Other risk factors are spasticity and contractures(20).

The general approach in the treatment of pressure ulcers is to ensure the redistribution of pressure with appropriate positioning and support surfaces, to reduce the underlying factors, to provide local wound care according to the characteristics of the ulcer. It is important to treat the infection in pressure sores. All open ulcers are colonized with bacteria, but only clinically significant infections should be addressed with culture and antibiotic therapy. Patients with deep wounds should be evaluated for the presence of osteomyelitis. Patients with pressure-related skin and soft tissue injuries are in a chronic catabolic state. Optimizing both protein and total caloric intake is particularly important for patients with stage 3 and 4 pressure injuries(20). Wound management includes debridement of necrotic tissue and appropriate dressings. Absorbent dressings serve to protect the wound from contamination and facilitate healing by absorbing exudate and protecting healing surfaces. Dressings with absorbent qualities include foams and alginates. Healing of pressure ulcers is promoted by dressings that maintain a moist wound environment while keeping the surrounding intact skin dry. Options for a dry wound include saline-moistened gauze, clear films, hydrocolloids, and hydrogels(21). Various adjunctive therapies have been investigated for the treatment of pressure ulcers, such as electrical stimulation, negative pressure wound therapy, therapeutic ultrasound, hyperbaric oxygen, topical oxygen, and application of growth factors to the wound, but the indications for use remain unclear.

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figure 1.Bilateral hip joint anklosis due to HO.(Patient has an intraabdominal baclofen pump for the treatment of spasticity)

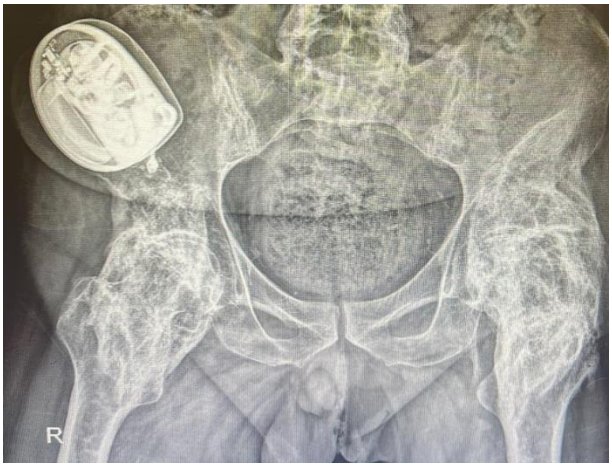


figure 2. a. Elbow joint ankylosis due to HO AP X-ray



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figure 2. b. Elbow joint ankylosis due to HO Lateral X-ray



figure 3. HO formations visualized by conventional radiography around the hip and knee joint



figure 4. Stage 1 Pressure sores are characterized by red, firm skin that is usually overlying bony tissue.



figure 5. In the stage 2 pressuresSores the area looks more damaged and may have an open sore, scrape, or blister. There is a disruption of the integrity of the dermis.



