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Chapter 1

GLIAL MARKERS USED IN IMMUNOHISTOCHEMISTRY

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1. GLIAL MARKERS USED IN IMMUNOHISTOCHEMISTRY

1.1. Glial Cells

The nervous system comprises two fundamental cell types, neurons, and glial cells. Each neuron plays a direct role in making hundreds of connections with other neurons, processing information ,and generating responses [1]. Although glial cells do not directly participate in cell-to-cell electrical communication, as in neurons, they are of great importance in that they are involved in the support and protection of neurons, as well as in many neural activities, neural nutrition, and defense of cells in the central nervous system (CNS) [1, 2]. Neuron cells make up half of the cells in the human CNS, while the remaining half is made up of glial cells, according to a new analysis. Glial cells surround and provide physical and metabolic support to the soma, axon, and dendrites of neurons [2].

In the CNS, there are four classifications of glial cells including oligodendrocytes, astrocytes, microglia, and ependymal cells all together are called central neuroglia. The supporting cells in the peripheral nervous system (PNS) are called peripheral neuroglia and include Schwann cells, satellite cells, and various other cells associated with specific organs for tissues. Examples of these cells are terminal neuroglia (teloglia) associated with the motor endplate, enteric neuroglia in ganglia in the wall of the digestive tract, and Müller cells in the retina [3].

1.1.1. Central Neuroglia

Astrocytes are the largest of the neuroglial cells. They communicate with neurons, forming a network of cells in the CNS to support neurons and regulate most of their activity. Some astrocytes prolong against the entire thickness of the brain, forming a skeleton for migrating neurons during brain development. Other astrocyte extensions extend from blood vessels to neurons. They also take on major task in delivering metabolites to neurons and removing waste products from neurons. They help maintain the tight junctions of capillaries that form the blood-brain barrier. Another important task is to regulate neuronal activities by buffering the K+ concentration in the extracellular area of the brain [3]. Oligodendrocytes form and maintain the myelin sheath that enables high-speed transferring of electrical signals in the CNS. They contain fewer extensions and are small compared to astrocytes. Microglial cells have phagocytic properties. They normally makeup about 5% of glial cells in the adult CNS, but proliferate at sites of injury and disease and become active phagocytic. It is the smallest of neuroglial cells and has small flat nuclei [3]. Finally, ependymal cells form the epithelial-like lining of the cerebral ventricles and spinal canal. They are single-layered cubic prismatic cells and have morphological and physiological characteristics of cells that transport

fluid. Microvilli on the apical surface of the cell are responsible for the absorption of cerebrospinal fluid [3].

1.1.2. Peripheral Neuroglia

The main glial cells in the PNS are Schwann cells which cover total axons in peripheral nerve fibers, together with there is two classifications of them are myelinated as well as unmyelinated. It is assumed that unmyelinated cells have metabolic and mechanical support due to their similarity to astrocytes. Myelination Schwann cells form insulator envelopes around the axes. Olfactory coat cells symbolize a particular predicament of glial similar to non-myelinating Schwann cells and associated with both the CNS and the PNS portion of the prime olfactory axons. Other significant cell of PNS glia is enteric glia which is located in the autonomic ganglia (enteric nervous system) of the intestine. Dissimilar to other segments of the PNS, although enteric glia is structurally and biochemically very similar to astrocytes, the enteric system has complicated synaptic interplays and a great deal of integration capability. While the straightforward satellite glial cells encircle the cell bodies of another autonomic and sensorial ganglia, telologia covers synapses amongst nerve terminals and skeletal muscles. They play a role in maintaining the stability of the neuromuscular connection and in the regulation of synaptic transference [4].

1.2. Glial Markers

1.2.1. Glial Fibrillary Acidic Protein (GFAP)

Originally, Glial Fibrillary Acidic Protein (GFAP) has been purified from fibrous astrocytes and demyelinated axons that form the MS plaque in the brains of patients with multiple sclerosis [5]. In 1969, the amino acid content of GFAP was defined firstly by Dr. Eric Shooter at the 2nd International Society for Neurochemistry [6]. In 1984, thanks to the Cowan laboratory, the mouse GFAP gene was cloned and led to further studies on the molecular biology of GFAP, [7] with the human GFAP gene cloned in 1989 [8].

Initially, GFAP was assumed to be an astrocyte-like intermediate filament [5]. Subsequent studies have shown that GFAP is also phrased in foetal and grown-up neural stem cells. Phrasing of GFAP mRNA and protein begins premature in development in the developing radial glia. For example, in mouse brains, GFAP starts to be expressed at embryonic day E9.5 [9] and in human radial glia at almost 13 weeks of gestation [10]. In the adult nervous system, GFAP is expressed in grey and white substance, while Muller glia in the retina, Bergmann glia in the cerebellum, and also grown-up neural stem cells in the subventricular and sub granular zones [10]. In addition, GFAP has been shown to have a wide distribution, where

it can be expressed by Schwann cells in the PNS, by mature glial cells in the gut, and by non-glial and insomuch as non-CNS cells [11, 12]. These are fibroblasts [13], chondrocytes [13, 14], myoepithelial cells [13, 15], lymphocytes [16], liver stellate cells [17].

GFAP is the member of the intermediate filaments family, along with microtubules and microfilaments from the cytoskeleton of most eukaryotic cells [18]. Among the important functions of GFAP are maintaining the mechanical strength and cytoskeletal structure of glial cells, supporting neighboring neurons and the blood-brain barrier [6]. The four proteins called type III intermediate filament proteins are GFAP, peripherin, vimentin, and desmin (Figure 1) [19]. All intermediate filament protein types consist of a 'head' with an amino-terminal, a 'rod' with a central helix, and a 'tail' domain with a carboxy-terminal [8]. The tail and head domains modify in size and amino acid sequences, while the rod domains (amino acid residues 310-350) are highly conserved between intermediate (Figure 1) [11].

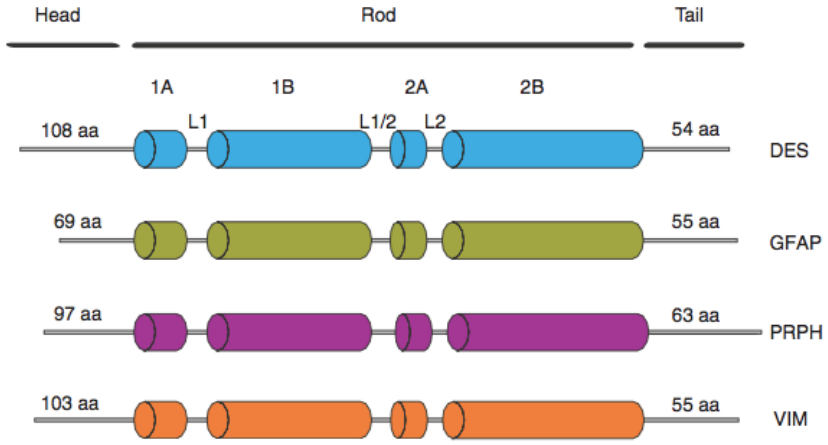

Figure 1. Diagram showing type III intermediate filaments structures including the head, rod, and tail areas (human format is demonstrated)

Also the GFAP gene comprise nine exons, 10 additional isoforms of the GFAP gene have been expressed in the mouse [20] and the human nervous system to date [11, 20, 21] In addition, recent studies have proven that these isoforms are expressed in prominent astrocyte subsets and can alter all the features of a cell's intermediate filament network. Thus, it is a marker for astrocytes known to be stimulated in cerebrum injury or throughout CNS disruption and is more expressed in the elderly brain [11]. It has been recommended that enhanced transcription of GFAP during aging may be due to the increment burden of oxidatively faulty proteins seen in tissues throughout the body, including the cerebrum [22].

Most studies using GFAP knockout mice suggest that GFAP is involved in the modulation of neural functions by astrocytes [23, 24]. By investigating diverse electrophysiological parameters in the cerebellum of GFAP knockout mice, Shibuki et al. discovered insufficient cerebellar long-dated depression at Purkinje cell synapses. It was also studied that the conditioned wink response in these mice was substantially disrupted. According to these researchers, GFAP is required for communication between Bergmann glia and Purkinje cells along the long-term induction and care of depression [24]. Also, another study showed increased pyramidal neuron loss in the hippocampus after ischemia in GFAP knockout mice exposed to injury conditions [25].

1.2.2. Ionized Calcium Binder Adapter Molecule (IBA1)

With the discovery of the Iba-1 protein and the determination of microglial specificity by the Japanese research group [26] in 1996, the popobability of establishing a protocol for the immunohistochemical finding of microgliocytes emerged [27]. The Iba-1 protein, an associate of the calcium-binding protein group, has a molecular weight of 17 kDa and consists of 147 amino acid remnants that form a compact domain containing two calcium binding sites rich in hydrophobic amino acids [28]. The Iba-1 protein is thought to be similar to Allograft Inflammation Factor, AIF-1;daintain and Microglia Response Factor, MRF-1, which are proteins characterized by other authors [29]. Nevertheless, there are conflicting studies concerning the similarity of these proteins with Iba-1 [30]. Iba-1 has a role in cytoskeletal reorganization as well as changing the form of plasmalemma processes that occur in phagocytosis [31]. Iba-1 protein is expressed in the cytoplasm of microgliocytes and studies show that fixatives [32, 33] used in immunohistochemical methods are effective in protecting antigenic markers.

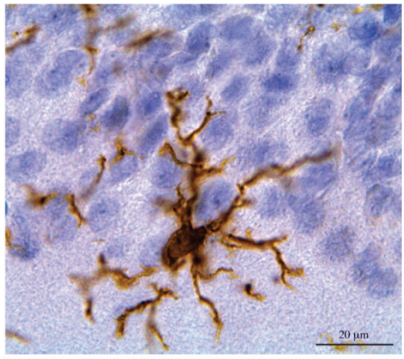

Figure 2. A microglioside for Iba-1 in the rat hippocampus [34].

Iba-1 expressions are activated in microglia following facial nerve axotomy, ischemia, inflammatory conditions, and viral infections and thus

play a role in active phenotypes of microglia [35].

A study of cell lines expressing Iba1 found that Iba1 is particularly overexpressed in cells of the monocyte/macrophage strain. They showed that the anti-Iba1 antibody also got to know several tissue-resident macrophages, including alveolar macrophages, splenocytes, and Kupffer cells. These findings suggest that Iba1 is a macrophage-typical protein and the anti-Iba2 antibody is an important marker for the acknowledgement of microglia and macrophages. And also, the anti-Iba1 antibody has a major benefit over other marker antibodies as it is just the polyclonal antibody so far applicable to double-labeling studies of macrophage/microglia lineage, even on paraffin sections [36].

1.2.3. Neuron Glial Antigen 2 (NG2)

Neuron glia antigen-2 (NG2), expressed by many CNS cells in the course of growth and differentiation, is involved in proliferation and angiogenesis stages [37]. Lack of NG2 during early development causes pericyte endothelial fusion and damaged basement membrane formation in the blood vessel [38]. The increase in NG2 expression is correlated with an aggressive disease course and low survival in human adult glioma. Gliomas, which are malignant tumors originating from glial cells, include oligodendroglioma (derived from oligodendrocytes or oligodendrocyte precursor cells), astrocytoma (derived from astrocytes), and oligoastrocytoma (complex glial cell origin) [39].

Invasion and migration of glioblastoma multiforme (GBM) tumor, a extremely invasive astrocytoma, to the CNS is based on the mutual effect of tumor cells with extracellular matrix molecules and host cells. According to 'The Cancer Genome Atlas' analysis of mRNA data of human GBM samples, NG2 is one of the highly regulated proteoglycans [40].

In murine models of glioma, NG2 expression induces necrosis resulting in increased vascular leakage, tumor volumes, vasogenic edema, and dysregulation of the host-derived tumor vasculature [41]. Although the expression and role of NG2 in adult gliomas are fine defined, studies on the role of NG2 expression in pediatric brain tumors are scarce. Yadavilli et al recently found that NG2 expression was associated with childhood diffuse intrinsic pontine glioma (DIPG), which occurs only in children. DIPG is one of the most offensive and infiltrative tumor forms, accounting for 10% to 20% of CNS tumors. Expression of NG2 was found in 78% of the DIPG samples analyzed in a cohort of 50 postmortem samples [42] (Figure 3).

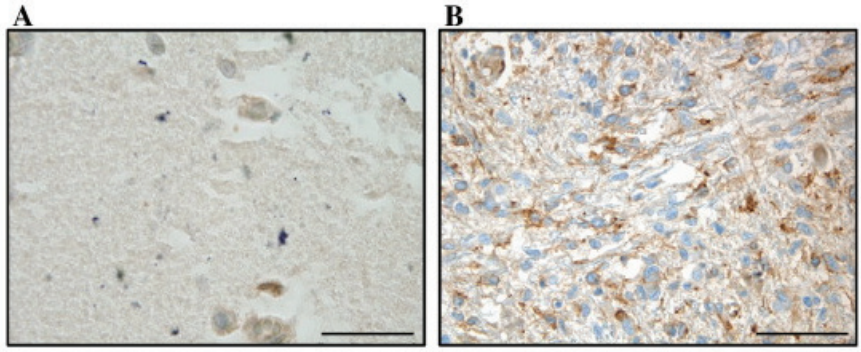

Figure 3. NG2 immunohistochemical staining images in pediatric diffuse intrinsic pontine glioma (DIPG). *A) Solid and B) tumour cerebrum samples are shown at autopsy from patients with DIPG. Increased immunoreactivity level of NG2 (Brown stained areas) is observed in the tumor sample [42, 43].*

1.2.4. Myelin basic protein (MBP)

The myelin sheath produced by oligodendrocytes in the CNS accelerates neuronal communication by enhancing the rate of impulse propagation in neuronal axons. One of the main ingredients of CNS myelin is the myelin basic protein (MBP), which has been called the 'executor molecule of myelin' [44]. Mice lacking MBP show, tremor symptoms, severe hypomyelination of the CNS, and premature death [45].

Classical MBP proteins consist of a gene complex named Golli, meaning genes of the oligodendrocyte strain, giving rise to the Golli (-MBP) family [46]. Golli (-MBP) proteins are expressed in other neural and non-neural cells, whereas classical MBP proteins are found only in myeliminating cells [47].

1.2.5. 2',3'-Cyclic-nucleotide 3'phosphodiesterase (CNPase)

2',3'-Cyclic-nucleotide 3'phosphodiesterase (CNPase) accounts for 4% of human CNS myelin protein, making it a possible autoantigen in multiple sclerosis and one of the most abundant proteins in non-compact myelin [48]. CNPase is expressed to a much lesser extent in Schwann cells and olfactory sheath cells in PNS [49, 50]. Oligodendrocytes, Schwann cells, and myelin of all species have two CNPase isoforms [51, 52]. These isoforms are synthesized on free ribosomes and enzymatically active [53].

The absence of CNPase by knockout in mice results in axonal swelling, resulting in premature death, and a myelin morphology very similar to wild-type animals continues to be preserved. In addition, the behavioral phenotype typically occurs between 6 and 12 months of age, with muscle weakness and consequent death and loss of motor abilities [54]. Further,

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Have presented that this enzyme participates in age-related regulatory changes that will be partially involved in age-related CNS degeneration [55]. This enzyme activity was found to be increased in active microglial cells used as models for cerebrum damage. When this enzyme is knocked down, an increase in inflammatory mediators IL-1β and TNF-α was observed, suggesting that CNPase may have an anti-inflammatory function in damaged CNS cells [56].

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Chapter 2

THE IMPACT OF WOMEN'S FEAR COVID-19 ON BREAST CANCER EARLY DIAGNOSIS PRACTICES

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INTRODUCTION

COVID-19 is a progressive disease that causes various illnesses with symptoms more severe than the common cold, such as respiratory failure (WHO, 2019). It has been declared a Public Health Emergency and recognized as a pandemic by the World Health Organization (WHO, 2020). According to studies on COVID-19, the disease has progressed in 10-15% of the cases and resulted in death in approximately 2% (Richardson et al., 2020).

Patients with cancer, who are in the risk group for COVID-19 mortality, and oncology centers have been affected by the pandemic process. Measures, such as the transformation of full-fledged hospitals into "Pandemic Hospitals", allocation of intensive care and inpatient services in these hospitals for COVID-19 patients, postponement of non-emergency diagnosis and treatment procedures, and commissioning all hospital personnel to COVID-19 clinics alternately have led to a slowdown or even disruptions in oncology services (Turkish Radiation Oncology Association Report, 2020). On the other hand, some patients diagnosed with cancer have tended to avoid going to oncology centers and refuse treatment due to anxiety about COVID-19 (Turkish Radiation Oncology Association Report, 2020).

During the pandemic process, difficulties have been observed in the management of breast cancer treatment, as in many types of cancer, due to the limited use of resources and the working system of healthcare personnel according to pandemic conditions (Citgez et al., 2020). Especially in regions where COVID-19 patients are concentrated, some changes have been made in breast cancer diagnosis and treatment processes with the recommendations of scientific associations. Routine screening programs have also been temporarily suspended in many countries around the world during this period.

The increase in early diagnosis of breast cancer, improvement in the prognosis of the disease, improvement in aesthetic outcomes, and increase in postoperative recovery rates are effective in decreasing the costs in the health care system (Welch, 2016). However, early diagnosis practices have decreased during the COVID-19 process. For example, it has been reported that the number of presentations to oncology outpatient clinics in the United States has decreased by up to 50% and that 80.000 undetected cancer diagnoses will be observed in just three months during the COVID-19 disease process (IQVIA, 2020). It has also been reported that most of these will be breast cancer cases (Vanni et al., 2020). As a matter of fact, the weekly average number of people diagnosed with breast, colorectal, lung, pancreatic, and stomach cancer in the USA during the

pandemic period has decreased to 46.4%. Breast cancer diagnoses, on the other hand, have been reported to decrease to 51.8%, that is, from 2,208 people to 1,064 people (Kaufman et al., 2020).

Erşen et al. (2020) stated that the fear of contamination experienced by patients with cancer during the pandemic negatively affected their preferences for receiving health services and that only 40.1% of the patients presented to the hospital in emergencies. Bagus et al. (2020) reported that the number of patients diagnosed with advanced stage cancer increased due to delayed emergency admissions during the COVID-19 process. However, the vast majority of patients with cancer face the risk for disease progression when they do not receive treatment. Reducing the number of patients presenting to health institutions and postponing treatments due to the uncertainty about how long the current epidemic will last may protect from COVID-19, but it may also cause cancer to progress. Therefore, it is important that the treatment of these patients should not be delayed (Örün et al., 2020).

It is thought that as the number of women who do not get a mammogram increases due to pandemic conditions, the burden on the health system, the cost of healthcare practices, and mortality rates will increase, as well (Vanni et al., 2020). When COVID-19 first appeared in Turkey, many medical procedures which were thought to be non-urgent were delayed or canceled. Over time, preventive procedures have been shown to slow down the spread of the virus. Breast cancer screening services have started again in many health centers since June 01, 2020 (Ministry of Health, 2020).

This study aimed to determine the effect of women's fear of COVID-19 on breast cancer early diagnosis practices. Accordingly, the study sought answers to the question "How does the fear of COVID-19 pandemic affect women's breast cancer early diagnosis practices?"

METHOD

The study used a descriptive design and was carried out between October-November 2020. The population of the study consisted of a total of 9639 ≥40-year-old women who had presented to the Obstetrics and Gynecology, Dermatology, Internal Medicine, Infectious Diseases, Urology, Ophthalmology, Otolaryngology (ENT), or General Surgery polyclinic of a state hospital located on the city for any reason in the last 6 months before the pandemic, and whose phone number was registered in the hospital database. The sample size was determined as 370 individuals as calculated on the G * Power 3.1 software package based on a 5% acceptable margin of error and a 95% confidence level. To make up for possible data loss, the sample size was increased and the study was completed with a total of 425 people who accepted to participate in the study, were at least

literate, and did not have any communication problems or a history of cancer.

Study data were collected using a questionnaire developed by the researchers in line with the literature and the Fear of COVID-19 Scale. The questionnaire was created on Google Forms (Google Inc, California, USA) due to the ongoing COVID-19 pandemic and was administered to women aged 40 or over via WhatsApp.

The Fear of COVID-19 Scale: The scale was developed by Ahorsu et al. (2020) (Ahorsu et al., 2020) and adapted to Turkish by Bakioğlu et al. (2020). It consists of one dimension and 7 items. There are no reverse items on the scale. The total score obtained from all items of the scale reflects the level of fear of COVID-19 experienced by the individual. The scores that can be obtained from the scale range from 7 to 35. High scores from the scale mean experiencing a high level of fear of COVID-19 (Bakioğlu et al., 2020).

Data Collection Tools:

The data of the study were evaluated using the SPSS 20.0 package program. Descriptive statistics, Kruskal Wallis Test, Mann-Whitney U and Post-hoc tests were used in the analysis of the data. The normal distribution of the responses given in the test was tested with Kolmogorov-Smirnov. Statistical significance level was accepted as $p < .05$.

Ethical Aspect of the Research

The research was conducted under the Helsinki Declaration of Ethical Principles for Medical Research on Human Volunteers. Approval of a local clinical research ethics committee (decision number: 2020/683) was obtained to conduct the study. Also, the written permission of the Scientific Research platform of the Ministry of Health was obtained. The individuals participating in the study were informed about the study, their verbal consent was obtained, and their volunteer participation was ensured.

RESULTS

The mean age of women in the study was 48.10 ± 6.30. It was determined that 71.1% of the participants were aged between 40 and 50, 26.6% were primary school graduates, and 71.5% were married. Also, 38.4% had a chronic disease and 62.4% had a family member who had a history of COVID-19. The mean total score of the participants from the Fear of COVID-19 Scale was 22.58 ± 8.16.