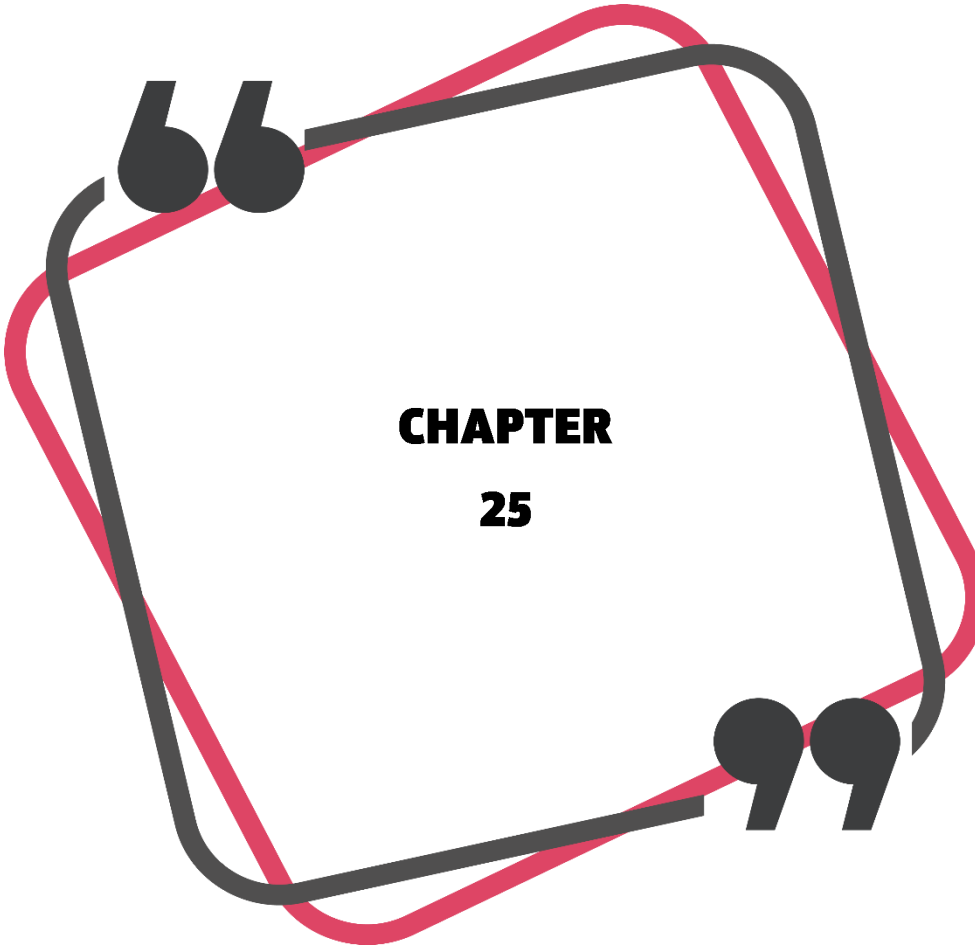
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figure 6.There are lesions with partial thickness and full thickness dermis loss in the stage 3 pressure sores.



figure 7. In the stage 4 pressure sores bone, tendon or muscle tissue is damaged.




CHAPTER
25

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CANCER AND MICRORNA: AN OVERVIEW OF HUMAN AND CANINE MAMMARY TUMORS

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Introduction

Cancer develops due to gene mutations that control the systems that carry out the cell's normal functions, resulting in uncontrolled cell proliferation and metastasis. The mutation often starts in a single cell at the cellular level, and the combined effect of many independent mutations in the process determines the type of cancer detected at the clinical level. Cancer, which is common all over the world, causes significant costs for countries. Despite advances in diagnosis and screening tests, cancer remains one of the leading causes of death (Bray et al., 2020). Cancer treatment is constantly evolving with the discovery of new markers in recent years (Ghasemi, Nabipour, Omrani, Alipour, & Assadi, 2016). For this, it is necessary to consider the differences in sensitivity, treatment, and prognosis for the individual (Penet, Krishnamachary, Chen, Jin, & Bhujwalla, 2014). One of the aims of medicine has been to advance targeted therapy instead of the classical approach to cancer management. In this review, the role of miRNAs in canine mammary tumors (CMTs), as well as human breast cancer (BC) will be discussed.

Human BC

BC is a clinically and genetically heterogeneous disease in women (Stingl & Caldas, 2007). About 2 million new cases are diagnosed each year around the world (Bray et al., 2020). With early diagnosis, advances in surgical techniques, and the use of targeted therapies, life expectancy is improved (Macià et al., 2012).

Despite all the advances, survival for metastatic-stage breast cancer is still low (approximately 25%). After the diagnosis of BC, classification based on histological and molecular typing determines the treatment plan and prognosis for the patient (Malhotra, Zhao, Band, & Band, 2010).

BC histological classification

BC is classified histologically as either invasive or in situ. In carcinoma in situ (CIS), malignant cells remain confined to the basement membrane of the terminal duct lobular units. These cancers can be subclassified as lobular or ductal (LCIS or DCIS). The most common is DCIS, which typically shows E-cadherin expression (Connolly, 1998). Hormone receptors and p53 are important in the histopathological classification of DCIS and LCIS (Gradishar, 2016). Invasive carcinomas include invasive lobular, infiltrating ductal, ductal/lobular, tubular, mucinous, papillary, and medullary types (Malhotra et al., 2010). Infiltrating ductal carcinoma accounts for 70-80% of all invasive lesions (Li, Uribe, & Daling, 2005). Evaluation of hormone receptor expression in invasive carcinomas may determine the use of targeted therapies (Gradishar, 2016). This approach aims to determine the specific treatment response for the tumor by molecular classification. Largely microarray-based gene expression analyses have identified differing treatment responses and some molecular types that have been shown to predict overall survival (OS) and disease-free survival (DFS). This group includes normal breast-like, HER2+, lumen A, lumen B, claudin-low, and basal (Prat et al., 2010). Subtypes (lumen A and lumen B) in ER+ tumors affect clinical outcomes and patient survival differently(Malhotra et al., 2010). While this molecular classification is crucial, the cost of genome sequencing or microarray analysis is a barrier. For this, the defined smaller gene sets can be used to identify BC types and predict the course of or response to treatment.