Table 1: Comparison Of Demographic Characteristics Of Women Participating And Mean COVID-19 Fear Scale Score

Characteristics	n	%	COVID-19 fear scale	Statistic
Age(Years)				
40-50 y	302	71.1	24 (7-35)	U=-0.673
51-65 y	123	28.9	23 (7-35)	p=.50
Education status				
No illiterate	16	3.8	26.5 (14 - 35)[b]	
Primary school	113	26.6	24 (7 - 35)[b]	**χ2 =31.05**
Middle School	65	15.3	28 (7 - 35)[a]	****p=.000**
High school	89	20.9	24 (7 - 35)[b]	
Associate degree	53	12.5	20 (7 - 34)[b]	
License	89	20.9	20 (7 - 35)[b]	
Marital status				
Married	304	71.5	24 (7 - 35)	U=18.405
Single	121	21.5	22 (7 - 35)	p=.99
Other Chronic disease				
Yes	163	38.4	27 (7 - 35)	**U=16585**
Non	262	61.6	22 (7 - 35)	****p=.000**
Number of children				
No children	126	29.6	25 (7 - 35)[b]	
1 child	62	14.6	26 (7 - 35)[ab]	**χ2= 15.08**
2 children	131	30.8	26 (7 - 35)[b]	***p=.002**
3 veya daha fazla çocuk	103	24.2	20 (7 - 35)[a]	
Being a relative with COVID-19				
There is	265	62.4	23 (7 - 35)	U=22743
No	160	37.6	26.5 (7 - 35)	p=.20

-Letters (a, b) indicate no difference between groups that have the same letter* = P <.05, ** = P <.001 U: Mann Whitney U test, χ2: KruskalWallis

The Kruskal-Wallis test, a non-parametric statistical test, was employed to test whether there was a significant difference between the mean total score of the participants from the Fear of COVID-19 Scale and their level of education and number of children. Post Hoc test was used to determine the source of the difference.

Among the women participating in the study, middle school graduates were found to experience higher fear of COVID-19 compared to participants with different levels of education ($p < 0.05$) (Table 1). It was found that women with chronic illnesses had higher fear of COVID-19 than those without a chronic illness.

Also, the number of children and total scores from the fear of COVID-19 scale were compared among the women participating in the study. Accordingly, it was found that women who had three or more children experienced less fear of COVID-19 than women who had no children and those who had 2 children.

Table 2: Comparison Of Women's Characteristics For Breast Cancer With The COVID-19 Fear Scale

Characteristics	n	%	COVID-19 fear scale	Test statistic p Value
Menopausal status				
Yes	113	26.6	24 (7 - 35)	U=17525
No	312	73.4	23.5 (7 - 35)	p=.92
Family breast cancer status				
Yes	97	22.8	27 (8 - 35)	**U=12207**
No	328	77.2	22 (7 - 35)	****p=.000**
Previously a problem with the breast				
Yes	102	24.0	26 (8 - 35)	**U=13397**
No	323	76.0	22 (7 - 35)	***p=.004**
Knowing about Breast Cancer Early Diagnosis applications				
Knows	273	64.2	21 (7 - 35)	**U=28457**
Does not know	152	35.8	28 (7 - 35)	****p=.000**
Believing that they will get rid of cancer with early diagnosis				
Believes	388	91.3	23 (7 - 35)	**U=8913**
Does not believe	37	8.7	28 (9 - 35)	***p=.015**
Knowing where to have mammography and CBE				
Knows	270	63.5	21 (7 - 35)	**U=28176**
Does not know	155	36.5	28 (7 - 35)	****p=.000**
Frequency of BSE				
Once a month	79	18.6	27 (8 - 35)[ab]	
At irregular intervals	160	37.6	22 (7 - 35)[a]	**χ2= 9.60**
I never do	146	34.4	25.5(7- 35)[b]	***p=.022**
I do not know BSE	40	9.4	24(10- 35)[ab]	

-Letters (a, b) indicate no difference between groups that have the same letter* = P <.05, ** = P <.001 U: Mann Whitney U test, χ2: KruskalWallis

The mean fear of COVID-19 scale total scores of the women who had a family member with a history of breast cancer was compared to those who did not. The fear of COVID-19 experienced by women who had a family member with a history of breast cancer was higher compared to those who did not. This finding was statistically significant.

The total scores obtained from the fear of COVID-19 scale in the study were compared among women who had a breast problem previously and those who did not. Accordingly, it was found that women who had a breast problem before had higher levels of fear of COVID-19 compared to those who did not have a problem and that this finding was significant.

The total scores of the women in the study from the fear of COVID-19 scale were also compared in terms of the knowledge of breast cancer early diagnosis practices. The fear experienced by women who knew about breast cancer early diagnosis practices were found to be lower compared

to those who did not, and this finding was statistically significant.

Table 3: Comparison Of Women's Characteristics For Breast Cancer And COVID-19 With The COVID-19 Fear Scale

Characteristics	n	%	COVID-19 fear scale	Test statistic p Value
Making early diagnosis applications for breast cancer				
Regularly performs BSE at home	133	31.3	18 (7 - 35)[a]	
Goes to the doctor for a breast exam	27	6.4	24 (8 - 33)[ab]	**χ2= 26.72**
Getting breast ultrasound	15	3.5	22 (7 - 33)[ab]	****p=.000**
Regularly having mammography	23	5.4	25 (8 - 35)[ab]	
Does not apply any early diagnosis	227	53.4	27 (7 - 35)[b]	
Previously had a mammogram				
Yes	147	34.6	24 (7 - 35)	U=21559
No	278	65.4	24 (7 - 35)	p=.34
Barriers to mammography and CBE				
COVID 19 is afraid of contracting disease	186	43.8	22 (7 - 35)	
Doesn't know where it was made	11	2.6	22 (10 - 35)	χ2= 14.18
Is ashamed	24	5.6	28 (11 - 35)	p=.07
Afraid that I will have a health problem	18	4.2	26 (8 - 34)	
Hearing that the breast hurts on mammography. I'm afraid	9	2.1	30 (14 - 33)	
Doesn't know that I have to get it done	24	5.6	26 (7 - 33)	
I wants to be a woman doctor in the institution I go to	8	1.9	22.5 (14 -33)	
Afraid of getting mammography	9	2.1	24 (12 - 33)	
There is no obstacle	136	32.0	24 (7 - 35)	
Fear of getting COVID-19 if I goes to the hospital				
Yes	261	61.4	22 (7 - 35)	U=23331
No	164	38.6	27 (7 - 35)	P=.11
Considering Mammography or CBE until COVID-19 is over				
Thinks	87	20.5	26 (7 - 35)	U=12.891
Not thinking	338	79.5	23 (7 - 35)	P=.07

-Letters (a, b) indicate no difference between groups that have the same letter* = P <.05, ** = P <.001 U: Mann Whitney U test, χ2: KruskalWallis

In the study, the total scores of the women from the fear of COVID-19 scale were compared in terms of believing that early diagnosis would help recover from cancer. It was found that women who believed that they would recover from cancer with early diagnosis experienced less fear of COVID-19 than those who did not believe it and that the relationship between them was statistically significant.

The total scores of the women in the study from the fear of COVID-19 scale were also compared regarding the knowledge of places where they could get mammograms and have CBE. It was determined that women who

knew where they could get mammograms and have CBE experienced less fear of COVID-19 than those who did not know it and that the relationship between them was statistically significant.

One of the comparisons was between the total scores of the women in the study from the fear of COVID-19 scale and the frequency of performing BSE. It was found that those who performed BSE at irregular intervals experienced less fear of COVID-19 than those who never did it.

The comparison of the total scores of the women in the study from the fear of COVID-19 scale in terms of doing breast cancer early diagnosis practices indicated that women who regularly performed BSE at home experienced less fear of COVID-19 than those who did not perform any early diagnosis practices at all.

DISCUSSION

Breast cancer is known to be the most frequently diagnosed type of cancer among women (24.2%) and the leading cause of cancer-related deaths (15.0%) (Bray et al., 2018). Maintenance of women's health during the pandemic process is of significance for the health system. In this section, the effect of fear of COVID-19 on breast cancer early diagnosis practices of women will be discussed.

The total score of the women participating in the study was found to be 22.58 ± 8.16 on the COVID-19 Fear Scale. The scores that can be obtained from the scale range from 7 to 35. High scores on the scale mean experiencing a high level of fear of COVID-19. In a study conducted in Russia with 850 participants, 73.2% of whom were female, the mean score from the fear of COVID-19 scale was found to be 17.7 ± 4.6 (Reznik et al., 2020). According to another study, the mean score of women from the fear of COVID-19 scale was found as 26.16 ± 5.73 (Mertens et al., 2020). In a study conducted on 2157 people in Bangladesh, the mean score of female participants obtained from the fear of COVID-19 scale was 19.07 ± 5.04 (Hossain et al., 2020). It is thought that similar results in different studies stem from the fact that women are affected biologically, psychologically, and socially during the COVID-19 pandemic process.

Among the women participating in the study, middle school graduates were found to experience higher fear of COVID-19 compared to participants with different levels of education ($p < .05$) (Table 1). According to a study, the fear of COVID-19 among illiterate people was found to be higher than those with primary school, high school, and undergraduate degrees (Hossain et al., 2020). This difference is thought to be caused by the differences in variables such as individuals' reading comprehension skills and ability to interpret the material read.

It was found that women with chronic disease had higher fear of COVID-19 than those without chronic disease ($p < .05$) (Table 1). In a study, the process of benefiting from health services about breast cancer was compared in terms of before and after the COVID-19 pandemic. It was found that women with chronic diseases benefitted from health services more in the pre-pandemic period than those who did not have a chronic disease (Altman, Levkovich, and Tavori, 2020). Some studies reported that COVID-19 was encountered more in individuals with chronic diseases and that the course of the COVID-19 was clinically more severe (Sandalcı, Uyaroğlu, & Sain Güven, 2020; Guan et al., 2020; Zhu et al., 2020; Mao et al., 2020). It is thought that this is because individuals with chronic diseases learn from news that the course of COVID-19 is more severe.

It was found that women with three or more children had less fear of COVID-19 than those who had no children and those who had two children ($p < .05$). According to a study consisting of 151 women, no relationship was found between the number of children and the fear of COVID-19 (Altman, Levkovich, and Tavori, 2020). This result, which is not consistent with the result of the current study, may be due to the study population.

Fears of COVID-19 were found to be higher in women who did not have breast cancer in their family compared to women who had no previous breast-related problem ($p < .05$) (Table 2). According to the literature, the delay in screening and imaging applications may have a much more serious effect in women with a very high risk of breast cancer due to genetic predisposition. A one-year interval in early diagnosis is stated as the maximum acceptable time in terms of benefit-risk ratio (Pilewskiw et al., 2019). This situation may explain why women who had breast cancer or who had a family member with a history of breast cancer were afraid of COVID-19 more due to the thought that there might be disruptions in their diagnosis and treatment during the pandemic process.

Women who knew about breast cancer early diagnosis practices were found to have lower COVID-19 fears than those who did not ($p < .05$) (Table 2). A study found that most women did not know what to do to avoid breast cancer and how to do BSE (Al-Naggar et al., 2011). In another study, 31% of the participants reported that they canceled their healthcare appointment due to the COVID-19 pandemic, and 93% of those who canceled their hospital appointments reported they had done it due to fear of contracting the virus (Altman, Levkovich, and Tavori, 2020). This is thought to be due to women's fear of a complex and unknown disease like COVID-19.

The fear experienced by women who knew about breast cancer early diagnosis practices were found to be lower compared to those who did not, and this finding was statistically significant ($p < .05$), (Table 2). In a study,

it was found that the treatment process was delayed because participants (n = 32583) did not perform breast cancer early diagnosis practices, and in this case, it was predicted that compared to the pre-pandemic period, the number of breast cancer-related deaths would increase by 2% along the first year and by 6-8.9% at the end of 3 years (Maringe et al., 2020). Cancer screening plays a critical role in early cancer detection, but COVID-19 is reported to significantly hamper the cancer screening infrastructure (Waterhouse et al., 2020). It is thought that this is because women's belief in early diagnosis practices may have increased their health motivation.

It was found that women who knew where mammography and CBE were performed had less fear of COVID-19 ($p < .05$), (Table 2). Of the breast cancer cases, 65-70% is diagnosed with radiological imaging (Puliti et al., 2017). In a study, women with a family history of breast cancer reported that they had better information and performed BSE more than those who did not have family members with a history of breast cancer (Koşgeroğlu, Ayrancı, & Özerdoğan, 2011). In another study, it was found that 36.8% of women over the age of 40 had at least had one mammogram (Aker, Öz, & Tunçel, 2015). This finding is thought to have arisen because women who know where they can get a mammogram and have CBE are aware that they can have early diagnosis procedures done whenever they want.

It was found that women who performed BSE at irregular intervals experienced less fear of COVID-19 than those who never did ($p < .05$), (Table 2). Studies have indicated that about 45% of the improvement in breast cancer prognosis in western countries in the last 10-20 years has been achieved with breast cancer screening (Vanni et al., 2020a). Studies have shown that there are several healthcare organizations implementing or establishing telehealth programs for breast cancer screening during the COVID-19 outbreak (Zhao et al., 2020; Vanni et al., 2020a; Cancino et al., 2020). This situation is thought to be because women who perform BSE know how to protect their health and that their awareness is high.

Those who regularly performed BSE at home were found to have less fear of COVID-19 than those who did not perform any early diagnosis ($p < .05$), (Table 2). In a study, it was reported that 80.5% of women knew about breast self-examination and that 12.6% of those who knew it regularly performed BSE (Aker, Öz, & Tunçel, 2015). In another study, it was found that women who were aware of the severity of breast cancer and saw themselves under the threat of the disease tended to perform BSE, have CBE, and get a mammogram more than other women of the same age (Baysal and Polat, 2012). According to a study conducted in the UK, it was reported that people postponed breast cancer early diagnosis practices because they were afraid of COVID-19 (Hamilton, 2020). It can be thought

that women who regularly perform BSE at home know about breast cancer, and this is thought to be because women have control over their health.

Considering the research findings, it was reported that 43.8% of women did not have Mammography and CBE because of the COVID-19 disease. A study found that 44.2% of the participants postponed breast cancer early diagnosis practices due to the COVID-19 outbreak (Papautsky and Hamlish, 2020). In another study, it was reported that the implementation of breast cancer early diagnosis practices and diagnostic activities needed continuing under necessary hygiene measures and physical distance rules (Ceugnart et al., 2020). According to another study, breast cancer early diagnosis practices decreased by 89.2% due to COVID-19 (London et al., 2020). This situation is thought to be because women are afraid of getting COVID-19 when they go to health institutions.

It was determined that 79.5% of the women participating in the study did not consider having mammography or CBE until the end of COVID-19. In a study conducted in Turkey, data from the three-month period after the normalization process during the COVID-19 pandemic process were compared with the 3-year breast cancer data before the pandemic. As a result of the comparison, it was determined that the rate of early-stage breast cancer detection decreased from 81.2% to 52.9% and that the rate of advanced-stage breast cancer increased from 18.8% to 47.1% (Yılmaz, Güldoğan & Arıbal, 2020). According to a study conducted in the UK, the number of breast cancer cases detected in the early stage in the first 6 months of 2020 was 28% lower than the number of cases in the same period of 2019 (Gathani et al., 2020). As a result of the delay in early diagnosis practices, the diagnosis of symptom-based cancer will increase (Hamilton et al., 2016). Besides, it has been stated that early diagnosis and treatment of cancer patients during a pandemic should not be delayed (Corsi et al., 2020). The fear of COVID-19 may have prevented women from going to healthcare institutions for early diagnosis applications.

Conclusions and Suggestions

Considering the findings obtained according to the results of the present study, women were found to be affected by COVID-19 disease. According to the findings, most of the women participating in the study did not want to go to the hospital until the pandemic was over, and they did not want to perform breast cancer early diagnosis practices. In addition, it was determined that most of the women knew about breast cancer early diagnosis practices but that they did not perform them. In this context, to maintain health protection under pandemic conditions, people should be informed using mass media possibilities, health services such as remote telemedicine methods should be planned, mobile mammography units

should be increased, and areas necessary to carry out early diagnosis practices safely should be created.

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Chapter 3

THE PREVALENCE OF CRYPTOSPORIDIOSIS IN RUMINANTS IN TURKEY

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1. Introduction

Cryptosporidiosis is a common protozoan infection in humans and animals caused by *Cryptosporidium,* and is characterized by a series of gastrointestinal disorders (Pumipuntu & Piratae, 2018; Shahiduzzaman & Daugschies, 2012). *Cryptosporidium* spp. Apicomplexa: Cryptosporidiidae are among the most important coccidian parasites of mammals, birds, reptiles, and fish, and are spread worldwide (M. Ö. Arslan, Gicik, Erdoğan, & Sarı, 2001; de Graaf, Vanopdenbosch, Ortega-Mora, Abbassi, & Peeters, 1999; Ramirez, Ward, & Sreevatsan, 2004; Sarı, Aktaş, & Arslan, 2008; Şimşek et al., 2012). These protozoan parasites mainly target the intestinal tract, but they occasionally infect the respiratory tract of animals and humans as well (M. Ö. Arslan et al., 2001).

Cryptosporidium spp. is recognized as one of the major enteropathogens associated with neonatal diarrhea in ruminants. High morbidity rates which are sometimes accompanied by high mortality rates in farm animals make cryptosporidiosis an important disease to watch out for animal owners (Kabir et al., 2020; Kaminjolo, Adesiyun, Loregnard, & Kitson-Piggott, 1993; Mamak, Özçelik, Değerli, Oğuztürk, & Akın, 2000; Ozdal, Tanritanir, GOZ, Deger, & Kozat, 2009; Ulutaş & Voyvoda, 2004).

Cryptosporidiosis is a zoonotic disease that is particularly prevalent in tropical and subtropical regions, which include Turkey as a country. The disease has severe effects on many animal species (particularly young members such as calves, lambs and kids), livestock workers, and humans with weakened immune systems (Ekinci, Sevinç, Coşkun, Işık, & Sevinç, 2011; Sarı et al., 2008; Sevinç, 2004; Şimşek et al., 2012). Some studies repot that the disease can have a life-threatening course in humans, particularly in AIDS patients (de Graaf et al., 1999). It's presence in water is the main factor for the prevalence of *Cryptosporidium* oocysts, followed by food and animal-human contamination (Sarı et al., 2008). Transmission occurs by ingestion of food and water contaminated with oocysts that were excreted through the feces of infected hosts (Sevinç, 2004).

Most of the data on the prevalence of cryptosporidial infection in farm animals are from cattle, and there is less information about the occurrence of cryptosporidiosis in sheep and goats (Ulutaş & Voyvoda, 2004). *Cryptosporidium* infection was first detected in cattle in 1971 (de Graaf et al., 1999; Panciera, Thomassen, & Garner, 1971), in sheep in 1974(Barker & Carbonell, 1974), and in goats in 1981 (Mason, Hartley, & Tilt, 1981). Cryptosporidiosis is accepted as a source of infection for humans as well as causing morbidity and mortality in lambs and calves (Sarı, Arslan, Gicik, Kara, & Taşçi, 2009). It is reported that cattle, sheep, and goats play role in human epidemics (Shahiduzzaman & Daugschies, 2012).

1.1. Etiology

Cryptosporidium spp. is classified in the Phylum Apicomplexa, and Family Cryptospordiidae (Ramirez et al., 2004). Major species causing disease are stated as *Cryptosporidium parvum*, *Cryptosporidium muris* and *Cryptosporidium hominis* (M. Ö. Arslan et al., 2001; Sarı et al., 2008; Sarı et al., 2009; Sevinç, 2004). The most common zoonotic species, *Cryptosporidium parvum,* effects a wide variety of mammals and is responsible for the majority of waterborne outbreaks (Shahiduzzaman & Daugschies, 2012). *Cryptosporidium parvum* is the only species that can progress in both humans and animals and is reported to be the main source of transmission to humans from ruminant animals (Sarı et al., 2009; Sevinç, 2004; Shahiduzzaman & Daugschies, 2012).

1.2. Symptoms

Transmission usually occurs through ingestion of food and drinking water contaminated with sporulated oocysts excreted in the feces of infected hosts (Ekinci et al., 2011; Sevinç, 2004). Infected animals excrete billions of oocysts with feces that are resistant to external environmental conditions. The rate of oocyst excretion depends on the severity of infection and the age of the animal. It has been reported that goats excrete oocysts for 3 weeks postpartum (Birdane, 2017). Hydrous and mucous diarrhea is the most typical clinical finding in the disease. Dehydration, weight loss, anorexia, tenesmus, weakness, tangled hair, muscle tremors and abdominal pain can also be seen (M. Ö. Arslan et al., 2001; de Graaf et al., 1999; Ekinci et al., 2011; Sevinç, 2004; Sevinç, Uslu, & Derinbay, 2005; Thompson et al., 2005). The disease is more severe and lethal when complicated with other enteropathogens such as *Escherichia coli*, *Salmonella,* Rotavirus, and Corona virus infections (M. Ö. Arslan et al., 2001; Ekinci et al., 2011).