Canine mammary cancer

Canine mammary tumors (CMT) are common in veterinary practice. It accounts for approximately 25-70% of all cancers (Merlo et al., 2008). The primary risk factors for this malignancy are advanced age (mean 9.2–10.4 years) (Burrai et al., 2020), breed (Canadas, Santos, Pinto, Medeiros, & Dias-Pereira, 2018), ovariohysterectomy after 2.5 years, progestogen and estrogen therapy, robust reproductive status, and early obesity (Canadas et al., 2018). The development of breast tumors is affected by the endocrine state (Pang, Cervantes-Arias, Else, & Argyle, 2022). Endocrine status strongly affects ovarian hormone exposure in the first two years of life. This has been confirmed in female dogs undergoing ovarian

hysterectomy at an early age, particularly before the first estrus. Surgical sterilization at a later age does not seem to result in a change in risk (Klopfleisch, 2011). However, in estradiol-dependent mammary tumors, it prolongs survival in mastectomized female dogs (Kristiansen et al., 2016). The frequent occurrence in some breeds indicates a genetic basis. However, these changes are not fully known (Canadas et al., 2018). The identification of mutations in tumor suppressor genes BC 1 and 2 (BRCA 1 and 2) in female dogs with CMT suggests a role in the carcinogenic process(Thumser-Henner, Nytko, & Rohrer Bley, 2020).

MicroRNAs

MicroRNAs (miRNAs) are single-stranded RNA molecules that are 21-23 nucleotides long. They are encoded by genes that are transcribed from DNA but are not converted into proteins. They play a role in a various physical and pathological states as post-transcriptional gene regulators in all living things. The role of miRNAs as microbiological agents is illuminated by studies on many organisms, including domestic animals. It was found that miRNAs are important regulators of gene expression, contributing to phenotypic variability and disease development(Pillai & Bhattacharyya, 2007). They induce sequence-dependent mRNA degradation or translational repression by binding miRNA to messenger RNA (mRNA)(Reddy, 2015). miRNAs suppress expression mostly by interacting with the 3'UTR of target mRNAs (Ha). However, it has also been shown to interact with other regions such as the 5' UTR, coding sequence, and gene promoters (Broughton, Lovci, Huang, Yeo, & Pasquinelli, 2016). It has been reported that miRNAs activate gene expression under definite conditions (Vasudevan, 2012). miRNAs, which have an important role in normal animal development, have functions in various biological events (Fu, Brkić, Hayder, & Peng, 2013). Abnormal expression of miRNAs has been implicated in many human diseases (Paul et al., 2018). In addition, extracellular miRNAs have been recognized as potential biomarkers in various diseases (Wang, Chen, & Sen, 2016).

miRNAs in cancer

The relationship between miRNA and cancer has been considered for the first time with the deletion of miR-15 and miR-16 clusters and low expression in chronic lymphocytic leukemia (GA, 2002). Some miRNAs inhibit the translation of proto-oncogenes in normal tissues. MiRNAs that control the expression of such an oncogene are called "tumor suppressor miRNAs" (TS-mir) (Lujambio & Lowe, 2012). Conversely, decreased expression of tumor suppressor miRNAs results in increased expression of oncogenes and tumorigenesis. This type of miRNA, called onco-mir, appears to increase cancer development. MicroRNAs

can see both oncogenes and tumor suppressor mRNAs as potential targets. Thus, the actual function of a given miRNA depends on the cellular content of either TS-mir or onco-mir (HOTHER, GRØNBÆK, & COWLAND, 2007).