1.3. Diagnosis

In farms, accurate diagnosis of the disease in its early periods is very important in preventing potential losses (Şimşek et al., 2012). Various tests have been developed for the diagnosis *Cryptosporidium*. Most of these involve direct detection by microscopic examination of sample tissues or fecal material using staining techniques. Many specific staining procedures have been described to facilitate reliable detection of oocysts (Ramirez et al., 2004). Acid-fast staining methods such as safranin-methylene blue, Kinyoun, Ziehl-Neelsen and DMSO-carbol fuchsin have been proposed since *C.parvum* oocysts are difficult to distinguish from other small particles in the stool (like fungus, mold, algae, and plant debris) (Ekinci et al., 2011; Sevinç, 2004; Sevinç et al., 2005). Some studies report that recently developed PCR protocols are very specific and highly sensitive (Ramirez et al., 2004), and can give more effective results in the diagnosis

of cryptosporidiosis compared to classical methods (Sungur et al., 2008; Şimşek et al., 2012).

1.4. Treatment

The lack effective prevention methods and treatment makes the disease a significant challenge. Hygienic measures alone are not sufficient, as oocysts are highly resistant to environmental conditions and many disinfectants (Shahiduzzaman & Daugschies, 2012). Various drugs have been tried in the treatment of the disease, but no fully effective drug has been found. Cryptosporidiosis treatment includes anti-cryptosporidial agents, immunization, and supportive treatment protocols. Studies report that paromomycin, spiramycin, halofuginone lactate, dequinate, lasaloside, fenbendazole and sulfadimethoxine are not effective enough for the treatment (Birdane, 2017; Ekinci et al., 2011; Sevinç, 2004; Shahiduzzaman & Daugschies, 2012; Yazar, 2018), while sulfonamide+diaminopyrimidine, albendazole, and macrolide antibiotics might be partially effective. It has been stated that halofuginone can be used at a dose of 0.1mg/kg (PO, SID, 7 days) or paromomycin at a dose of 50-100 g/100kg (PO, BID, 5-11 days), in ruminants (Yazar, 2018). These drugs reduce the number of oocysts excreted and the severity of diarrhea even though they do not eliminate the infection immediately and/or completely (Sevinç, 2004). Liquid electrolyte therapy should be applied for support in the disease treatment (Batmaz, 2010). In addition to these, separation of patients, cleaning of the shelters (using hydrogen peroxide or chlorine dioxide), and implementation of strict sanitation rules should be performed (Batmaz, 2013).

1.5. Prognosis

Spontaneous recovery is seen in calves after 6-10 days of course. Death is usually related to insufficient feeding and/or cold temperatures during the diarrhea period (Batmaz, 2010). Most cases in lambs recover gradually in 5-7 days unless complications occur. The disease can be more severe in kids. Prognosis might be poor in cases of mixed infections and severe dehydration (Batmaz, 2013).

1.6. Prophylaxis

Since infections are initiated by the ingestion of oocysts, control strategies should aim not only at treatment but also at reducing the number of oocysts in the environment (Shahiduzzaman & Daugschies, 2012). Immunity is the most important factor in protection from cryptosporidiosis. It is thereby very important for newborns to receive enough colostrum immediately after birth in the control of the disease (Ekinci et al., 2011; Sevinç, 2004). A suitable management system (barn cleaning, trough and feeder cleaning, ventilation, etc.) should be implemented in the farm

(Sevinç, 2004). Hygienic measures are very important in terms of cleaning and disinfection in order to reduce the infection load in the environment. Humid heat between 45 °C to 60 °C can be applied for 5–9 minutes to completely inactivate the oocysts (Shahiduzzaman & Daugschies, 2012).

2. Prevalence of Cryptosporidiosis in Turkey

Studies were conducted in different cities (Figure 1) with various methods to determine *Cryptosporidium* prevalence in animals in Turkey (Table 1). The highest positivity was reported in the Aegean Region (30.39%), and the lowest positivity was reported in the Southeast Anatolia Region (10.00%) (Table 2).

In studies carried out on calves in Bursa, *Cryptosporidium* has been detected in 15 (26.7%) of 56 stool samples (Burgu, 1984). *Cryptosporidium* spp. oocysts were encountered in 36 (25.7%) of 140 calves in Kars province, and the prevalence was reported as 29.5% in calves up to one month old and 10.7% in calves older than one month old (M. Ö. Arslan et al., 2001). In another study conducted in Kars province, 49 (32.9%) of 149 stool samples were reported to be positive for *Cryptosporidium* (Çitil, Arslan, Güneş, & Erdoğan, 2004). *Cryptosporidium* prevalence was found to be 22.8% (43/189) by Sarı et al. (2008) in Erzurum province. The prevalence of the disease was determined as 30.3% (36/119) in calves with diarrhea and 10.0% (7/70) in those with normal stools. Cryptosporidiosis positivity was detected in a total of 31 (20.7%) samples as a result of the analysis of stool samples taken from 150 calves with diarrhea from different districts of Nevşehir (Şimşek et al., 2012). In two seperate studies carried out in Sivas, 70.3% (Değerli, Çeliksöz, Kalkan, & ÖZÇELİK, 2005) and 7% (Kuliğ & Coşkun, 2019) prevalence was reported for calves respectively, while 31.4% (Değerli et al., 2005) and 4.5% (Mamak et al., 2000) prevalence has been reported for cattle, respectively. *Cryptosporidium* prevalence was found to be 11.21% in the feces of 107 neonatal calves with diarrhea in the Tokat region (Kaya & Alparslan, 2018). In Siirt province, 110 calf feces were examined using a rapid diagnosis test kit and 10% prevalence was determined (Kozat & Tuncay, 2018). In Konya, *Cryptosporidium* was found in 145 (39.4%) of 368 diarrheal stool samples (Ekinci et al., 2011), while in a different study carried out in Konya, 27.4% prevalence was determined (Kabir et al., 2020). In a study conducted with 123 calves aged 3-12 months and 17 cattle aged 12-36 months, a prevalence of 22.14% was determined in Hakkari by Göz, Gül, and Aydin (2007). In Van, 24 (13.19%) of 182 stool samples were reported to be positive for *Cryptosporidium* in a study carried out on calves (Gül, Ciçek, & Kilinç, 2008). Özer, Erdoğmuş, and Köroğlu (1990) reported a prevalence of 7.2% in a study conducted in Elazığ. In a study conducted in Ankara, 12 (37.5%) of 32 stool samples were reported to be positive for *Cryptosporidium* (Sakarya et al., 2010).

In 172 diarrheal and 130 normal bovine feces samples collected in Ankara *Cryptosporidium,* prevalence was 63.3% and 69.2% in diarrheal and normal cattle, respectively (Emre, Alabay, Fİidanci, Düzgün, & Çerçi, 1998). In a study carried out by Sungur et al. (2008) 27 calf feces samples were examined and positivity was found in 3 (11.2%) samples in examinations performed with Carbol fuchsin, and in 8 (29.7%) samples with Nested PCR method.

In studies carried out on lambs and kids in Aydın region, it was reported that *Cryptosporidium* oocysts were encountered in 67 (46.5%) of 144 lamb feces samples collected from lambs with and without diarrhea, and the infection rate was found to be significantly higher in lambs with diarrhea (79.1%, 53/67) compared to lambs without diarrhea (18.2%, 14/77) (Ulutaş & Voyvoda, 2004). In a study conducted in Konya, a prevalence of 19.4% was reported in lambs and 13.4% in kids (Kabir et al., 2020). In a different study carried out in Konya, a 2% prevalence was found in lambs, while no positivity was found in sheep (Handemir, Gozun, & Kamburgil, 1999). 400 diarrheal lamb stools were examined and a prevalence of 38.8% was determined in a study Kars, carried out by Sarı et al. (2009). In a different study conducted in Kars, a prevalence of 21.5% was found (Gökçe, Ünver, & Erdoğan, 2010). In Konya, 471 lamb feces were examined using the Modified Ziehl Neelsen (MZN) staining method and ELISA technique, as a result, a prevalence of 2.97% was determined by MZN method and 9.13% positivity was determined by ELISA method (Sevinç et al., 2005). In a study conducted in the Marmara Region, a prevalence of 9.9% in lambs and 11.54% in kids was determined (S. Arslan et al., 2016). In a study conducted in İzmir, a prevalence of 23.3% was reported in lambs with diarrhea, which was 2% in lambs without diarrhea, 46% in kids with diarrhea, and 14% in kids without diarrhea (Erman, Beyazıt, & Öz, 2000). A prevalence of 13.63% was determined in stool samples taken from 132 diarrheal lambs in Van province (Ozdal et al., 2009). In a study carried out in Konya intestinal samples were used which were necropsied from 60 lambs that showed diarrhea symptoms while they were in agony, and 85 lambs that died with diarrhea symptoms (145 in total). In another study, 13.3% *Cryptosporidium* spp. positivity was determined by a modified Ziehl-Neelsen staining where paraffin blocks were prepared from the sections cut from a total of 145 stool samples. In the same study, 13.8% *C. parvum* positive reaction was encountered in the immunofluorescence and immunohistochemistry stainings (Akpınar & Oruç, 2019). A prevalence of 13.5% was found in the study conducted by Aciöz (2018) in Isparta region. 397 goat feces samples were examined in different regions of Aydın province and the total prevalence was reported as 18.6%. Infected goats ratios were detected as 10.6% in kids younger than three months old, 3.5%

in kids 3-6 months old, 2% in kids 6-12 months old and 2.5% in goats older than 12 months (Paşa & Ulutaş, 2003).

Table 1. Distribution of studies carried out in Turkey by province, method and results

Province	Breed	Examined	Positive		Method	References
		(n)	(n)	(%)		
Ankara	Cattle	302	199	65.89	Safranin-methylene blue	(Emre et al., 1998)
	Calf	56	15	26.7	Carbol fuchsin Modified $ZnCl_2$ NaCl centrifugal flotation	(Burgu, 1984)
	Calf	32	12	37.5	Nested PCR	(Sakarya et al., 2010)
		32	7	21.88	Carbol fuchsin	
Aydın	Goat kid	397	74	18.64	Heine's carbol-fuchsin	(Paşa & Ulutaş, 2003)
	Lamb	144	67	46.53	Heine's carbol-fuchsin	(Ulutaş & Voyvoda, 2004)
Bursa	Calf	56	15	26.7	Carbol fuchsin Modified $ZnCl_2$ NaCl centrifugal flotation	(Burgu, 1984)
Erzurum	Calf	189	43	22.75	Modified acid-fast	(Sarı et al., 2008)
Hakkâri	Calf	123	29	23.58	Modified Ziehl-Neelsen	(Göz et al., 2007)
	Cattle	17	2	11.76		
Isparta	Lamb	74	10	13.51	Giemsa, Autopsy, ELISA, Flotation, Sedimentation	(Aciöz, 2018)
İzmir	Lamb+Goat kid	150	69	46.00		(Erman et al., 2000)
Kars	Lamb	400	155	38.8	Modified acid-fast	(Sarı et al., 2009)
	Lamb	38	8	21.05	ELISA	(Gökçe et al., 2010)
	Calf	140	36	25.71	Modified acid-fast	(M. Ö. Arslan et al., 2001)
	Calf	149	49	32.9	Modified acid-fast	(Çitil et al., 2004)
Konya	Lamb	471	43	9.13	ELISA	(Sevinç et al., 2005)
		471	14	2.97	Modified Ziehl-Neelsen	
	Lamb	60	8	13.33	Modified Ziehl-Neelsen	(Akpınar & Oruç, 2019)
		145	20	13.79	immunohistochemistry immunofluorescent	
	Lamb	67	13	19.40	Nested PCR	(Kabir et al., 2020)
	Calf	333	91	27.33		
	Goat kid	15	2	13.33		
	Calf	368	145	39.40	Modified Ziehl-Neelsen	(Ekinci et al., 2011)
	Lamb	50	1	%2	Autopsy Zinc sulfate flotation, Giemsa, HE, PAS	(Handemir et al., 1999)
	Koyun	50	0	%0		
Nevşehir	Calf	150	31	20.67	Real time PCR Nested PCR	(Şimşek et al., 2012)

Siirt	Calf	110	11	10.00	Rapid diagnostic test (BoviD-5 Ag Test Kit)	(Kozat & Tuncay, 2018)
Sivas	Calf	138	9	6.52	Rapid diagnostic test (Bio-X Diagnostics)	(Kuliğ & Coşkun, 2019)
	Calf	387	272	70.28	Modified acid-fast	(Değerli et al., 2005)
	Cattle	70	22	31.43		
	Cattle	200	9	%4.5	Kinyoun acid-fast	(Mamak et al., 2000)
Tokat	Calf	107	12	11.21	Rapid diagnostic test (BoviD-5 Ag Test Kit)	(Kaya & Alparslan, 2018)
Van	Lamb	132	18	13.64	Modified Ziehl-Neelsen	(Ozdal et al., 2009)
	Calf	182	24	13.19	Modified acid-fast	(Gül et al., 2008)

Table 2. Distribution of studies by regions

Regions	Examined	Positive	
	(n)	(n)	(%)
The Mediterranean Region	74	10	13.51
Eastern Anatolia Region	1370	364	26.57
Aegean Region	691	210	30.39
Southeast Anatolia Region	110	11	10.00
Central Anatolia Region	3397	913	26.88
Black Sea Region	107	12	11.21
Marmara Region	56	15	26.79
Overall	5805	1535	26.44

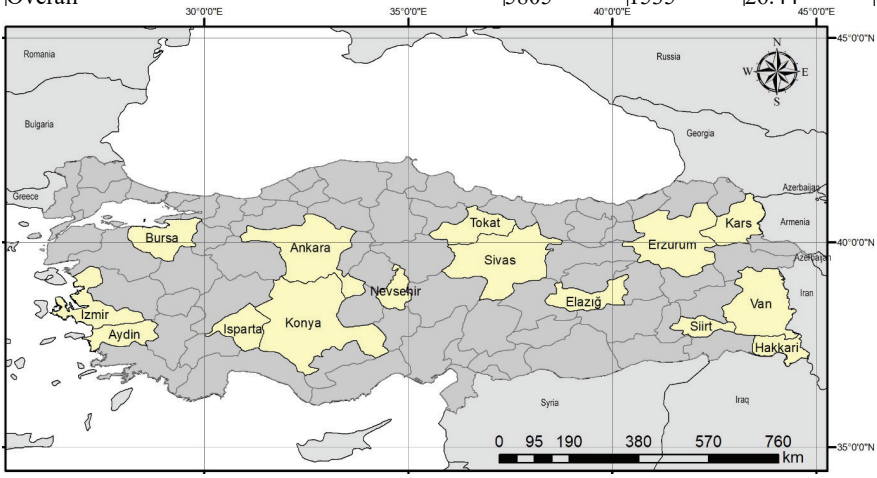

Figure 1. Turkey map where Cryptosporidiosis studies carried out

3. Conclusion and Recommendations

Cryptosporidium sp. existence is reported in different provinces of Turkey. The reasons for the differences observed between the studies may include geographical conditions, the species and number of animals studied, the methods used, and the difference in seasons. The disease

causes high morbidity and death in farm animals. In addition, sick animals suffer from diarrhea, dehydration, and growth retardation. The treatment costs associated with the disease are also significantly high. The disease therefore causes economic losses, especially considering the challenging treatment and prevention conditions such as special care of animals, anticoccidial drug treatments, liquid electrolyte treatment, and necessary improvements of the hygiene conditions. As a result of the findings of this study, it has been concluded that in addition to taking protective measures after birth, implementation of good farming management practices (calves receiving colostrum, proper shelter hygiene, etc.), water-borne outbreak risks associated with the zoonotic nature of the disease should also be monitored carefully by the relevant authorities.

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Chapter 4

A PHYSICIAN'S THERAPEUTIC POWER

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A physician's therapeutic power

Introduction

It was a situation that we observed a positive effect of the patient's belief in the physician or the patient's satisfaction with the physician on the treatment, but there has been no clear evidence until recently. In recent times, while some people accept the therapeutic power of a positive patient-physician relationship, some focus on new and more effective methods for diagnosing and treating patients. Although these two views seem to be contradictory, they are actually intertwined, as they contribute to increased power of the physician leading to the increased treatment effectiveness. When patients trust the competence of the physician, their chances of compliance with the treatment and the subsequent positive results increases. In this article, by reviewing the literature and research, the importance of the strength of patient-physician relationship and patients' belief in physicians leading to increased well-being is explained.

The therapeutic power of physicians from past to present and their healing feature

The healing power of physicians can also be described as a type of healing or even therapeutic healing. Physicians have always been aware of this healing power, and it is well known that physicians are most effective treatment available (Bensing & Verheul, 2010; Thomas, 1978). In fact, this power has been known since the time of Hippocrates, and over the years spirituality and science have continued to coexist in healing. Hippocrates expressed this spiritual power as "some patients, though conscious that their condition is perilous, recover their health simply through their contentment with the goodness of the physician" (Dixon et al., 1999). In contrast, Balint stated that it was not only medicine that was important, but the way and the atmosphere in which the medicine was administered was effective in the treatment, and even described this power of a physician as a "medicine doctor" (Balint, 1955).

Although physicians are expected to gain many competencies and skills in their training, patients and people see them primarily as clinical healers. Most physicians entering medical school express their intentions as wanting to relieve patients' pain and suffering and help them heal beyond the cure of their illnesses. When a patient seeks professional skills for the diagnosis and treatment of medical diseases, the physician takes on the role of a healer (Thirumoorthy, 2012). Patients likewise see physicians as "healers" and a referral to a physician for recovery provides them with hope and assurance. Hence, healthcare professionals must not only learn technical skills, but also develop appropriate social skills to better interact and communicate with their patients (Benedetti, 2013).

Rapid developments in science and technology at the beginning of the 19th and 20th centuries brought technological advances in medicine, especially in diagnosis, and effective and evidence-based treatment practices. As a result,

physicians started to train in treating diseases only with drugs instead of healers who were aware of their own strength (Sturmberg, 2002).

Another dimension of these developments is that placebo studies, which are increasing with evidence-based medicine practices, have helped reveal the therapeutic power of a physician and patient-physician communication (Dixon et al., 1999). Evidence provided by placebo studies shows that the characteristics of the patient, physician, treatment given, and treatment environment affects the success of the treatment. In this context, physicians and physician-patient relationships constitute a very important component (Benedetti, 2013).

The effects of healthcare interactions include factors common to all medical, alternative, and psychological treatments. These include factors such as attention, empathy, respect, compassion, hope, enthusiasm, trust, availability, availability, and continuity. A skilled physician with these attributes can alter a patient's perception of their illness, thereby improving symptoms in the short term and possibly affecting the physiological processes in the long term. Another long-term skill is the ability to create a positive attitude towards the illness and coping style that can change the course of the illness as well as the patient's life (Benedetti, 2013).

In this review, the effect of physicians' beliefs on treatment will be discussed with the titles including effective patient-physician communication, empathy, trust, and positive conditioning which affect the therapeutic power of a physician.

Effective Patient-Physician Communication

Effective communication with patients is at the center of medicine and is accepted as the cornerstone to ensure, maintain, and improve health. For this reason, it has been known for years that physicians should provide effective communication and develop social skills (Mistiaen et al., 2016; Papakostas & Daras, 2001). Communication skills training has become a part of the medical curriculum in medical schools in western countries which indicates that the central role of patient-physician communication on the delivery and effectiveness of care is generally accepted (Bensing & Verheul, 2010).

Effective patient-physician communication contributes positively to treatment in many ways which directly or indirectly affects the success of the treatment. It is effective directly in reducing stress and anxiety and indirectly by affecting the patient's commitment, satisfaction, physician trust, and self-efficacy (Bensing & Verheul, 2010; Street et al., 2009).

Effective communication primarily increases patient satisfaction. A study investigated the relationship between patient-physician communication and satisfaction, and it was found that patients were quite satisfied if their physicians had a harmonious orientation or were more patient-centered. However, patients whose physicians were not patient-centered were significantly less satisfied (Krupat et al., 2000).

Many studies have shown that good communication positively affects patient satisfaction and related treatment outcomes. A training program to improve communication skills was implemented for healthcare professionals (physicians, physiotherapists, and nurses) in the heart surgery department in a hospital. Results of the training showed increased patient satisfaction in the intervention group, decreased duration of hospital stay, and reduced incidence of post-surgery tachyarrhythmia (Trummer et al., 2006). In patients with acute tonsillitis, those given more attention, detailed information, and a comprehensive physical examination showed subjective improvements (regression of symptoms, feeling better) more frequently (Olsson et al., 1989).

Effective communication can also result in reduced use of health services. A study with families of children receiving asthma treatment evaluated the relationship between physician communication behavior and performance and the patient's asthma office visits, emergency room visits, and hospitalization. Specific patient-physician communication behaviors, such as careful listening, non-verbal attention, and interactive speech resulted in decreased healthcare use, and led to a positive perception of care quality (Clark et al., 2008). Another study showed that homeless adults who were frequently admitted to the emergency department and received compassionate care had fewer repetitive visits and increased satisfaction compared to patients who received standard care (Redelmeier et al., 1995).

Owing to effective communication, patient compliance with the treatment increases. If the conversation between the physician and the patient helps determine the correct diagnosis and the appropriate treatment plan, communication can provide better physical health in the continuation of this determined treatment (health beliefs of the patients may also be affected) (Street et al., 2009). The physician's clear and understandable expressions increase the patient's compliance with the treatment because they understand the patient's disease and treatment more clearly, participate in medical decisions, and empower the patient emotionally (Mistiaen et al., 201; Street et al., 2009). It also increases the accuracy of the diagnosis, prevents the risk of misapplication, and can improve clinical outcomes with many effects ranging from adherence to treatment (Howick et al., 2017). It has been observed that collaborative communication led to better treatment compliance in patients with hypertension (Schoenthaler et al., 2009).

The findings showed that effective physician-patient communication not only improved patient satisfaction, but contributed to the improvement of physically determined health outcomes through greater patient adherence to medications and medical advice, keeping appointments, and the use of medical services, in a so-called "therapeutic alliance." It has been observed that effective patient-physician communication in chronic diseases (ulcer disease, hypertension, and diabetes) creates a "therapeutic alliance," leading to better physical recovery and subjective evaluations in follow-up examinations. In the evaluated patients, researchers found that diabetic patients had better control of their blood sugar levels, and those with hypertension significantly improved diastolic blood pressure (Blease, 2012).

Evidence that an effective patient-physician relationship increases the therapeutic power of physicians have been presented above. Physicians can improve this through communication-related skills. On this subject, in a study conducted with in-depth interviews in primary care, what needed to be done was collected in three basic processes: a non-judgmental emotional bond, the conscious management of the physician's power in a way that would benefit the patient the most, and stick to the commitment to care for patients over time. Trust, hope, and a sense of knowing emerge from these processes (Scott et al., 2008). Effective communication contributes to the healing power of a physician by increasing confidence and hope, which we will discuss in the following sections, as well as patient satisfaction.

Empathy

Empathy refers to an internal process in which someone else's cognitive and emotional experiences are shared without losing the original source of the experience (Benedetti, 2013). A physician's tone of voice, expressing and conveying empathetic concern on behalf of the patient, and a level of hope helps develop the expectation that the patient can expect recovery (Blease, 2012).

Many studies have shown that empathy positively affects patient treatment. One study treated patients with irritable bowel syndrome with placebo acupuncture with empathic or neutral communication. It was found that treatment was twice as successful in practitioners who established empathic communication (Kelley et al., 2009). The empathic attitude of the anesthesiologist at a preoperative visit to the gynecologist reduced the patient's anxiety compared to the attitude of a neutral anesthesiologist. Patients who received continuous care by the same anesthesiologist in the operating room exhibited higher levels of satisfaction in terms of anesthesiologist behavior and quality of care (Soltner et al., 2011).

Verification is a communication method that can be evaluated within the scope of empathy. Even if patients do not have serious health problems, they expect a serious explanation from their physicians and it is disappointing to be told that they do not have any serious diseases. In a study of patients with back pain, patients were divided into two groups, validating and invalidating. Patients in the validation group were found to have reduced pain and frustration. In addition, the validation group was more satisfied with the interview (Vangronsveld & Linton, 2012).

Trust

The patient's trust in the physician is also one of the factors that increases the therapeutic features of the physician. Trust is a determining factor in any interpersonal relationship and is especially at the center of the patient-physician relationship. Patients develop a sense of trust based on the competence, compassion, confidentiality, and reliability of the physicians. Patient confidence increases the likelihood of patient satisfaction, compliance with treatment, and improved health status (Benedetti, 2013; Pearson & Raeke, 2000). In addition, physicians can be effective in shaping the way patients think and feel about their disease or treatment

with the information and assurance they provide (Blasi et al., 2001).

It has been suggested that trust in physicians is the basis for effective treatments. In addition to a deontological imperative, an important relationship between trust in physicians and health outcomes has been observed. An increasing number of studies confirm that there is a significant relationship between patients' trust in healthcare professionals and health outcomes (Birkhäuer et al., 2017). Empirical studies have confirmed that physician-patient interaction based on trust increased patient satisfaction, compliance with treatment, and improved health outcomes (Birkhäuer et al., 2017; Ommen et al., 2011). When patients find the physician competent, if the physician is not optimistic about the treatment, their treatment is negatively affected (Howe et al., 2017).

Looking at studies that show the relationship between trust and health outcomes, a study conducted in Taiwan on patients with type 2 diabetes provided evidence that trust was associated with both subjective health and objective (decrease in blood lipids) health outcomes (Lee & Lin, 2009). Another study of patients with type 2 diabetes also found that trust was positively associated with both objective (glycemic control) and subjective health outcomes (physical HRQoL and satisfaction at 12 months) (Lee & Lin, 2011).

Trust is built on the competence of the physician and their communication skills. Inadequate physician communication skills can have extensive negative effects on patients' trust in their physicians. Thus, medical support requires both biomedical and psychosocial skills (Ommen et al., 2011). However, a study showed that breast cancer patients were not primarily concerned with the communication skills of their physicians. Instead, they emphasized on the physicians' enduring qualities. In particular, they valued physicians who they believed were technically experts and whom they respected (Wright et al., 2004). This shows that competence is the most important component of building trust, especially in serious health conditions.

Positive Conditioning

Words coming out of a physician's mouth also affect patient's treatment. Physicians trying to influence patients' beliefs about the effects of the treatment have been shown to have a significant impact on the patients' health outcomes. A change in patient expectations can lead to positive and negative health consequences (Street et al., 2009). While positive words for treatment affect the patient's expectation for treatment positively, negative words can lead to negative results (Blasi et al., 2001). Studies have shown that clinicians can increase pain relief by giving positive messages that change patients' expectations and pain experiences (Howick et al., 2017).

The power of physicians to manipulate patients' expectations is based on the classical conditioning mechanism. Conditioning can have a placebo effect on health outcomes when associated, consciously or unconsciously, with any treatment or previous experience. Conditioning applied by the physician reveals

the possibility of promoting benefit expectations or negative outcome expectations with teaching and observational learning (Bensing & Verheul, 2010).

However, the characteristics of a physician are also important. After inducing an allergic reaction in participants through the histamine skin prick test, a healthcare professional applied a cream containing no active ingredients and set positive expectations (the cream reduces the reaction) or negative expectations (the cream will increase the reaction). The effect of the expectations on the allergic response increased when the provider behaved both warmer and more competently (Howe et al., 2017). In one of the first studies in this area, Thomas et al demonstrated the benefit of positive consultation in a positive way (Thomas, 1978).

Another study investigated whether positive consultations given to patients shorten the duration of the disease. It was found that patients with positive consultation were given a clear diagnosis recovered soon, while patients with negative consultation did not have a serious underlying disease and the physician did not know exactly what was wrong. Positive and negative consultations had no effect on the results. This result was based on the fact that patients wanted to know that there were no serious health problems (Knipschild & Arntz, 2005). As seen, the conditioning has to be performed after the patient has been clearly explained the treatment for it to be more effective.

Although positive conditioning studies have focused on drug treatments, especially pain, some studies have shown that invasive procedures had an effect on long-term results. It has been shown to improve the long-term outcomes of invasive medical interventions by optimizing these expectations to improve outcomes in patients undergoing coronary artery bypass graft (CABG) surgery. Further studies are needed to generalize this approach to other fields of medicine (Rief et al., 2017).

Changes in patient expectations can lead to positive and negative health consequences. Studies have shown that when pain relievers or anti-anxiety medications were administered covertly compared to overtly, their effectiveness was significantly reduced (Street et al., 2009). In other studies, covert treatment (Benedetti et al., 2003) and covert injections (Amanzio et al., 2001) were found to be less effective than open administration. These studies both show the patients' desire to know the treatment and reveal the therapeutic effect of positive conditioning from another aspect.

In some cases, patients may feel better because of positive conditioning, even if their symptoms do not improve. Saline solution was administered to a group of patients with chest pain, and it was said that this solution would cause vasodilation. Despite vasoconstriction in these patients, the pain was reduced (Ronel et al., 2011). A study evaluated post-cesarean pain, and found that positive conditioning reduced the incidence of pain by using more positive words, but it did not affect its severity when measured by pain scores (Chooi et al., 2011). Optimistic drug presentation increased the placebo effect for patient-reported outcomes (asthma control) but did not affect lung function (Wise et al., 2009).

Although positive conditioning sometimes does not directly affect the treatment, it may indirectly contribute to the treatment by increasing physician satisfaction. Experimental manipulation of physician behavior in suppressing airway hyperreactivity in asthmatic patients positively altered the physician's perception, but not the magnitude or frequency of the placebo response (Kemeny et al., 2007).

Another dimension of positive conditioning is to give hope to the patient. Hope is a target-oriented positive motivation in the treatment of the patient and the positive effects of hope on disease outcomes have been shown in some health problems such as arthritis, burn injuries, fibromyalgia and pain (Benedetti, 2013).

Conclusion

Current advancements in medical technology have created a barrier between science and the art of medicine, although it has caused many breaks in both diagnosis and treatment. Our belief in the latest technical possibilities has shifted our focus to determinants of health (Sturmberg, 2002). In this context, although physicians may not be adept at improving all aspects of human beings, they should at least know the cultural, social, spiritual, and psychological characteristics of health. When it comes to problems, health, and healing in their patients, they should be aware of the effects of ignoring these aspects (Ross, 2009).

For effective recovery, patients need compassionate and reliable physicians who care about the care of their patients. The therapeutic patient-physician relationship is an alliance marked by mutual respect, trust, empathy, and sincerity (Thirumoorthy, 2012). Such an alliance results in the patient's trust, and what the physician says about the treatment affects the outcome of the treatment.

Although the factors affecting the power of the physician are classified in this article, they are intertwined and mutually reinforcing factors. The physician themself can be as effective as the treatment given, sometimes even more than the treatment, with their competence, communication, words, and behaviors. This increases patient satisfaction, compliance with treatment, stability, and improved health status both objectively and subjectively. The data is clear and shows that what needs to be done is not only give medicine but also train physicians who are aware of their power and who are self-educating for this improvement. Medical educators have a great responsibility in this regard.

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Chapter 5

HYALURONIC ACID AND RELATED ANALYSIS IN FOOD SUPPLEMENTS

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1. INTRODUCTION

Functional foods are foods or food components that express conventional food forms, meet the general needs of our body, as well as provide additional benefits on physiological and metabolic functions, and positively affect our health (1). Food supplements are solid, liquid, capsule, soft gel etc. to be taken orally. It contains products such as vitamins, minerals, amino acids, enzymes and metabolites in the form of vitamins, and they are products that are proven to have protective or physiological benefits against a chronic disease, but are not considered drugs. Products used as food supplements contain fatty acids, carotenoids, antioxidant vitamins, phenolic compounds, terpenoids, steroids, indoles, organic acids, fibers, etc. There are scientific studies showing their beneficial effects on human health (2).

HA, the amount of which is increasing day by day in parallel with the breadth and importance of its usage area, has a market volume of approximately 1 billion dollars in the world (3). Thanks to its characteristic structural properties, HA provides high viscoelasticity and water holding capacity (4, 5). Due to these features, HA is used in the production of cosmetic products and drugs for wounds, tissue regeneration and treatment, delivering drugs to the target tissue, the controlled release of target chemicals, eye drops, hip or knee implants, orthopedic prostheses, skin tissue reconstruction and soft tissue treatment. It is used in MR imaging, healing of periapical tissue after periradicular surgery and microsurgery. HA provides the healing of wounds or scars with effects such as extracellular matrix regeneration, epithelial tissue regeneration, keratinocyte transfer, epidermal regeneration. In eye surgery, in the treatment of dry eye, HA gel is both a good carrier for the antibiotic to be applied and prolongs the effect of the drug because it is well adapted to the environment and is difficult to separate. In order to increase the efficiency of drugs, systems containing HA are used as carriers and reaching the target is facilitated. It is also widely used in malignant tumor, lung pathology studies, joint pathology studies, aesthetic surgery (6,7).

2.FOOD SUPPLEMENTS

2.1. Definition of Food Supplements

Food supplements are defined as products containing nutrients such as vitamins, minerals, amino acids, herbs or herbal dietary components that are used to supplement a human’s diet. Food supplements are products containing nutrients and excipients and available in the form of capsules, tablets, powders or liquids They are used in situations where nutritional deficiencies may lead to deterioration of health (8). Food supplements are only complementary and are not intended to be used as a substitute for a

normal diet. These supplements often contain chemical compounds that act as nutrients in the body, and they can help meet the specific nutritional needs of people with certain health conditions. For example, protein supplements are used to prevent joint pain and help tighten the skin (9). It is known that food supplements should not be considered as drugs, but when consumed correctly, they affect people's health positively (10). The Dietary Supplement Health and Education Act (DSHEA), which was adopted by the US Senate in 1994, includes food supplements, one or more nutrients (minerals, vitamins, amino acids and herbal drugs) used to support the diet. It qualifies as products prepared in tablet, capsule and liquid form to be taken orally containing (11,12). DSHEA; categorizes food supplements under the general name of 'foods' rather than medicine (13).

2.2. Use of Food Supplements

As the beneficial properties of foods on human health (antioxidant, dietary fiber, etc.) are known, the consumption of food supplements containing these beneficial properties is increasing due to the effect on the human body. Antioxidants such as anthocyanins and phenolic substances, especially found in fruits and vegetables. The positive effects of food components such as dietary fibers and milk proteins in cereals on health are known. The use of these components or foods as food supplements is becoming more common day by day and helps diet (10). It is stated that food supplements are used for purposes such as improving performance, providing a cosmetic or balanced diet, strengthening the immune system and curing some diseases (14). The reasons such as the desire to lose weight, elimination of the feeling of fatigue and having a fit body are the main reasons. In addition to these reasons, these types of products are frequently used for reasons such as stress, menopause, strengthening the immune system, skin and hair care. However, in recent years, it has been observed that the sales values of products that are claimed to be especially effective as aphrodisiacs have risen to the top (15). It is reported that the food supplements with the highest usage/consumption amount are multivitamins with or without minerals (12). The World Health Organization recommends that at least five servings of vegetables and/or fruit should be consumed each day. Increasing consumption of fruits and vegetables increases the intake of antioxidant vitamins and other components in the body. As an alternative to consuming five servings of fruit a day, some manufacturers have recently suggested food supplements that aim to increase the daily intake of antioxidants (16). The use of dietary supplements is common due to successful sales strategies and advertisements in the Internet. The market value of products defined as "Food Supplements" reached a figure of 50.6 billion dollars in the early 2000s all over the world. This figure constitutes more than 80% of the herbal products market, which is 60

billion dollars in total. The distribution of different product groups in this market share has been determined as 40%, 39% and 21% for vitamins and minerals, herbal drugs, sports and weight loss products, respectively. From the point of view of countries, the largest market share in the nutritional supplements market is North American countries with 16.3 billion dollars. (37%). European countries (33%) follow with 15 billion dollars and Japan (18%) with 7.2 billion dollars (15). The prevalence and frequency of use of food supplements has been increasing in recent years. In a study conducted in 2001, 61.2% of 376 adults randomly selected used food supplements; on the advice of some of these patients from their doctor; it has been reported that most of them use food supplements through their family, friends or the internet (17). According to the study by Durante et al. (2001) about the use of vitamin, mineral or herbal supplements, it is detected as 44% in men and 70% of the users under the age of 50 and 26% of the users over the age of 50 benefit from food supplements without the knowledge of their doctors. It has been determined that especially young patients find food supplements safer and more effective than using the drugs recommended by the doctor. It is known that the use of food supplements has become widespread and popular, especially in recent years, through sales strategies, advertisements and the internet (11).

3.HYALURONIC ACID

3.1. General Properties of Hyaluronic Acid

HA is a naturally occurring substance in all living organisms, from the simplest bacteria to the most advanced. HA is considered natural because it is obtained from bacteria or yeast cultures, but it is produced in a laboratory environment and is not considered as organic. HA is critical in the formation of the organism. During the fertilization of the egg, the sperm can pass through the layer, 98% of which is HA, thanks to the enzyme hyaluronidase it carries (18). HA is formed as a result of the combination of the Greek terms hyalos (glassy, glassy) and uronic acid. HA and hyaluronan are terms that can be used interchangeably. The term hyaluronan is preferred for this purpose, especially in order not to have a negative effect on the patient after eye surgery.(19). HA was first obtained from the vitreous fluid of the cattle eye by Meyer and Palmer in 1934 (1). It was isolated from other animal species in the 1940s; and its chemical structure was determined by Meyer et al. (20) Thanks to Balazs' studies, it was revealed that it plays a role in many important diseases such as rheumatoid arthritis, degenerative arthritis, cancer and some skin diseases in the 1950s (21). Its therapeutic use was started in the 1950s; the first product containing HA was developed in the 1980s. This visco-elastic product (Healon®, Pharmacia) was used in eye surgery procedures.

Then, products used in the treatment of osteoarthritis and wrinkles were produced (22).

3.2. Chemical Structure and Synthesis of Hyaluronic Acid

HA is a high molecular weight, negatively charged linear polysaccharide found especially in the extracellular matrix of connective tissue (1). It consists of repeating chains of N-acetyl-glucosamine and D-glucuronic acid connected to each other by β1.3 -1.4 glycosidic bonds. This substance, with an average molecular weight of 0.1-10 million Daltons, has a special feature due to its high molecular weight, polyanionic character and branching side chain structure. It has rheological character (20,23).HA; It consists of carbon, hydrogen, nitrogen and oxygen. Its molecular formula is C14H21NO11. The chemical form of HA is shown in Figure 1.

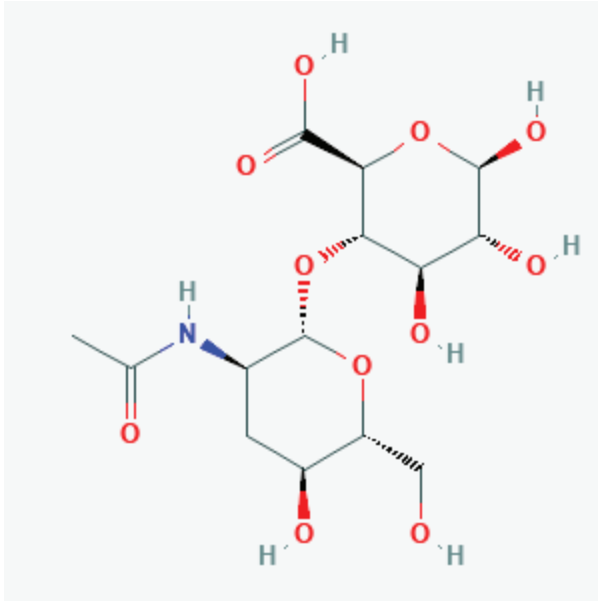

Figure 1. Hyaluronic acid (PubChem)

HA; It is a hygroscopic sugar that can hold 1000 times more water than its own weight. Its molecular volume increases approximately 10,000 times when it is hydrated compared to the dry state. It is stated that the formation of edema along with any inflammation in the tissues is due to this (19). HA is produced by an enzyme complex in the cell membrane. It is produced by fibroblasts and is broken down by the enzyme hyaluronidase. HA exists in biological tissues and fluids of various molecular sizes and can be degraded by both normal physiological and pathological conditions (24). It consists of linear macromolecular chains of repeated glucuronic acid and N-acetyl-D-glucosamine subunits that form glucosamine when

hydrolyzed (25). HA molecules of different weights have different rheological and biological properties, and this, combined with its intrinsic biocompatibility and viscoelastic physical character, means it is used as a treatment for osteoarthritis (26). For this reason, HA is often used in food supplements to maintain joint health and control tissue hydration.

3.3. Hyaluronic Acid Production

HA is obtained either from animal sources or by fermentation or direct isolation from bacteria (20,27). The animal sources from which it is obtained are amaranth, spinal cord, skin and joint fluid. Because of its high HA content compared to other animal tissues, amaranth is the most widely used source. HA used in skin care products and injection products used to be obtained from amaranth. However, most of the HA in skin care products today is of bacterial origin and is produced in a laboratory environment. Fermentation technique from microorganisms. HA obtained with high purity (23). While HA, which has a wide usage area and economic importance, was purified from amaranth with expensive methods in the early days, when it was revealed that HA could be produced by fermentation, production on an industrial scale shifted to this technique (28). The main factors in the shift of the production technique to fermentation are that the production method can be standardized and that the desired amount of HA with the targeted molecular weight can be produced throughout the year. Recent studies have shown that it is possible to extract HA from egg shells. Its molecular size varies according to the source from which it is obtained. There are three different molecular weight HAs commercially available. They are named as high molecular weight (10 million Dalton), medium molecular weight (500,000-800,000 Dalton) and low molecular weight (160,000-240,000 Dalton) HA. By making some changes in the molecular structure of HA, more resistant HA derivatives, both mechanically and chemically, have been obtained without changing its compatibility and degradability in the biological environment (20).

3.4. Functions of Hyaluronic Acid in the Organism

It is present in all organs of vertebrates, mostly in the extracellular matrix of soft connective tissue. It is found mostly in the skin of mammals. The amount of HA in human skin is about five grams, and this value corresponds to one third of the amount of HA in the human organism (20). It is found at the lowest level in human blood serum (1). HA is the most important and basic element of the extracellular matrix in connective tissue. It is found in the vitreous fluid of the eye, hyaline cartilage, joint fluid, dermis and epidermis. It increases the volume of the vitreous fluid,

acts as a lubricant in the tendons and muscles, gives lubrication to the joints, increases the stability of the spine and provides a relationship between the mother and the fetus in the umbilical cord. It has regulatory roles in cell motility, cellular proliferation, morphogenesis, embryonic development, cancer metastasis and inflammation (29,30). It has a much higher water holding capacity than other natural and synthetic polymers (23.) HA plays an important role in the hydration and moistening of tissues, the passage of substances from tissues, the movement and differentiation of cells. Therefore, it is used in orthopedics, rheumatology, ophthalmology, dermatology and cosmetology (29,31).

3.5. Uses of Hyaluronic Acid in Treatment

HA, a dermal component responsible for attracting water and giving volume to the dermis, is found freely in the dermis and is concentrated in areas with fewer cells. HA is used in medical aesthetic applications in dermatology to remove wrinkles. Topical HA is used as a popular additive in skin care products in various fields from under-eye bags and wrinkles reduction to skin tightening. It is responsible for the fullness and volume of the skin. Therefore, it is used for cosmetic purposes and as a filler in soft tissue surgeries, in various surgical procedures and in many clinical applications such as tissue engineering, in the form of oral supplements and injections (20). Due to its highly viscoelastic nature, biocompatibility and non-immunogenity, it is used to increase HA in synovial fluid in patients with arthritis. HA is often used in supplements because it can nourish bones and joints (8).