MicroRNAs in canine breast cancer

The CMT global miRNA expression profile in in-vitro models has been evaluated by various investigators (Lutful Kabir, DeInnocentes, & Bird, 2015; Osaki et al., 2016). Some researchers have evaluated miRNA expression in canine mammary tissue at different phases of tumorigenesis. It has been reported that few miRNAs are expressed (Boggs, Wright, Stickney, Porter, & Murphy, 2008; von Deetzen, Schmeck, Gruber, & Klopfleisch, 2014). miRNA expression was investigated in co-cultures of tumor-associated macrophages and CMT cells, and it was reported that the tumor microenvironment may affect miRNA expression (Klopfleisch, 2011). Another study examined the expression profiles of 277 miRNAs in three CMT cell lines. It was discovered that 41 miRNAs showed increased expression and 24 miRNAs showed decreased expression in the cell lines studied. In two cell lines, the tumor suppressor gene INK4A was found to be regulated by increased miR-141 expression (Lutful Kabir et al., 2015). Osaki et al.'s study, which compared the miRNA profile in a CMT cell line and normal canine mammary tissue, found that the expression of 291 miRNAs was altered. It was shown that miR-143 expression increased while miR-138a expression decreased in the examined cell line (Osaki et al., 2016). CMT stem cell-like cells from three CMT cell lines were also investigated. When expression in well-differentiated tumor cells was compared to this cell culture, twenty-four miRNAs showed decreased expression and nine showed increased expression.

Furthermore, miRNA target prediction and signaling pathway analysis were carried out, and it was discovered that the transforming growth factor beta (TGFβ-) pathway was potentially altered (Rybicka et al., 2015). There are not many studies evaluating miRNA in CMT tissues. In one study, six malignant breast tumor specimens and 10 normal tissue specimens were examined. They evaluated the levels of ten miRNAs previously reported to be associated with human BC. MiR-21 and miR-29b expression were found to be increased in these tumors (Boggs et al., 2008). There was also a significant decrease in miR-15b and miR-16 expression. A different study looked at the global miRNA expression profile in 146 CMTs, including 30 benign, 116 malignant, and 25 normal breast specimens.10 miRNAs and target genes were confirmed by real-time PCR. Four miRNAs were also validated from plasma samples of non-metastatic CMTs, metastatic CMTs, and non-tumor female canines. However, the researchers could not confirm the relationship. Based on this, they suggested that miRNAs primarily have a regulatory role in the process of metastasis, but do not affect malignant

transformation. It is well argued that miRNAs could be molecular markers for metastases (Bulkowska et al., 2017).

Clinical applicability of miRNAs and future perspectives

Understanding the fundamentals of cancer biology may aid the development of specific therapies that target molecular pathways in tumor cells. MiRNAs regulating the expression of many target genes may enable the development of new therapeutic strategies in the future (Kasinski & Slack, 2011). Considering the function of a miRNA in tissue and its expression in the tumor, agents and miRNA-based therapies may be developed in the future (Kasinski & Slack, 2011). In the therapeutic setting, by inhibiting miRNAs with increased expression, molecules with antagonistic effect can be used, and for miRNAs with loss of function or decreased miRNAs, molecules with mimetic effect can be used. MiRNAs could be factors influencing chemotherapy resistance in patients. It has been suggested that it can be used as a future therapeutic target for human breast cancer (Campos-Parra et al., 2017). In a study on the use of miRNA inhibitors in cancer, increased miR-10b expression was found in metastatic breast cancer. showed that positive miR upregulation in non-metastatic BC cell lines could provide potential for metastasis formation (Ma, Teruya-Feldstein, & Weinberg, 2007). It has been shown that lung metastasis can be highly prevented by IV administration of miR-10b, an antagomir derivative inhibitor, in mice. However, no change was observed in the primary tumor. As with all drugs, the efficacy and safety of miRNA-derived drugs need to be rigorously evaluated.

Conclusion

In both human and veterinary oncology, distinguishing healthy individuals from sick individuals is important. In summary, regardless of the histopathological type of the tumor, the canine BC miRNA profile was found to be differentially expressed according to tumor type, metastasis factor, and malignancy grade. Differences in miRNAs expression were found between metastatic and non-metastatic groups. These are very interesting results. While miRNAs are not associated with malignant transformation, they are highly associated with the metastasis process. In addition, miRNA can be used as a molecular marker for metastasis. It has predictive importance for the course of the disease. Because histopathologically identical malignancies may have inconsistent clinical outcomes. Moreover, because of the similarities between canine BC and human BC, metastasis biomarkers for dogs can be examined to see if they are useful for humans as well.

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