3.6. Formulation Properties of Hyaluronic Acid

Although HA is a very important skin component, its large size and inability to penetrate the dermis have prevented its topical use. However, if the appropriate size of HA is used, it can penetrate the epidermis. If it has a short half-life, it hydrates the skin and improves fine lines quickly. It should be known that the isolation stage and chemical processes greatly affect the biological activity of HA (32).

HA is available in oral forms in food supplements. However, when taken orally, it is broken down by the stomach and renders useless for the skin. However, glucosamine supplements (HA, dermatan sulfate, heparin, keratin sulfate) can help increase the skin's HA production (33).

The use of topical HA has been shown to increase skin hydration. Its hydration-enhancing effect is better in a humid environment. In a dry environment, it can dehydrate the skin by drawing water from the skin. Therefore, HA should be used together with an occlusive agent

in dry environments. Applying HA to the skin in a humid environment draws water into the skin and wrinkles instantly However, HA, which is traditionally found in many moisturizers; Contrary to manufacturers' claims, due to its large size, it cannot penetrate the epidermis and enter the dermis when applied topically. There are no published studies showing the penetration of topical HA into the dermis (34).

The Food and Drug Administration (FDA) has approved barrier repair products with HA as the main component. Topical medications such as diclofenac contain HA as an additive (20).

3.7. Literature Studies of Hyaluronic Acid Formulations

HA has been shown to be effective in many combined products. In 2010, Guevara et al. Evaluated the safety and efficacy of a new cream containing hydroquinone, glycolic acid, and HA for the treatment of Malesma. 15 Latin American women applied this formula to both sides of their faces twice daily (35). In 2012, Joksimovic et al. HA conducted a small, double-blind placebo-controlled clinical trial in 36 patients with hemorrhoids to evaluate the efficacy of a medicinal gel containing tea tree oil and methyl sulfonyl methane. The 14-day treatment regimen findings in all symptoms were significantly reduced compared to placebo. The gel containing HA was the same. It was found to be reliable and tolerable at the same time (36). HA is also used in combination with an iodine complex to provide significant clinical benefit in different wound types (32). In 2012 Schlesinger et al. conducted a small safety and efficacy study in 15 patients aged 18 to 75 years in which they used a topical anti-inflammatory formulation containing low molecular weight HA (HA sodium salt gel 0.2 percent) to treat percent seborrhoic dermatitis. and that it has an anti-inflammatory effect due to its effects on cytokine formation. Study results showed that HA treatment improved all measured findings, including erythema pruritus and reduced scaling. This study is the only publication evaluating the use of HA in inflammatory skin diseases (37).

4. ANALYTICAL METHODS

Harmita et al. developed two methods for the determination of HA and methylsulfonylmethane in food supplements. HPLC method with fluorescence detector was used for the determination of HA. Fluorenylmethyloxycarbonyl chloride was used as derivatizing agent. The excitation wavelength was 255 nm and the emission wavelength was 330 nm. Acetonitrile-acetate (1:4) mixture (pH 4.2) was used as mobile phase at a flow rate of 1.0 mL/min. The linear range was 5-50 ppm ($r = 0.9983$). LOD value was found as 3.55 ppm and LOQ value as 11.84 ppm. GC-FID method was used for MSM analysis. The stationary phase was

studied in G2 column at 110 °C. The method was linear in the range of 4000–15,000 ppm (r = 0.9988). LOD and LOQ were found to be 332.90 ppm and 1109.67 ppm, respectively (38).

In the study of Restain et al., chondroitin sulfate, keratan sulfate and HA were analyzed simultaneously by high performance capillary electrophoresis. Detection was made with a photodiode array detector. The maximum absorbance was determined as 193 nm. Five chondroitin sulfate standards and 13 food supplement samples of animal origin from six different suppliers were analyzed in the study. According to the results of the study, capillary electrophoresis may be a valid quantitative alternative method to separate HA. The new method, the HPCE method, can also precisely detect HA intentionally added to food supplements (from 2.45% to 6.96%) and which cannot be analyzed by HPAE (high performance anion exchange chromatography) (39).

Park et al. developed a suitable HPLC-UV method using enzymatic digestion for the determination of HA in food supplement formulations. Appropriate sample preparation procedures have been prepared for various matrices of food supplement formulations. In this study, an anion exchange type HPLC column was used for the analysis of disaccharides after enzymatic degradation of chondroitin and isocratic study. For the selectivity test, the Di-HA of the enzymatically digested HA compound was clearly separated from the matrix with a 0.05 M NaCl (pH 3.5) mobile phase solution on the SAX column with a retention time of 5.6 min (40).

5. DISCUSSION AND CONCLUSION

HA, which is used as a food supplement, is also widely used in medicine and biomedical fields. Within the results to be obtained, there is no doubt that the area of use of the product, which increases the production amount and decreases the costs, will increase day by day. As a result of the literature research, it has been observed that various analysis methods are applied for HA. Several methods such as hyaluronic acid analysis with fluorescent detection liquid chromatography, hyaluronic acid analysis with high performance capillary electrophoresis, and hyaluronic acid analysis by HPLC are available in the literature. Some difficulties have been identified for the qualitative and quantitative determination of HA in dietary supplement formulations. Due to the polydispersity and high molecular weight (106-107 Da) of HA, direct HA determination without degradation is difficult. Therefore, indirect methods using enzymatic degradation have been investigated to determine HA without affecting polydispersity. Although the studies in the literature for the analysis of HA in food supplements are few, they are promising. Among the analytical methods (HPLC) reported in the literature for HA analysis, it is the most

convenient and effective method; therefore, various HPLC methods (direct UV and mass spectrometry detection and derivatization by enzymatic digestion of HA after indirect fluorescence detection) have been reported. Considering that the analysis studies in the literature have been carried out since 2020, we can evaluate HA in food supplements qualitatively. Safe and effective methods are gaining importance day by day for quantitative analysis.

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Chapter 6

RELATIONSHIP BETWEEN PERIODONTAL DISEASES AND VITAMIN D

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1. INTRODUCTION

Periodontal and gingival health is defined as the condition that is either present without periodontal disease or prevents consequences of the past disease and that allows the individual to function normally. (Accessed March 26, 2018.)

Gingivitis is a disease, which does not have alveolar bone and attachment loss in periodontal support tissues and is characterized by chronic inflammation of the gingiva, with clinical findings such as color change, bleeding on probing, structural gingival disorder, and gingival edema (Newman et al., 2011).

Periodontitis is an inflammatory disease of the dental support tissues, caused by the complex interaction of some microorganisms with the host immune response, which is clinically seen with gingival recession and periodontal pocket formation, led by periodontal attachment loss and destruction of alveolar bone (Genco and Borgnakke, 2013).

Despite the fact that they have microbial effects, periodontal diseases actually have multifactorial etiology. Although the primary etiological factor is the bacteria, several environmental and genetic factors play a part in the progression of the disease (Hassel et al., 1995).

Vitamin D is a lipophilic hormone that can be synthesized in the body. Vitamin D engages in the regulation of bone metabolism and inflammatory response and the maintenance of serum calcium and phosphate levels. Because of its effect on periodontal health, mainly on bone metabolism; the significant role it plays in many inflammatory diseases characterized by the release of cytokines associated with inflammation; its immunomodulatory effects; and the fact that its relationship with some autoimmune diseases is shown, it has become a research topic for periodontal diseases.

Recent studies have suggested that vitamin D has positive effects on periodontal disease, tooth loss, and gingival inflammation (Diethtich et al., 2004). In addition to that, some other studies reported that vitamin D supplementation could reduce alveolar bone resorption and tooth loss (Wical et al., 1974; Baxter, 1984). The supplementation with vitamin D resulted in an improvement in reduced mineral density in the jawbones and increased alveolar bone resorption (Hildebolt et al., 2004; Miley et al., 2009).

For these reasons, the prevention and treatment effects of the disease with the changes in the serum level of vitamin D in periodontal diseases, which are multifactorial diseases, have become a matter of curiosity.

2. Periodontal Disease

Periodontium is a whole consisting of the gingiva, periodontal ligament, cementum, and alveolar bone. Periodontal disease occurs as a result of damage to these tissues (Lindhe et al., 2003). Periodontal disease is a chronic infectious disease caused by pathogenic microorganisms, characterized by progressive loss of attachment, bone resorption, pocket formation and/or gingival recession (Offenbacher S. 1996).

Despite the fact that the etiological factor of the disease is primarily plaque, the response of periodontal tissues to these microorganisms determines the formation and progression of the disease. It has been shown that periodontal disease occurs as a result of the disruption of the balance between the microbial dental plaque and the host response; and that it is affected by various risk factors and determinants, including systemic diseases, genetics, smoking, and stress (Sahingur & Cohen, 2004).

2.1. Gingivitis

Gingivitis: It is a disease, which does not have alveolar bone and attachment loss and is characterized by chronic inflammation accompanied by clinical findings, such as color change limited to the gingiva only, edema, bleeding on probing, structural gingival disorder (Newman et al., 2011b).

As a result of the vasodilatation of the veins in gingival tissues, the gingiva's color turns red, and bleeding occurs in drilling. Because of the edema in the gingiva, healthy gingiva in the form of a knife-edge swells and gains a rounded form. With the collagen loss in the connective tissue and deterioration of the stipling structure, the gingiva loses its tight, compact form and becomes soft (Newman et al., 2011b). No clinical attachment loss or bone loss is seen in gingivitis (Mariotti, 1999).

2.2. Periodontitis

Periodontitis is a bacterial infection that is characterized by the inflammation and destruction of the tissues supporting the tooth (Taylor, 2001). Clinically, it is characterized by the formation of periodontal pocket formation, clinical attachment loss, and alveolar bone loss, in addition to gingival inflammation. The destruction in the alveolar bone can also be observed radiographically and is horizontal or vertical bone destruction. Depending on the severity of the disease, mobility and migration in teeth are among clinical findings as well (Greenstein, 2000; Newman et al., 2007).

Despite the fact that plaque is the primary factor as the local factor in the start and progression of periodontal disease, restorations, the anatomical structure of the tooth, caries, and root resorptions, etc., and as systemic factors, diabetes mellitus, smoking, osteopenia and osteoporosis, neutropenia, agranulocytosis, hypophosphatasia, Ehlers- Danlos stress,

diet, etc. are considered among the factors (Rose et al., 2000).

2.3. Pathogenesis of Periodontal Diseases

The factors that lead to the formation of the disease and play a role in its progression are called pathogenesis. There are two mechanisms that play a role in the periodontal tissue destruction, and these are endotoxins produced by the bacteria in the microbial dental plaque, toxic substances and the direct destructive effect they create with protease enzymes; and cytokines created by the host against these destructive bacteria, and the indirect destructive effect they create with enzymes (Listgarten, 1987). In addition to this, systemic diseases affecting the host's defense mechanism, bad habits, and environmental factors also play a role in the pathogenesis of periodontal diseases. (Page ve Kornman, 1997).

For periodontal microorganisms to show pathogenicity, they must have at least three characteristics. These are the microorganisms' ability to form a colony in periodontal tissues, the host's ability to overcome antibacterial defense mechanisms, and the ability to secrete substances that can directly lead to tissue destruction (Flemming TS 1999, Praveen N, Rajesh A 2014).

Pathogen microorganisms detected in the periodontal disease consist of gram-negative anaerobic bacilli, some cocci, and largely anaerobic pirochetes (Kinane DP 2000, Newman MG and Takei H 2011).

Microbial dental plaque is a complex ecosystem that is organized and contains aerobic and anaerobic bacteria species (Socransky SS, Smith C, Haffajee AD 2002). At first, a bacterial biofilm consisting of gram (+) cocci is formed, and then gram (-) anaerobic bacteria species become dominant with the maturation of the dental plaque. Gram (-) anaerobic bacteria are closely associated with periodontal tissue destruction. These bacteria particularly include the several species of Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Bacteroides, and Porphyromonas (Page RC, Kornman KS 1997).

They trigger the innate immune response and lipopolysaccharides (LPS) found in the cell wall of bacteria with virulence factors in periodontal tissues (Dixon DR, 2004). The LPS in the biofilm increases the microcirculation in the gingiva, and cytokines such as IL-1, IL-3, and TNF-α are secreted from the endothelial cells. With the secretion of these cytokines, firstly neutrophils, and then monocytes and lymphocytes leave the blood vessel wall and form the inflammatory cell infiltrate. After they are activated, the matrix metalloproteinase enzymes secreted from the cells cause the destruction of collagen and connective tissue extracellular matrix, and a periodontal pocket emerges. With the progression of the periodontal lesion, fibroblast, epithelial and endothelial cells, which are the host cells,

also begin to secrete cytokines and enzymes that lead to tissue destruction. As a result of cytokines such as prostaglandin-E2, IL-1, IL-3, IL-6, and TNF-α released from activated macrophages and fibroblasts, alveolar bone destruction is seen (Kinane DF 2001).

2.4. Association Between Periodontal Diseases and Systemic Diseases

In periodontal diseases, systemic and local conditions change the progression pace and course of the disease.

Today, diabetes mellitus, osteoporosis, premature birth and low weight at birth, HIV infection, neutropenia, leukemia, multiple myeloma, leukocyte adhesion defects, blood diseases such as histiocytosis, genetic diseases such as Down syndrome, Chediak-Higashi, hypophosphatasia, Papillon-Lefèvre syndrome, and Ehlers-Danlos syndrome, respiratory system diseases and cardiovascular diseases have been reported to systemically affecting the periodontal disease (Kinane and Marshall, 2001; Öztekin et al., 2014; Reddy et al., 2014; 2015; Wu et al., 2015).

Diabetes mellitus may play a significant role in the start and progression of the disease and is one of the systemic risk factors of periodontal diseases. Periodontitis is accepted as the sixth complication of diabetes (Löe, 1993). Whether diabetic diseases are under the increased risk of periodontal diseases has been the subject of several researches. In their study, Grossi at al. revealed the association between systemic diseases and diabetes' attachment loss increase. Diabetes mellitus is associated with periodontal ligament destruction, which can be associated with individuals' tooth loss. It was established that in individuals with periodontitis, gingival groove fluids and saliva have higher concentrations of inflammatory mediators including different cytokines including different cytokines in individuals with periodontitis, compared to periodontally healthy individuals (Parker et al., 1993). Likewise, in another study, it was seen that poorly controlled diabetic patients had more gingivitis and periodontitis compared to well-controlled diabetic patients (Seppala and Ainamo, 1994).

The studies regarding the evaluation between osteoporosis and periodontal disease reported that patients with osteoporosis have more alveolar bone and tooth loss (Yoshihara et al., 2005). In many cross-sectional studies made in post-menopausal women, a study was conducted in 227 healthy post-menopausal women to see the association between osteoporosis and periodontitis, and it was observed that the rate of tooth retention in the presence of periodontitis was higher in post-menopausal women with high bone density, compared to women who are osteoporosis patients (Klemetti et al. 1994). Tezal et al. stated that among 70 post-menopausal women, in individuals between ages 51 and 78, osteopenia

could be associated with periodontal disease (Tezal et al. 2000).

In developed countries, cardiovascular diseases, along with malnutrition and malnutrition, are among the leading causes of premature death in the male population. The main reason for cardiovascular diseases is shown as atherosclerosis. Hemostatic and rheological variables are associated with cardiovascular diseases, and it is known that risk factors, such as smoking, hyperlipidemia, and infections (including periodontal disease), may trigger vascular incidents (Rees, 1994).

The increase in the atherosclerotic incidents leads to an increase in the free oxygen radicals, similar to the periodontal disease (Loscalzo J. 2003).

C-reactive protein (CRP) is a risk factor for atherosclerosis. Slade et al. (2000) showed the increased CRP levels in patients with periodontitis, even after the elimination of the identified risk factors for increasing CRP.

In their study conducted in 1999, Chui et al. isolated periodontopathogenic bacteria in atherosclerotic plaque samples. Dorn et al. (1999) showed that periodontal pathogens such as S. sangius and P. gingivalis colonize in coronary artery endothelial cells.

As mentioned above, several systemic diseases like these were associated with periodontal diseases. The local treatment methods for periodontal diseases have been proven in our day. Professionally performed scaling, subgingival curettage, local antibiotic application, air-flow, laser antimicrobial treatment, etc., methods are used locally. However, the fact that periodontal diseases are multifactorial leads to new pursuits in treatment methods.

3. Vitamin D

Vitamin D is a lipophilic hormone that can be synthesized in the body. The physiological duty of Vitamin D is to adjust the level of calcium and phosphor in the blood and to realize the mineralization of the bone matrix. Despite the fact that the primary duty of vitamin D is the bone and calcium metabolism, it also takes part in such mechanisms as cell differentiation and proliferation, cardiovascular functions, cellular humoral immunity (Nagpal S, Na S, Rathnachalam R 2005).

There are two forms of vitamin D, one of which is called ergocalciferol (vitamin D_2), taken into the body by eating plants that get sunlight and turned into cholecalciferol in the human body (Vieth R. 2004).

Another form of cholecalciferol is synthesized in our body through our skin by 95% with the effect of UV-B, by transforming from 7-dehydrocholecalciferol to pre-D_3, then to D_3 (cholecalciferol) (Holick MF 2007). In the human body, the main synthesis place of vitamin D

is the skin. The one that is actually active in human is cholecalciferol (Vitamin $D_{3)}$. D_3 in the blood is hydroxylated in the liver cell and turns into vitamin 25-OH-D. This form turns into vitamin 25(OH)2 D with 1-alpha hydroxylation in the other tissues of ours, particularly the kidney. Its most active form is 1-25(OH)2 D. It binds to the receptors in the cell cytoplasm and shows its effect thereby (Vieth R 2004).

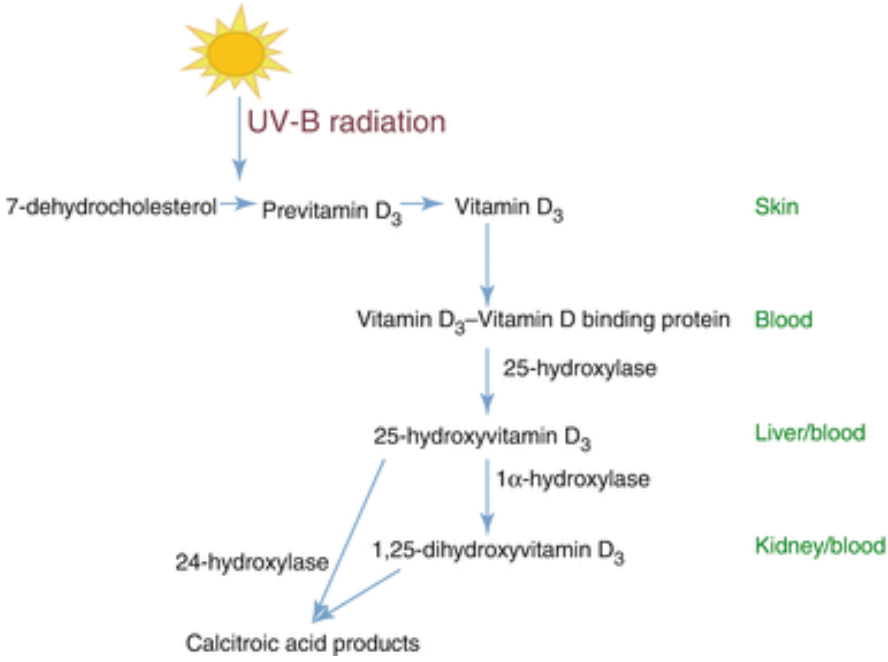

Figure 1*: transformation of vitamin D into an active form in the body*

3.1. Receptor of Vitamin D

Vitamin D needs receptors to become biologically active, and the receptor is with high affinity (Brow A 1999). This receptor of vitamin D is in cells' cytoplasm and nuclei (Michigami T 1999). An mRNA synthesis is realized with VDR gene activation, and then the protein synthesis realizes after vitamin D (Rosen CJ 2012).

In VDR gene transcription, first ligand binding occurs, and then heterodimerization with the retinoid X receptor is seen. These heterodimers bind to the response elements of Vitamin D, the regulators are activated, and at the end, protein synthesis occurs (Christakos S 2016).

Due to VDR protein's effect with gene transcription, the genetic change in VDR protein may lead to problems affecting calcium metabolism, cell proliferation, and immune functions (192).

VDR gene transcription starts with ligand binding first, and then heterodimerization with the Retinoid X receptor (RXR) occurs. As a result

of this, heterodimer's vitamin D binds to the response elements (VDRE), and regulators become active. Lastly, protein synthesis realizes (Moon S. J 2005).

Because VDR protein shows an effect by controlling the transcription of different genes, potential genetic changes in VDR gene may lead to significant defects that affect calcium metabolism, cell proliferation, and immune functions (Holick MF, Chen TC 2008). The effect on such mechanisms may affect the progression and recovery patterns for periodontal disease.

3.2. Determining Vitamin D Level

To detect the vitamin D in the body, 25-OH vitamin D is used in serum. 25OH-D is the most important circulating metabolite of vitamin D. It shows the amount of vitamin D in the body, which is received from plants and also synthesized by the skin (Hollick MF., 2006).

The active form of vitamin D is 1,25(OH)-D. However, its half-life (4-6 hours) is short, and the amount of 1,25 OH-D in circulation is relatively low. For this reason, 1,25(OH)-D is not used in the ideal measurement in serum (Hollick MF., 2009).

The optimal 25OH-D_3 level in circulation is in an amount that does not lead to hyperparathyroidism by adjusting the Ca absorption and PTH level (Heaney RP., 2004). The optimal level in children has been determined as ≥30 µg/l, and the level of 25(OH)D3 in adults is around 50 µg/l. The levels above 30 µg/l are considered optimal for vitamin D (Dawson-Hughes B., 2005, Misra M., 2008). In cases where vitamin D is not sufficient, no clinical finding is observed, and the level of the hormone PTH increases. In long-term deficiencies, a decrease in bone tissue occurs (Heaney RP., 2004).

In general, the levels of serum 25(OH)D3 are between 15 and 30 µg/l (Heaney RP., 2004). Nevertheless, 25(OH)D levels between 10 µg/l and 30 µg/l or between 20 µg/l and 30 µg/l for vitamin D deficiency are considered as vitamin D deficiency in different sources (Lips P., 2007).

3.3. Vitamin D Deficiency

Vitamin D deficiency is a term used for patients with a very low level of vitamin D in serum. There are several risk factors for vitamin D deficiency. These are as follows:

- Geographical region;
- Premature and dysmature births;

- Pigmented skin and skin age (aged skin produces less vitamin D compared to younger skin);
- Using suncreen;
- Insufficient exposure to sunlight;
- Obesity and malnutrition;
- Using medical drugs;
- Cases of malabsorption;
- Granulomatous diseases;
- Tumor-induced osteomalacia;
- Hyperphosphatemia and nephrotic syndrome;
- Liver failure (Hollick, 2004).

There are different opinions on how much daily vitamin D intake should be because of the fact that there is a synthesis of vitamin D in the body with sunlight intake. In individuals who cannot benefit from sunlight sufficiently, a diet not fortified with vitamin D cannot prevent vitamin D deficiency (Hollick, 2005).

The fact that vitamin D metabolism and physiology are different due to genetic reasons leads to different vitamin needs for different individuals.

Different skin pigmentations result in different vitamin D needs of different individuals. Melanin absorbs sunlight at wavelengths of 290 nm and above. Melanin competes with epidermal provitamin D3 for UVB photons and effectively absorbs UVB photons, reducing the photosynthesis of procholecalciferol. Melanin pigmentation is more in individuals with dark skin, but its amount synthesized in the skin is low (Norman AW.,2008). The capacity of synthesizing vitamin D varies depending on age. In elderly individuals, the concentration of 7-dehydrocholesterol in the skin reduces, and its capacity to synthesize vitamin D decreases.

Sunscreens are used for the purpose of preventing undesired effects of the sun, such as skin cancer and skin burns. However, they reduce the amount of vitamin D synthesized by the skin. Sunscreens containing protective factor 8 reduce vitamin D synthesis in the skin by 95%, while those containing skin protective factor 15 reduce it by 99% (Fidan F., 2014).

Seasonal differences and latitude differences affect the synthesis of vitamin D in the skin as well. In latitudes above 37°, the number of UVB photons reduces in winter. In latitudes below 37° and regions close to the equator, there is more vitamin D synthesis in the skin throughout the year.

Due to the oblique angle, vitamin D production is also low in the afternoon and early morning because fewer photons reach the earth with the oblique angle. Some hours of the day, seasons, and latitude are among the factors that affect the oblique angle. The most ideal sun exposure time interval is between 10:00 AM and 03:00 PM (Norman AW., 1998). An impenetrable style of clothing prevents UVB rays from passing through the skin and reduces the synthesis of vitamin D in the skin.

In obese individuals, the amount of adipose tissue is high, and vitamin D is stored in the adipose tissue. For this reason, vitamin D deficiency can be observed in such individuals. In addition to this, because obese individuals are not outdoors frequently, they have a low amount of vitamin D due to the fact that the duration of indirectly benefiting from sunlight is short for them (Hippönen E.,2007).

Vitamin D is directly associated with UVB light; the duration and frequency of UVB exposure are important. For sufficient synthesis of vitamin D, it is recommended to take sunlight for 5-30 minutes from 10:00 AM to 03:00 PM (Decick MF.,1994).

The natural form of vitamin D is present in some foods (salmon, mackerel, herring, fish oil, offal, egg yolk). Some foods are supported with vitamin D. Milk, some fruit juices, bread, yogurt, and cheese are fortified foods with respect to vitamin D (Hollick MF.,2009).

Vitamin osteomalacia is observed in adults as a result of D deficiency. The clinical picture of osteomalacia is pain in the skeletal system, and proximal muscle weakness, and the level of vitamin D, calcium, and phosphorus in the serum is low. In osteomalacia, the levels of PTH alkaline phosphatase are generally high. Due to this fact, a decrease in bone density can also be found in fractures with the radiographic examination. As a result of the biopsy, a reduction in the density of minerals in the bone is seen (Bhan A.,2010).

In children, rickets is observed as a result of vitamin D deficiency. Furthermore, it shows clinical signs such as tetany in muscles, hypocalcemia, craniotabes, pectus carinatum, kyphoscoliosis, tibial curvatures, and delayed tooth eruption (Joiner TA.,2002).

3.4. Vitamin D and Its Biological Effects

3.4.1. Vitamin D and Its Effect on the Immune System and Inflammation

Vitamin D has significant effects on cell differentiation, proliferation, and immune modulation. Vitamin D has no direct effect on lymphocytes, but there are no calcitriol receptors in lymphocytes. First, the receptors are

released upon the activation of lymphocytes, and then macrophages and lymphocytes that encounter vitamin D realize the expression of the MHC antigen. As a result of this, IL-1 production takes place, and an increase in phagocytic activity is observed. Vitamin D also has an effect as an inhibitor in the production of IL-2, and immunoglobulin synthesis of lymphocytes B in T cells' proliferation, and as a result of this effect, infection is frequently observed in individuals with vitamin D deficiency (Aarskog and Harrison, 1994).

Vitamin D activates monocytes, regulates cell-mediated immunity, suppresses Ig production and cytokine synthesis with lymphocyte proliferation (Van Etten, 2005). In most immune cells, there are vitamin D receptors. Vitamin D ensures the transformation of immature monocytes into mature macrophages (Manolagas et al., 1990). The effect of vitamin D deficiency at the cellular level is observed on the macrophage cells, with the disruption in the macrophages' function. As a result of this chemotaxis, phagocytosis, and proinflammatory cytokine production cannot be performed (Aarskog and Harrison, 1994; Manolagas et al., 1990; Van Etten, 2005).

Association of vitamin D with inflammation and its protective effect against various inflammatory and chronic diseases by affecting the expression of some cytokines have been reported (Guillot et al. 2010 and Tang et al. 2013). In another study evaluating the anti-inflammatory effect of vitamin D, it was observed that there was less IL-8 when vitamin D was added to human periodontal tissue cell cultures affected by *Porphyromonas gingivalis* (Tang et al. 2013). In another study supporting this study, it was determined that high vitamin D serum concentrations lead to less IL-6 and leptin as markers regulating immune responses, and the anti-inflammatory properties of vitamin D were revealed (Teles et al. 2012).

Additionally, vitamin D supplementation has been found to be associated with reduced pain and C-reactive protein (CRP) levels (Andjelkovic et al. 1999). Likewise, it has been reported that vitamin D can inhibit periodontal inflammation by reducing the expression of IL-6, IL-8, and TNF-α, which increase inflammation and bone loss (Tang et al. 2013).

3.4.2. Vitamin D's Effect on the Bone

Vitamin D's effects on the bone are among its most known effects. Vitamin D stimulates the production of osteoblast bone matrix, binds bone resorption into formation/turns bone resorption into formation, optimizes bone's remodeling, stimulates the bone metabolism, and maintains bone mass with the stimulation of alkaline phosphatase (ALP) activity and the increase in the bone matrix proteins (osteocalcin and osteopontin)

(Haussler et al. 2013). In addition to this, it also increases the absorption of calcium in the intestine and leads to a low amount of bone resorption with a decrease in the PTH secretion by inhibiting osteoclasts (Holick 2007, Zhou et al. 2012).

By looking at its effects, vitamin D in bone metabolism has been associated with periodontal disease and low serum vitamin D concentrations, and it has been suggested that the optimal vitamin D level prevents the progression of periodontal disease (Dietrich et al. 2004, Jimenez et al. 2014). Chen et al. (2012b) Vitamin D and calcium take part in controlling the formation of the dental alveolar bone. It was reported that the low level of 25(OH)D_3 in serum might be associated with periodontitis independent of bone mineral density (Dietrich et al. 2004).

Recent studies also show that in individuals with optimal serum 25(OH)D_3, it can reduce the risk of gingival inflammation (Dietrich et al. 2005), periodontitis (Millen et al. 2013), decay (Grant 2011), and tooth loss (Jimenez et al. 2014).

3.5. Association Between Vitamin D and Periodontal Disease

Vitamin D has several effect mechanisms, either directly or through receptors. It was shown that vitamin D could be synthesized from fibroblast cells and periodontal ligament cells (Liu et al. 2012). Because vitamin D can be synthesized from these tissues and also has effects on the bone mechanism, an association can be established between periodontal diseases and vitamin D. It has been shown that vitamin D regulates inflammatory responses in periodontal ligament cells both directly and via VDR, and may affect inflammatory processes in periodontal disease (Andrukhov et al. 2014).

The effect of vitamin D in preventing tooth loss is shown with the studies conducted (Krall EA. et al. 2001, Jimenez et al. 2014). Another study established that there is a negative correlation between vitamin D levels and attachment loss which suggests that the increase in the levels of vitamin D has a positive effect on periodontitis (Dietrich et al. 2004).

In a study they conducted in 2005, Dietrich et al. found that the low levels of serum 25(OH)D_3 are associated with periodontitis and gingival inflammation.

In an animal study, it was established that in mice with 1,25(OH)D_3 deficiency, defects were established in dental and mandibular bone mineralization, and as a result of this, it was established that vitamin D is more dominant than PTH in the formation of hard tissues (Liu et al. 2009a). Another study on mice showed that mice with 1,25(OH)D_3 deficiency had more alveolar bone loss, increased gene expression levels of IL-1β,

TNF-α, MMP-3, and MMP-8, and increased bone mineral density, and it was observed that bone mineral density significantly decreased regardless of extracellular calcium and phosphorus levels, and age (Gong et al. 2018).

1,25(OH)D_3 deficiency ensures higher numbers of NF-κB p65, and CD3 + cells as well as a higher inflammatory response stimulation in gingival tissues. This stimulation supports the studies which show that 1.25(OH)D_3 regulates the biosynthesis of proinflammatory molecules, which mediate oral infections and periodontitis, and has inflammatory effects (Lin and Li 2016).

1,25(OH)D_3 deficiency inhibits osteoblastic bone formation regardless of phosphor and age and accelerates bone loss by increasing the periodontal tissue regeneration. These results show the harmful effects of vitamin D deficiency on the periodontium, and this way, they support that vitamin D has a protective role in periodontal tissues (Gong et al. 2018).

The biological mechanisms of vitamin D can be explained by its function of regulating calcium maintenance, which plays an essential role in bone metabolism, and its anti-inflammatory or antimicrobial effects (Zanetti et al. 2014). Despite the fact that the association between vitamin D and the development and progression of periodontal diseases is reported, there are still uncertainties whether vitamin D deficiency contributes to the severity of periodontitis (Kral et al. 2001, Dietrich et al. 2005, Liu et al. 2009b, Millen et al. 2013, Jimenez et al. 2014, Millen et al. 2014, Pavlesen et al. 2016).

Additionally, studies showing that vitamin D is inversely associated with tooth loss, increased alveolar bone loss and gingival bleeding and probing depth and that patients with low 25(OH)D_3 levels can be kept periodontally stable for 5 years, and studies which show that these data do not support the association between vitamin D and tooth loss are available in the literature (Millen et al. 2014, Pavlesen et al. 2016)

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Chapter 7

ANALYSIS OF ENDOSCOPY REPORTS USING TEXT MINING ALGORITHM

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1- Introduction

An important part of the medical cases consists of files originating from misdiagnosis and treatment (Malpractice). The main reason for these malpractice files is that the necessary tests are not requested, the patient's medical history cannot be accessed, the physician does not examine the patient in sufficient detail, and the existing records are not examined in sufficient detail. When the physician fails to diagnose the patient's condition accurately and quickly, the patient's treatment is delayed or unsuccessful, and may even lead to death. These errors in the diagnosis of the disease can be caused by hard work, aging, fatigue, etc. may be caused by various reasons. [Anonymous 2020][1].

According to one report, 74 percent of medical error cases are due to physicians' perceptual errors. Studies show that misdiagnosis occur in 10-30% of all medical cases. Unfortunately, not all physicians have the same experience, education and knowledge. According to the medical literature, between 60 and 70 million American patients suffer from gastrointestinal problems, and approximately 250 000 patients die each year. According to the National Institute of Diabetes, Digestive and Kidney Diseases (NIH/ National Institute of Diabetes, Digestive & Kidney Diseases), close to 50 million patients visit clinics annually and 21.7 million are hospitalized for these conditions. Moreover, the US healthcare system pays the bill at more than $141.8 billion to treat and manage digestive diseases. [Anonymous 2020][2].

The goal of this study is developing a software analyzes upper gastrointestinal system endoscopy reports with text mining methods as a clinical decision support system and to evaluate the suitability of the routinely used endoscopy reporting technique for text mining during these development studies. For this purpose, we used natural language processing libraries in Python in this project. Since the texts in our data are in Turkish, we used zeyrek, which is part of the Zemberek library, to analyze the word.

The results of this study may have a positive impact in the field of medicine. Therefore, doctors will be able to more accurately describe the disease by combining the medical science and technology presented in this project. Artificial intelligence is able to improve health care outcomes in terms of productivity and efficiency.

2- State-of-Art

With artificial intelligence, data on various chronic diseases such as

1 Anonymous, 2020, İmaware, 13 Most Common Gastrointestinal Conditions and What to Do About Them, https://www.imaware.health/blog/most-common-gastrointestinal-conditions

2 Anonymous, 2020, https://www.meta.org/feed-previews/brain-computer-interface/dc5bbc99-fe4c-46b8-a130-a409bcf1dc10

Alzheimer's, diabetes, cardiovascular disease, and various cancers can be identified and interpreted. Various automated systems and tools such as brain-computer interfaces (BCIs), arterial spin labeling (ASL) imaging, ASLMRI, Biomarkers, Natural language processing (NLP), and various algorithms help minimize errors and control disease progression. [Mishra SG,2017][3].

• Brain-computer interfaces (BCIs)

Brain-computer interfaces (BCIs) receive brain signals, analyze them and then send to output devices by performing desired actions (Figure 1). The main purpose of BCI is to support people who are constrained by neuromuscular disorders [Jerry.J. Shin, 2012][4]. A wide range of BCI-based AI applications has emerged. Intelligent BCs such as motor and sensory BCs have improved the quality of life of paralyzed patients. [Xiayin Zhang, 2019][5]

• Arterial Spin Labeling Images (ASL-MRI)

Arterial spin labeling (ASL) is a magnetic resonance imaging technique to measure tissue perfusion using a freely diffusible intrinsic tracer (Figure2). Various post-processing methods have been proposed for ASL MRI, but only moderate improvements have been achieved, Deep learning (DL) is a highly complex and analytically indescribable new technique that can learn the most representative signal from data without prior modeling.

3 Mishra,SG. Vd., 2017, Role Of Artificial İntelligence in health Care, BioChemistry Indian Journal, Volume 11(5),

4 Jerry, J. Dean, J. Krusienski, vd., 2012, Brain-Computer Interface in Medicine, National Center and Biotechnology information, 87(3), 268-279

5 Xiayin.Z, Ziyue.Ma, vd., 2019, The combination of brain-computer interfaces and artificial intelligence: applications and challenges, NBCI, 8(11), 712

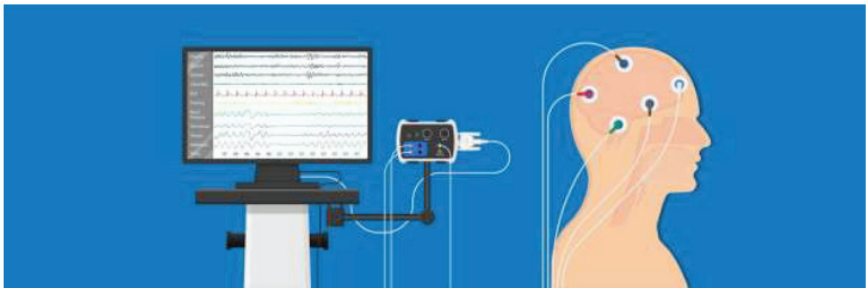

Figure1*: A brain-machine interface is a two-way communication path between an external device and a wired brain. (Anon 2020)*

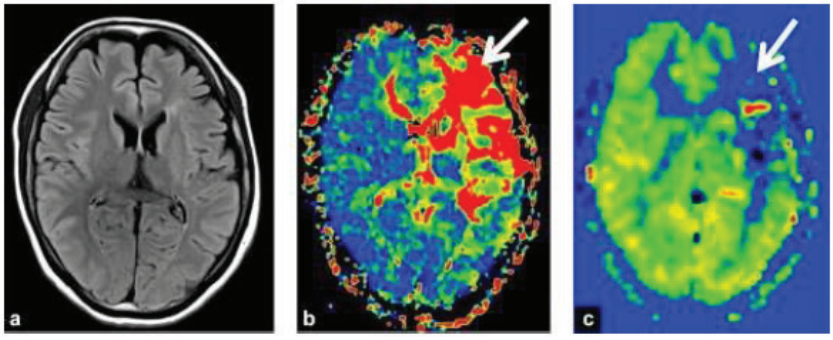

Figure 2*: Arterial spin labeling (ASL) perfusion is an MRI technique for measuring tissue blood flow (J-C.Ferre, 2013)*

• Biomarkers

By definition, a biomarker is "molecules that indicate a normal or abnormal process in your body and may be a sign of a condition or disease." Biomarkers such as blood pressure or cholesterol level are measures used to make assessments and to monitor and predict health conditions in individuals so that appropriate therapeutic intervention can be planned. The integration of biomarkers with artificial intelligence techniques has the potential to prevent age-related diseases [Ananya.M,209][6]

• Natural Language Processing

With features of NLP, computers can interpret, identify, and process human language and speech. Natural language processing refers to both active and passive modes of using algorithms, focusing on both the spoken and written part of human languages. Natural Language Generation (NLG) processes and decodes sentences and words that people use to speak

6 Ananya.M, 2019, What is Biomarker?, News Medical Life Sciences, https://www.newsmedical. net/health/What-is-a-Biomarker.aspx

(verbal communication); Natural Language Understanding (NLU), on the other hand, is the highlighting of written words to translate language in text or pixels.

With the development of NLP, businesses can internationalize their transactions, develop business relationships and thus promote global trade. The application of NLP can be found in a number of business contexts, such as Trade, E-Management, E-Training, E-health care. As mentioned, the goal of this study is to use artificial intelligence (text mining) techniques to analyze upper gastrointestinal system endoscopy reports textual reports. So, let's have a brief overview of what endoscopy is and why it is done.

Endoscopy is the examination of a closed body cavity by entering a natural or artificial opening. It can be used for diagnostic and therapeutic purposes. Endoscopy systems basically consist of a light source, a scope that provides dynamic and stable image acquisition, a processor for image processing, a database and user interface and monitor. The most important advantage of these systems is that the images obtained during the process can be stored in the form of image and video files, they provide archiving with the database they are associated with, and the user interface and reports containing text and images related to the endoscopy procedure can be prepared. Upper Gastrointestinal Endoscopy and Lower Gastrointestinal Endoscopy techniques are used in the examination of the gastrointestinal tract. Upper Gastrointestinal Endoscopy is usually abbreviated as "endoscopy" while "Colonoscopy" is used for Lower Gastrointestinal Endoscopy. Gastrointestinal endoscopy in cancer screening and many other It is used to better understand and diagnose gastrointestinal problem.

1.1. Related Studies

▪ Although serrated polyposis syndrome (SPS) is common in the population, it is under-recognized. Parthasarathy et al. used natural language processing (NLP) to extract colonoscopy and pathology data from electronic medical record (EMR).

▪ according they studies, It was determined that 71 of 255,074 patients met 1 WHO criteria for SPS.Manual review confirmed that the diagnosis of SPS was correct in 66 cases (93%). NLP correctly identified SPS in over 90% of most previously unrecognized cases.. [G.Parthasorathy vd. 2020][7].

▪ Electronic patient-written text (ePAT) is a critical component of making sense of symptoms and experiences. Dreisbach et al. In their review, they reviewed the literature on NLP and text mining applicable to

7 G.Parthasarathy, A natural language–based tool for diagnosis of serrated polyposis syndrome, Gastrointestinal Endoscopy, Volume 92, Issue 4, October 2020, Pages 886-890

symptom extraction and processing in ePAT. They have planned studies accessing the ePAT from sources such as Twitter and online community forums or patient portals focusing on diseases including diabetes, cancer, and depression. NLP and text mining have been used to extract and analyze patient-written symptom data in a wide variety of online communities. As a result of the study, it should take into account the needs of patients expressed through the ePAT and its relevance to symptom science. Understanding the role ePAT plays in health communication and real-time assessment of symptoms is critical for a patient-centered healthcare system through NLP and text mining [C.Dreisbach,2019][8].

▪ Natural language processing (NLP) is a field of computer science in which programs are trained to extract relevant information from text reports in an automated manner. NLP-based colonoscopy demonstrates the effectiveness and potential of quality measurement. In a cross-sectional study design, Mehrotra et al. used a pre-approved NLP program to analyze colonoscopy reports and associated pathology notes. The data obtained were used to support the performance of colonoscopy quality measures. A sample of studies involving nine hospital workers in the University of Pittsburgh Medical Center healthcare system consisted of 24,157 colonoscopy reports and related pathology reports from 2008 to 2009. Their work has shown how NLP can explore free

text data in electronic records to measure and report quality of care [A.Mehrotra, 2012][9]

▪ Little is known about NLP's ability to extract meaningful information from free-text gastroenterology reports for secondary use. Imler et al. (2013), 500 linked colonoscopy and pathology reports were randomly selected from 10,798 non-sur-sur-surce colonoscopies to train and test the NLP system. They evaluated the accuracy of an open-source NL Engine that processes and extracts clinically relevant concepts, using descriptions by gastro-enterologists as the reference standard. The primary outcome is a high level of pathology. Secondary outcomes are the location of the most advanced lesion, the largest size of an adenoma removed, and the number of adenomas removed. The NLP system determined the highest level of

8 Dreisbach. C., Koleck, A., vd., 2019, A systematic review of natural language processing and text mining of symptoms from electronic patient-authored text data, International Journal of Medical Informatics, Volume 125, Pages 37-46

9 Mehrotra, A., Dellon, E., vd., 2012, Applying a natural language processing tool to electronic health records to assess performance on colonoscopy quality measures, PubMed.gov

pathology with 98% accuracy compared to the gastroenterologists' triple notes (standard). (T.D.Imler,2015)[10].

3- Material and Method

Data Set

Since we could not find an anonymous database for this study, 2018 patient story forms archive records of Kütahya Health Sciences University, Department of General Surgery, Kütahya Evliya Çelebi Training and Research Hospital Endoscopy Unit were used. The volume of the recording set to be used in the research was determined according to the number and rates of monthly endoscopy in 2018. After extracting personal data, metadata, and image data from a total of 148 endoscopy reports archive records, the data were received. A research database including age, gender, report text and diagnosis sections was prepared and the data were transferred to this database. 90 (60.82%) of 148 patients in the 18-79 age group were female and 58 (39.18%) were male. Data were saved as *.doc, *.rtf and PDF files. To work with the data, all its information in word and pdf was transferred in an Excel (*.xlsx) file. Since the data is in Turkish, the Excel file was saved in CSV UTF-8* format. Different methods were used to create a suitable database and work on the data. We worked on 3 types of datasets.

Dataset 1. This database consists of 2 columns and 148 rows. Endoscopy report texts in the first column and diagnosis of the disease diagnosed by the doctor in the second column are included. (Figure 3)

Database 2. After our work on the first database, we realized that the results were not very good. The number of diagnosed diseases in patient reports was 50, which caused us to have problems with machine learning algorithms during training and not getting good results. After our work on the first database, we realized that the results were not very good. The number of diagnosed diseases in patient reports was 50, which caused us to have problems with machine learning algorithms during training and not getting good results. Since 50 types of diseases were abundant in 148 reports, we created a second database consisting of 148 rows and 50 columns (Figure 4). We used labeling 0 and 1 to identify which diagnosis each disease type belongs to. Zero indicates that the disease is not in the diagnosis and 1 indicates that the disease is present in the diagnosis.

10 Imler, T., 2015, Natural Language Processing System Accurately measures colonoscopy quality,

https://www.healio.com/news/gastroenterology/20150311/natural-language-processingsystem-accurately-measures-colonoscopy-quality

EndoscopyReportdescription	Tanı
SEDASYON ANESTEZİ ALTINDA İŞ	AL POLİPOZİS
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	MİDE FUNDUSTA POLİPLER, 2 SİNDEN BX ALINDI
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT,BULBİT,HİATAL YETMEZLİK
Servikal, torakal ve abdominal öz	HİATAL HERNİ, PANGASTRİT, NATRAL POLİP
Servikal, torakal ve abdominal öz	ANTRAL GASTRİT, ANTRUM VE FUNDUSTA POLİPLER
Servikal, torakal ve abdominal öz	ANTRAL GASTRİT , ANTRUMDA ÜLSER , KORPUSTA POLİPOİD LEZYONLAR
Servikal, torakal ve abdominal öz	SALİM ANASTOMOZ HATTI
Servikal, torakal ve abdominal öz	EROZİV GASTRİT, HİYATAL YETMEZLİK, POSTBULBER POLİP
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	KORPUSTA MALİGN GÖRÜNÜMLÜ ÜLSER
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
Servikal, torakal ve abdominal öz	PANGASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	HİYATAL YETMEZLİK, PANGASTRİT
Servikal, torakal ve abdominal öz	ANASTOMOZ ÜLSERİ, HİATAL HERNİ
Servikal, torakal ve abdominal öz	FUNDAL GASTRİT, HİYATAL HERNİ
Servikal, torakal ve abdominal öz	HİATAL YETMEZLİK, ANTRAL GASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
Servikal, torakal ve abdominal öz	TANI YOK
SEDASYON ANESTEZİ ALTINDA İŞ	ANTRAL GASTRİT
SEDASYON ANESTEZİ ALTINDA İŞ	BULBİT,ANTRAL GASTRİT
SERVIKAL, TORAKAL VE ABDOMIN	ANTRAL GASTRİT
Servikal, torakal ve abdominal öz	ANTRAL GASTRİT
Servikal, torakal ve abdominal öz	ANTRAL GASTRİT

Figure 3: *First database (2 columns 148 rows)*

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	Endoscopy	AL POLİPO	ALKALEN F	HİATAL YE	ANASTOM	HİATAL HE	ANTRAL G	ANTRUMC	KORPUSTA	KARDIADA	LES GEVŞE	ANTRUM Y	ANTRUMC	GEVŞEK M	HİPOTONİ	HİYATAK H	POLİP	BULBİT	DUODENA	REFLÜ ÖZ	ANTRAL PI	ATROFİK G	BULBUSDI
2	SEDASYON	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	SEDASYON	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	SEDASYON	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	SEDASYON	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	SEDASYON	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
7	Servikal, tc	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Servikal, tc	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
9	Servikal, tc	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Servikal, tc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	Servikal, tc	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	SEDASYON	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	SEDASYON	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	SEDASYON	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	SEDASYON	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	Servikal, tc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	SEDASYON	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	SEDASYON	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Servikal, tc	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	Servikal, tc	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	Servikal, tc	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	SEDASYON	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	SEDASYON	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 4: Second database (148 columns 50 rows)

Database 3. Since the number of diagnoses of the disease was 50, diagnoses were classified as 7 prediagnoses in order to facilitate and accelerate the training and testing process. This procedure was performed by an experienced and educational endoscopist (Prof. Dr. Faik YAYLAK) during the submission of independent data from other researchers (Figure 5).

	EndoscopyReportdescription	POLİP	GASTRİT	HİATAL YETMEZLİK	ÜLSER	HİATAL HERNİ	DİĞER
0	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SERV...	1	0	0	0	0	0
1	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SER...	0	1	0	0	0	0
2	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SER...	0	1	0	0	0	0
3	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SER...	1	0	0	0	0	0
4	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SER...	0	1	1	0	0	1
...	...	...	...	...	...	...	...
143	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SER...	0	1	0	0	0	0
144	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SER...	0	1	0	0	0	0
145	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SER...	0	1	0	0	0	0
146	Servikal, torakal ve abdominal özofagus normal...	0	0	0	1	0	0
147	SEDASYON ANESTEZİ ALTINDA İŞLEME BAŞLANDI. SER...	0	1	1	0	0	0

148 rows × 7 columns

Figure 5: *Third database (50 types of diseases 7 types of patients)*

4- Methods and Tools

We used a range of python libraries (Anaconda) to work on the data, test, train and evaluate the results. for instance,

- Pandas/Numpy library: NumPy stands for "Numeric Python". It is an open-source Python module that provides mathematical computation on arrays and matrices. Pandas is an open-source library that provides high performance, easy-to-use structure, and data analysis tools for Python programming. [Anonymous, 2017][11].

- NLTK: NLTK stands for Natural Language Toolkit. This toolset is one of the most powerful NLP libraries with packages that enable machines to understand human language and respond accordingly. Tokenization, Stemming, Lemmatization, Punctuation, Character count, word count is some of these packages.

- Zemberek/Zeyrek: It is an open source, platform independent, general purpose Natural Language Processing library and a toolkit designed for Turkish languages, especially Turkish language. [Anonymous, 2020][12].

4.1. Preprocessing

One of the most important steps to improve data quality and extract useful data is preprocessing. We can clean and organize raw data through data preprocessing, then prepare them to build and train machine learning models.In NLP, also text preprocessing is the first step in a model building process. The data preprocessing flow diagram we performed in this study is shown in Figure 6.

11 Anonymous, 2017, https://cloudxlab.com/blog/numpy-pandas-introduction/
12 Anonymous, 2020, https://pypi.org/project/zemberek-python

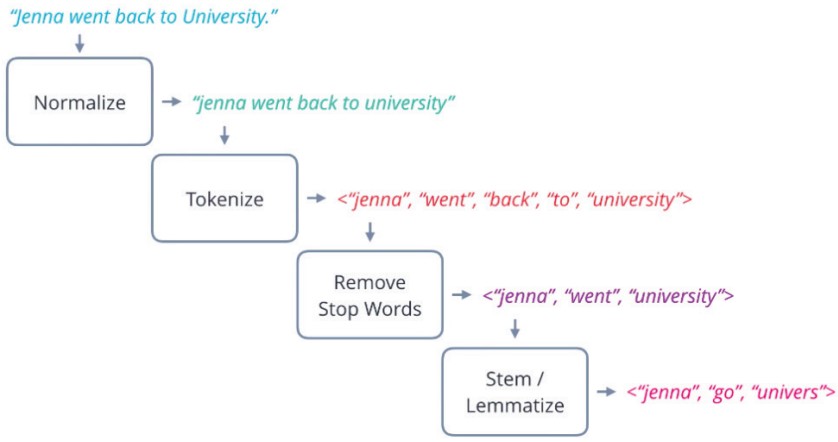

Figure 6: *Steps of Preprocessing*

Tokenization

Tokenization is basically breaking down a sentence, paragraph or an entire text document into smaller units such as individual words or terms. Each of these small units is called a token.

Lower casing

This task performs the process of converting a word to lowercase. Words like Book and book (in English) mean the same thing, but when converted to lowercase the two are represented as two different words in the vector space model.

Punctuation

It is the function of removing all punctuation marks from a sentence

Stop Words

At this stage, the stop words library is used to remove stop words from our dataset. When we used this library, we realized that this library does not contain all Turkish stop words. So to be able to use all the stop words we created a text file and added all the Turkish stop words into a Chart and then loaded that text file during the program.

Stemming and Lemmatization

Stemming and Lemmatization are Text Normalization techniques in natural language processing used to prepare text, words and documents for further processing. But in the process of finding the roots of English words, the root of the word is found directly as a word, but in the process

of finding the roots of words in Turkish, we encountered two or three roots. When we looked at the roots found in general, the first roots were more correct, so we took the first roots into consideration.

4.2. Modeling and Evaluation of Models

4.2.1. Modeling

We cannot work directly with text when using machine learning algorithms. Instead, we need to convert the text to numbers. Algorithms take number vectors as input, so we need to convert documents to fixed length number vectors. Several models are used to convert text to vector. for instance: Bag-of-Words Model (BoW), CountVectorizer, Tf-Idf Vectorizer, Hashing Vectorizer.

4.2.2. Model Evaluation

Training a model is a key step; How the model generalizes to unseen data is an equally important consideration in every machine learning phase. Model evaluation aims to predict the generalization accuracy of a model for future (unseen/out of sample) data. Methods of evaluating the performance of a model 2 divided into categories:

1- Holdout: The purpose of the holdout evaluation is to test a model on different data than it was trained.

2- Cross-validation: is a technique that involves splitting the original observation dataset into a training set used to train the model and an independent set used to evaluate the analysis.

4.2.3. Performance metrics for classification

The usual feature engineering is after making the selection and of course applying a model and getting some output in the form of a probability or class, the next step is to find out how effective the model based on some criteria is using the test datasets. Different performance metrics are used to evaluate different machine learning algorithms. such as Classification Accuracy, Confusion Matrix, Precision, AUC, F-Test. In this study, we used Tf-Idf Vectorizer method to convert our data to vector and Accuracy_Score, F1-Score, Precision _Score, and Hamming_loss concepts to evaluate our Model.

4.2.4.Training and Test

After converting the data from text to vector, we used the train-test split library to test and train our data. Train-test split is a quick and easy implementation procedure and its results allow us to compare the performance of machine learning algorithms for our predictive modeling problem. Since our dataset was limited in this study, we reserved 10% of

the dataset for testing and 90% for training. After separating the test and the training split ratio, we used three algorithms to train our dataset.

We used the Gaussian Naive Bayes algorithm to train the data in the first database (2 columns and 148 rows). Gaussian Naive Bayes is a variant of Naive Bayes that follows the Gaussian normal distribution and supports continuous data. As we mentioned earlier, we used 4 methods to evaluate our model. The results obtained from training on the first dataset are shown in Table 1.

Algoritma Adi	Accurac_Score	F1_Score	Precision_Score	Hamming_loss
Gaussian Naive Bayes Test: %10 Eğtim: %90	%30	%30.7	%32	%70

Table1: *Result from first type database*

We used the MultiNominal Naive Bayes algorithm to train the data in the second type of database (50 columns and 148 rows). Naive Bayes classifier is used in text classification, spam filtering and sentiment analysis. It has a higher success rate than other algorithms. The results obtained from training on the second dataset are shown in Table 2.

Algoritma Adi	Accurac_Score	F1-Score	Precision_Score	Hamming_loss
Multi Nominal Naive Bayes Test: %10 Eğtim: %90	%46	%50	%75	%2.4

Table2: *Result from second type database*

We used MultiNominal Naive Bayes and Logistic Regression algorithms to train data in the third type dataset (7 columns and 148 rows). Results from training on the third database Tables 3 and 4.

Algoritma Adi	Accurac_Score	F1_Score	Precision_Score	Hamming_loss
Multi Nominal Naive Bayes Test: %10 Eğtim: %90	%53.33	%71.79	%93.33	%12.22

Table 3: *Using MultiNominal Naive Bayes above the third type database and the results obtained*

Algoritma Adi	Accurac_Score	F1_Score	Precision_Score	Hamming_loss
Logistic Regression Test: %10 Eğtim: %90	%66.66	%81.08	%100	%7.77

Table 4: *Logistic Regression using on the third type database and the results obtained*

In this study, we used 3 types of datasets and three types of algorithms to train the data, and summarized all the results we obtained (Table 5).

Veritaban ve Algoritma	Accurac_Skor	F1_Skor	Precision_Skor	Hamming_kayıp
1.tip veri taban (Gaussian Naive Bayes)	30	30.7	32	70
2.tip Veri tabanı (Multi Nominal Naive Bayes)	46	50	75	2.4
3.tip veri tabanı (Multi Nominal Naive Bayes)	53.33	71.79	93.33	12.22
3.tip veri taban (Logistic Regression)	66.66	81.08	100	7.77

Table5: *Comparison of results from each algorithm applied to 3 types of databases*

5- Result and Discussion

Working with text data in machine learning falls within the realm of natural language processing (NLP), a field devoted to algorithms and methods for processing human languages for computers. In general, data must be in a structured form to build a machine learning model; that is, our data must have rows and columns. This will be a big problem when working with text data, as they come in an unstructured format, i.e., sentences, paragraphs, and words. As a result, a machine learning algorithm recognized We need to perform a large amount of preprocessing on the raw text data to convert it to a structure. This study is done showing how to analyze text data with NLP and how to extract features for a machine learning model. Regarding the available data types, we generally used tree types of classification algorithms to build our models. According to the preliminary research conducted with English texts during this thesis study, generally better results were found on English text data using the NLP toolkit. Since the language of our dataset is Turkish and the amount of data in the dataset is low, we could not get the near-perfect result we wanted. By creating three types of databases from our dataset, we were able to increase the accuracy from 30% (in the first dataset) to 46% (in

the second dataset) and finally 66.66% (in the third dataset). The fewer the number of pre-diagnosis types (disease type) of our disease, the better results we can get. We believe that by increasing the amount of data in the data set, the accuracy rates of training and test results will increase even more. Classification as the basis for evaluating the current study accuracy rate is used. Future studies will also focus on criteria such as classification speed and cost. In addition, it is planned to analyze with better performance enhancing methods by comparing them with natural language processing algorithm (BERT) or ALBERT techniques using the same parameters and values.

Chapter 8

GENETICALLY MODIFIED ORGANISMS AND EFFECTS ON HUMAN HEALTH

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1.INTRODUCTION

The use of biotechnological processes in human life has a long history. Biotechnological applications such as bread and wine making have held an important place in human life for ages. Biotechnology, which does not require the use of modern information and technologies and has been developed through trial and error throughout human history, is called traditional biotechnology (1).

In recent years, policies regarding genetically modified organisms have become one of the most hotly debated issues in both trade and environmental negotiations. Some; Concerned that unconvincing regulations, genetic engineering, and genetically modified organisms pose a fundamental threat to human health and the stability of ecosystems, others feel that they do not pose a threat to human development and that no limitations should be placed on sound science.

Again, some perceive free market initiatives for genetically modified organisms as a new mode of imperialism that seeks to impose its own environmental, cultural, ethical and social values, while others are of the view that restrictions on GMOs should not be imposed through protectionist measures. Due to conflicting fears, hopes, values and interests, it is thought that the debate and policies regarding GMOs have become overly emotional. It is clear that there is a need for a new, more constructive and collaborative approach that strives to reflect and respect the different interests and concerns on the subject (1). Regulations regarding genetically modified organisms (GMOs), foods produced from genetically modified organisms or containing genetically modified organisms; requires governments to fight more in areas such as health, food safety, environment, trade and ethics (1).

A significant portion of the public debate on genetically modified foods has focused on labeling policies for products derived from genetic modification processes (1).

Consumers have the right to make an informed choice among the goods and services offered to them. The information provided through the label is considered an important factor in consumer protection (1).

In addition, it is thought that health problems and environmental pollution will decrease as a result of the decrease in the use of pesticides such as herbicides and pesticides, with more use of biotechnology in agriculture (2-4). In addition to the potential benefits of GMO products, it is thought that there may be potential harms or risks that may adversely affect human health.

In this context, one of the most controversial issues is the transfer

of the gene encoding the allergic protein of a product to another product, which increases this feature of a food that is already known to be allergic, or the emergence of new allergic proteins. In a study conducted; A gene from Brazil nut, which is known to be allergenic, has been transferred to soy to enrich its nutritional content.

However, the protein synthesized by this gene turned out to be one of the allergic proteins in Brazil nut, and the development of this transgenic soybean was discontinued (2, 5, 6). Another controversial issue about GMOs is the use of resistant genes, which are used as marker genes, together with the original gene to be transferred to select organisms in which gene transfer is successful. For example, antibiotic-resistant genes are used for this purpose. However, it is thought that it will be difficult to control the infections that will occur if these genes are passed on to pathogenic microorganisms, and even if these genes used in the production of transgenic plants spread to nature, they will pose a great danger.

In addition, it has been stated that genetically modified foods can be toxic and have many effects such as immune system disorders and susceptibility to viral infections. Until recently, it was thought that DNA would be digested in our intestines, but recent studies have shown that foreign DNA that we take through food can be carried into our cells. It was determined that there were significant changes in white blood cell counts, kidney weights, and albumin/globulin ratios in rats fed pest-resistant corn (2, 7, 8).

In some studies, it has been emphasized that the pesticides to be used to get rid of weeds will cause many environmental problems such as soil pollution, and they also carry an important potential risk such as the malicious use of GMOs as bioterrorism agents (9). There is not much research on GMOs, which is a very new technology in the world. In some studies conducted in recent years, opinions of professional chambers have been included, but no comprehensive research has been done.

In the light of the above and in general, Modern Biotechnology allows to produce products and services in many fields. The production and consumption of these products and services for the benefit of all humanity; At the same time, minimizing the existing risks is only possible with a safe control mechanism, in other words, a Biosafety System.

2. GENETICALLY MODIFIED ORGANISMS

Although GM foods are among the current and controversial food products in the public opinion, it is thought that consumers have little knowledge about GM foods (14).

It can be thought that the fact that the concepts related to GMO foods

contain scientific and technical terms at a high level may have an effect on the formation of this situation.

2.1. Definition of GM, GMO and GMO Food

The idea of genetically modifying foods has only recently gained popularity, although it has been around for about 8,000 years (15). In this case, it is undoubtedly effective that these foods come to supermarkets where consumers can reach them, and that consumers are deprived of information about these products and acceptance of these foods (14).

The use of new technological methods such as genetic modification (GM) in the food industry has awakened consumers' interest in food production (16).

Genetic modification (GM) is a general term used for several specific techniques that make changes in the genetic material, i.e. DNA, of organisms. DNA contains the instructions that the organism uses to build and operate itself. By altering DNA, genetics can alter an organism's physical properties and functions (17).

Genetically Modified Organism (GMO), on the other hand, is defined as a plant, animal or microorganism whose specific characteristics have been modified by transferring genes from another species with the use of biotechnological methods (10).

According to the definition of the World Health Organization (WHO) (2009); "Genetically Modified Organisms (GMOs) are defined as organisms in which genetic material (DNA) has been modified in such a way that it does not occur/occur naturally.

Genetic modification requires the assembly of a gene of interest from a donor organism and affixing it to the recipient in order to display desired traits. Foods obtained from genetically modified plants or animals are called Genetically Modified Foods (GMO foods). GMO foods in another source; It is defined as "foods produced by gene technology that changes the DNA structure of foods of grain, vegetable or animal origin" (18).

Genetically modified organisms that involve the transfer of foreign DNA are also called transgenic. Genetic modification is a general term used to name many techniques for creating new organisms. However, many terms are also used to describe these new organisms.

Among these terms: genetically modified, genetically engineered, genetically manipulated, bioengineered and biotech (17) are used as the basis.

While traditional genetic modification (GM) occurs between organisms close to each other, genetic engineering (GE) allows transfer between any

two organisms (19). Although there is a fine distinction between them, it is seen that these terms are considered synonymous (20).

In this study, the two terms were considered synonymous. In addition, the terms transgenic and genetic alteration (GD) can be used synonymously (21).

2.2. GMO Products and Foods

It is seen that GMOs have wide usage areas and agriculture and biotechnology are the leading ones. GMOs; It is used in agriculture, food production, as well as medicine (pharmaceuticals, vaccines…), biomolecule production, and studies on industrial and environmental products are continuing (22).

GMOs are also used in chicken and cattle breeding; It is used to obtain vaccines, animal feeds, vitamins, amino acids and enzymes used in veterinary medicine (23).

Food products that are likely to be GMOs include; biscuits, crackers, puddings, vegetable oils, baby foods, candies, chocolate and wafers, instant soups, chicken and similar animals that consume corn and soy as feed are thought to be foods (24).

However, it is of commercial importance; Plants such as corn, soybean, canola, potato, rice, pumpkin (23), wheat (25), papaya, sugar beet, tomato are counted among GMO foods (23).

It is claimed that the following foods are among the food products in which GMOs can be used (26).

· Products made entirely from GMO corn itself: Corn oil, corn flakes, corn flour, corn chips, corn starch, canned corn.

· Products using starch-based sweeteners (glucose syrup, fructose syrup) obtained from GMO corn: Colas, sodas, fruit juices, cakes, biscuits, baby biscuits, pretzels, baby corn breakfast.

Products using GMO corn starch: Waffles, baby biscuits, regular biscuits, instant soups, broth tablets, mayonnaise, chocolate puddings, pasta sauce, ketchup.

· Products produced entirely from GMO Soybean itself: Soybean oil, soy meat minced meat, soy chips, soy flour, ready-made soy patties.

· Products using GMO soy flour: Waffles, biscuits.

· Products using soy lecithin obtained from GMO soy: Cakes, biscuits, wafers, baby biscuits, pretzels, puddings, chocolate cream, baby breakfast with milk cheese and molasses, follow-on milk, chocolate, margarine.

· Products using GMO soy protein: Ready-made hamburger patties, salami, sausage, sausage, chocolate.

· Products using GMO canola: Canola oil, margarine.

· Products using GMO cotton: Margarine, cottonseed oil obtained from GMO cotton and various oils.

2.3. Production of Genetically Modified Organisms

2.3.1. Gene transfer techniques in plants

2.3.1.1. "Shot-Gun" Method

The ballistic weapon method developed to bombard plant tissues with gene particles is used successfully in many plants.

After the newly transferred plants are grown by tissue culture, the gene or genes are determined to be functional for several generations and are transferred to the desired data by natural crossover. Thus, the plant carrying these new gene(s) is defined as a genetically modified (GM) plant (12).

2.3.1.2. *Agrobacterium tumefaciens* Transfection

It is the mechanism by which *Agrobacterium tumefaciens* is used as a mediator. This bacterium can leave its own tumor-forming genes to the genetic structure of plants through its plasmid. These genes began to be used as a manipulation tool after it was understood that they were functional in plants. By means of genetic engineering techniques, the tumor-forming genes of the bacteria are changed with the gene that is targeted to be in the transgenic living thing, and this gene is transferred to the plant cell by the bacterium (12).

2.3.1.3. Protoplast Transformation

It is a gene transfer mechanism in which viruses are used instead of bacteria as a mediator (12).

2.3.2. Gene transfer techniques in animals

2.3.2.1. DNA Microinjection

The method is defined as the direct transfer of foreign genetic material to the pronuclei of one-celled embryos.

2.3.2.2. Embryonic stem cell mediated gene transfer

It is defined as the transfer of stem cells that have been transferred by electroporation or transfection to another embryo at the morula or blastula stage.

2.3.2.3. Retrovirus-mediated gene transfer

It is a gene transfer method based on the principle of infecting early developmental embryos (8-16 cells) with recombinant retroviral vectors carrying the gene region to be transferred (13).

3. POSSIBLE EFFECTS OF GENETICALLY MODIFIED ORGANISMS

3.1. Effects on the Environment

Genetic improvements in plants may lead to new generations called super weeds. Just as genes for antibiotic resistance can theoretically pass from plants to bacteria, so can genes for pesticide or herbicide resistance to weeds. Because many plants, including squash, canola, and sunflower, are closely related to weeds, crosses sometimes occur that cause genes in one plant to mix with genes from another plant. But few experts predict any spike in genetically enhanced wild species. A large number of studies are needed to measure the full extent of this threat and to develop ways to minimize these risks.

The ecological potential harms of biotechnologically enhanced plants must be weighed against their clearly defined benefits. The first and foremost benefit is that it significantly reduces the use of chemical pesticides.

One of the key aspects of the environmental benefits of biotech plants is the reduction in crops, insecticide and herbicide applications. In countries where biotech crops are grown, pesticides used on four biotech crops – soybean, corn, cotton and canola – have decreased by £791 million per year. This means a 17.2% reduction with environmental impacts.

Forests of genetically modified trees can extract billions of tons of carbon from the atmosphere each year and reduce global warming, according to researchers at Lawrence Berkeley and Oak Ridge National Laboratories. These researchers argue that it is possible to genetically modify trees to expend more carbon in their roots. This change could greatly increase the amount of carbon the vegetation naturally takes up from the air. All things considered, biotechnology is unlikely to push us to the brink of ecological catastrophe. On the contrary, it really offers some solutions to environmental problems. The National Academy of Sciences has recently reported that biotechnologically enhanced plants pose no more environmental threat than conventional plants (83).

3.1.1. Effects on Biodiversity

Biotechnology has played a role in the protection and enrichment of biological diversity, which is the basis of agricultural sustainability in its historical development. Biotechnology has been used and continues to be

used in the protection of genetic resources of plants that are difficult or impossible to preserve with classical methods. In this way, biotechnology is an indispensable tool in terms of ensuring the continuity of plant genetic diversity, which is the insurance of sustainable agriculture, and creating new sources of diversity (40).

While the change in practices such as the use of herbicides and pesticides in agriculture is emphasized among the indirect effects, the following threat areas are emphasized as direct effects:

1. The possibility of harming beneficial organisms in the ecosystem through the food chain, during the cultivation of products carrying the gene developed to provide resistance to harmful substances and climatic conditions,

2. Changes in soil structure and nitrogen conversion, resulting in deterioration of the natural structure of ecosystems,

3. The mixing of newly developed -foreign-gens with wild plants existing in nature or with products grown by classical or organic farming method through "cross pollination", which is called "genetic pollution". Because mixing genes from organisms in foreign structures to an organism accustomed to living in a certain ecosystem is seen as contamination by these receptive organisms. Another risk that comes with this pollution is the emergence of the possibility of "genetic compatibility" in terms of organisms in nature and the decrease in biological diversity in parallel with this increase. Thus, biodiversity is essentially threatened by providing the core material of modern biotechnology on the one hand, and is at risk of degradation through genetic contamination, on the other. In this context, lethal effects of insecticidal gene transferred Bt corn pollen on the larvae of the Monarch butterfly, which is common in North America, were determined. Although these butterflies do not feed on maize, it has been stated that the pollen of Bt maize reaches the milkweed, which is the main food source of the butterfly, with lethal results and will lead to a decrease in biodiversity in the future.

4. Another risk that can be caused by all these possible negative effects is the negative effects on organic farming practices. Undoubtedly, the focus of these risks is the inability to eliminate most of them in case of potential negative effects (41).

Biodiversity is necessary for high agricultural productivity to be effective and to continue production without being dependent on a place. Turkey is a country rich in biodiversity. In terms of biodiversity, it has more than two-thirds of the biodiversity of all countries in Europe. Turkey's endemic species diversity is over 4000 (42). Our country is rich in farmer

varieties of cultivated plants as well as wild plants (40). One of the serious dangers in terms of the environment is that genetically modified plants may cause loss of genetic diversity in natural species after they are released into the environment, and the distribution and balance of species in the ecosystem may deteriorate, causing the wild species that make up genetic resources to deviate from natural evolution. In this respect, the gene resources of countries with rich genetic resources may be under threat (43).

3.1.2. Effects on Environmental Pollution

There are very few studies on the effects of GM crops on the soil ecosystem. However, current information indicates that there are risks related to the uncontrolled spread of genetic traits to the environment due to gene escape during hybrid generation. As a result of the emergence of resistant weeds and insects in the long term due to gene escape, in parallel with the increase in the use of pesticides, the possibility of the disappearance of biodiversity, especially in the soil, may arise. It is thought that the resistance properties of cultivated plants that have been made resistant to pesticides will pass to other organisms, and these plants will lose their genetic properties and their resistance will disappear in time, as well as harm the plant-soil cycle in ecological terms (44).

Researches and observations show that GM crops developed against agricultural pests and yield-restricting factors also cause damage to non-target organisms in the soil ecosystem (45, 46). In addition, it is stated that butterflies and other non-target organisms that feed on plants that are resistant to insects as a result of transferring the gene responsible for the production of a poisonous protein against certain insects obtained from Bt bacteria are at risk of poisoning (47, 48).

On the other hand, the ability of transgenic proteins to accumulate in the soil is an important factor that threatens the soil ecosystem. While the persistence of these proteins in the soil is affected by the physical and chemical properties of the soil, environmental factors also affect their bioavailability in the soil. For example, it has been stated that the persistence of Bt toxins is longer in soils with high clay composition and low pH. In this case, the role of transgenic proteins in changes in soil microflora and fauna, which is not yet known, will emerge in the long term (44).

Another issue to be investigated regarding GM plants is the use of glyphosinate-derived herbicides. Glyfosinate ammonium herbicide has an important place in the fight against weeds, especially in soybean and corn. This herbicide, as a result of transferring the pat gene to products such as soy and corn, only prevents the reproduction of weeds and provides great benefits to the farmer, especially in terms of labor and economy. However; This situation causes environmental pollution as it causes the intensive use

of herbicides by the farmers (49).

Although it is thought that GM plants will reduce the use of herbicides, pesticides and artificial fertilizers in the near future, it is thought that they may cause the emergence of resistant weeds and insects in the long run. (43). There is a possibility that the production with less variety in large areas may pose a risk for environmental pollution as a result of more machinery and therefore more oil (42).

3.1.2.1. Environmental Cleaning with Genetically Modified Organisms

Although bioremediation traditionally relies on the activation of microorganisms found in nature, many specific microbes are unable to decompose particularly highly toxic chemicals.

For example, some organic chemicals and resins that occur in the production of plastics are resistant to biodegradation and can remain in nature for centuries. In addition, many radioactive substances prevent biodegradation by killing microbes. So for some of the stubborn and especially toxic pollutants we may need genetically modified bacteria and plants.

3.2. Effects on Agriculture

3.2.1. Increasing Efficiency

In parallel with the increase in population in the world, ways to get higher yields from plants have begun to be scientifically researched. As a matter of fact, the increase in agricultural productivity achieved in the last 50 years has been achieved as a result of using modern breeding methods together with appropriate breeding techniques. Despite this, when it is considered that the world population is increasing day by day on the one hand and the areas used in agriculture are on the last limit on the other hand, it becomes clear that the increase in productivity should continue in the future. In fact, studies show that today's yield level is well below the potential yield (50).

Considering that the increase in biological yield that can be achieved with classical breeding methods is now at its limits, the use of gene transfer technology in plant and animal breeding studies seems inevitable (51, 52, 53).

Genetically modified plants can be used to increase crop yield and reduce crop loss by producing plants that are resistant to a variety of environmental factors such as insects, weeds, herbicides, viruses, salinity, pH, temperature, frost, drought and weather (51, 52, 53, 54) . Increase in global product production can be achieved by increasing efficiency and

reducing product loss. Important annual grain crops can be genetically modified to turn into perennial crops. Thus, less soil treatment (double plowing, etc.) reduces erosion and also yields crops throughout the year (54, 55). In addition, the drought resistance of genetically modified plants may reduce the use of water in agriculture, making these plants suitable for cultivation in some tropical and arid regions where water is insufficient.

Increasing the resilience of crops to other environmental stresses (eg, extreme pH, salt, insects, temperature, etc.) can help reuse cropland around the world that is currently unsuitable for crop production. Thus, pressures on non-recoverable natural resources such as rainforests can be reduced (54). Resilience to environmental stresses may be the result of complex interactions of many genes. Therefore, it may take time for plants to acquire these properties (56).

In animals, cloning leads largely to the production of livestock to meet the demand for protein products and meat. Milk production was increased in dairy cows given rSBH (recombinant bovine growth hormone), which was approved by the US Food and Drug Administration (US FDA) in 1993. Therefore, it is thought that these products can be produced in abundance in order to export these products to countries with insufficient meat and milk supply. genetically modified animals; They can be used for purposes such as lactose-free milk, low-fat milk, low-fat meat, special protein meat, special quality meat and milk production (54).

Researchers use gene transfer to increase the productivity of pets. By transferring the genes responsible for faster growth or slower growth rates, animals can be brought to market faster. Recently, producers have discovered that hens that come to market in less than 42 days from chick formation do not produce eggs. Although the goal of chickens raised for meat is not laying eggs, it is not possible to raise chickens without egg hatching.

It is debatable that chickens are produced so quickly. However, poverty and lack of food necessitate the discovery of cheaper agricultural methods. Each extra day means additional cost. Americans consume 10 billion chickens a year, and any reduction in the time it takes to produce these billions of chickens represents significant savings for the producer, and thus passed on to the consumer. These potential savings are fueling some recent research into transferring genes from fast-growing carnivorous hybrid chickens to eggs produced by laying hybrids. The eggs themselves can also be made healthy for human consumption by transgenics. Although eggs can be inexpensive, high-quality sources of protein, most people avoid them due to their high cholesterol content. By playing with the genes responsible for cholesterol production, healthier, lower cholesterol eggs

can be produced.

It is also the target of genetic developments in the dairy industry. Researchers use transgenics to increase milk production, enrich milk with protein and reduce fat content.

Another development. reduction of diseases in animals raised for food. During the foot-and-mouth disease epidemic in England in 2000, dairy and beef cattle, as well as sheep and goat herds, were destroyed. The extinction of entire herds has been devastating for farmers and the country's agricultural industry. Due to the suspicion of spreading foot and mouth disease in the USA, all sheep imported from England, whether they carry the disease or not, were confiscated and destroyed. Panicked for fear of future disease spreading, researchers hope to develop FMD-resistant animals by working on disease-preventing genes. Similar processes may one day help prevent cholera in pigs and Newcastle disease in chickens.

Researchers at the University of Guelph in Ontario, Canada, recently developed a transgenic pig called Enviropig, which has the phytase enzyme in its saliva. This pig is known to be environmentally friendly as it produces less phosphorus in urine and faeces than non-transgenic pigs. Phosphates are the main pollutants formed in pig farms. Phytase pig breaks down the phosphate in its food, resulting in a 30% reduction in the amount of phosphate waste.

3.2.2. Increasing the Adaptation of Plants to Environmental Conditions

As a result of changes that occur over generations, phenotypic changes occur that will better adapt to environmental and stress conditions. For example, those of different species of the same genus grown in cold regions are relatively shorter and flatter. Similarly, plants develop natural defense mechanisms such as thickening the cell wall, feathering, and forming a waxy layer on the outer surface, during the natural evolution process that occurs in regions where any pest is concentrated. Meanwhile, disease-resistant genotypes are also emerging. On the other hand, pests renew themselves in their natural evolutionary processes and continue their development in a way that overcomes the natural resistance mechanisms developed by plants. Disease factors are also developing new races in a way that will exceed the resistance genes. For this reason, some cultivars registered for resistance to a particular disease are broken by newly developing races of the same disease, sometimes within a few years (40).

Plants exposed to environmental stress develop strategies to protect themselves. Developing plants tolerant to extreme conditions using gene technology is still in its infancy. A higher tolerance to stress is achieved

by activating genes encoding enzymes that produce antioxidants. By transferring the Mangan-SOD gene to Nicotiana tabacum, ozone damage can be reduced 3-4 times.

Mannito-Hydrogenase provides drought resistance, and by transferring a gene to the plant for its formation, mannitol, a sugar alcohol, accumulates and this creates drought resistance in many plants. Genes that contribute to saturation of chloroplast membranes with lipids are appropriate to increase tolerance to cold (58). The 'antifreeze' gene found in a fish living in the poles has been transferred to plants such as tomatoes and strawberries, giving these plants resistance to cold (57).

In transgenic rice, for example, by overproducing the Glutamine-Syntatase enzyme, tolerance to salt has been increased. By transferring the methalotionein genes to tobacco, tolerance to cadmium in this plant has been increased. Tolerance to toxic mercury concentrations was achieved by transferring the mercury reductase gene from a mercury-resistant bacterium to Liriodendron tulipifera (58).

3.2.3. Increasing Insect and Weed Resistance

Most of the agricultural plants can be genetically modified to gain resistance against viruses, insects, weeds, herbicides, diseases and various environmental factors. For example, Bt insecticidal gene has been transferred to most of the crops such as potato, soybean and corn, resulting in insect resistant Bt plants. Although Bt protein is toxic to insects such as corn worms and potato beetles, it is not toxic to humans and is broken down by stomach acid (46). Giving plants the ability to produce this protein can eliminate the need for chemical insecticides, and thus damage to insects such as bees and predators, which are not the target of these insecticides, can be prevented (49).

In addition to insecticide resistance, some plants are genetically modified to make them resistant to herbicide applications (46). It is thought that increasing herbicide resistance may help reduce soil erosion and water loss and protect soil microfauna and microflora by ensuring that the soil where the plants grow is less or not treated (50, 51).

Today, GM plant production has increased so that plants can directly take up more nitrogen from the soil themselves. This, in turn, may be beneficial for the environment as it will reduce the need for chemical fertilizers that threaten the environment by evaporating or drifting into river mouths and causing water pollution (46).

GM plants or microorganisms can also be used for bioremediation as they ensure the removal of toxic wastes in the environment. Some researchers have reported promising use of mustard greens, lucerne, river

reeds, poplar trees and special weeds for cleaning up industrial, agricultural and oil production waste. In some cases, plants can decompose and render harmless the poisons transmitted to the environment (54).

With the production of transgenic cotton in China, the use of pesticides has decreased and positive developments have been observed in the health problems of the producers. It is also noted that drug residues are less mixed with drinking water (59).

3.3. Effects on Nutrients

3.3.1. Changes in Nutritional Value

The quality or nutrient level of GM foods that have been given new characteristics may differ compared to traditional species (60). Adequate data on gene-nutrient interaction and metabolism are not available. As a result of all these unknowns, nutrient imbalances and a decrease in biodiversity may result in a uniform diet. Transgenes transferred into food products can increase the level of some nutritional values and decrease the level of others. Horizontal gene transfer of recombinant DNA to humans and its human health consequences is an important issue (60). An example of a change or decrease in nutritional values comes from the study of Lappe and Bailey in 1999. It reports that the phytoestrogen concentration beneficial for heart health is less in GMO soy (60).

3.3.2. Enrichment of Nutrient Content

Increasing the quality and nutritional content of plants is thought to be one of the most important contributions of GM foods to human health. However, it is quite common to think that there may still be risks, since no definitive conclusion has yet been reached in this regard (50).

It is predicted that the rice variety (Golden Paddy) with high vitamin A and iron content, obtained by biotechnological studies carried out for the production of food with high nutritional value, can be used to eliminate the disorders that occur in rice-based nutrition. Since the countries where vitamin A deficiency is common are also countries where rice consumption is high, scientists have transferred genes to rice and ensured the synthesis of beta-carotene, the precursor of vitamin A. Through studies carried out in this direction, it is expected to produce sweet potatoes and rice with high protein content, canola with high vitamin A content, vegetables and fruits with high antioxidant content in the near future (50).

With gene transfer technology, protein quality – for example, the methionine and lysine content of the protein – can be increased, thereby increasing the essential amino acid content of the products (35). By increasing the carbohydrate content of GMOs, ketchup, tomato sauce, etc.

Intense content can be added to tomatoes to be used in food processing. With Russert Burbank potatoes with increased starch content produced by Monsanto Company, potato production with less oil absorption during frying, reduced cooking time and cost was achieved (35).

Lactose-free milk was obtained by genetic modification. In addition, decaffeinated coffee could be produced by inactivating the gene responsible for caffeine in the coffee plant. The iron content of rice was increased by transferring ferritin from soybean to rice. With recent studies, the calcium content of potatoes has been increased 3 times (52). These plants can be genetically modified to further increase the level of unsaturated fatty acids in vegetable oils such as canola, soybean, sunflower, and peanut (43). Kassava is an important food source in the diet of over 500 million people in many third world countries. In recent years, these plants have been genetically modified to produce cassava that is resistant to African cassava mosaic virus and general mosaic viruses and has high nutritional value (43). As an example of increasing the amount of nutrients, fish that secrete more growth hormone and increase meat production thanks to transgenic methods can be given (54).

3.3.3. Development of Organoleptic (Sensory) Characteristics

Flavr Savr tomatoes from Calgene Corporation are the first genetically modified crop to be approved by the US Food and Drug Administration (US FDA). These tomatoes are plants that have a long shelf life by delaying ripening, softening and rotting processes. Ripening and softening are largely dependent on ethylene production by fruit cells. By controlling the genes involved in ethylene production or, as a different strategy, by suppressing the enzyme polygalacturonase, an enzyme that disrupts the cell wall, by delaying pectin degradation, ripening in fruits and vegetables can be delayed. Slowing or delaying ripening can also be done on crops such as raspberries, strawberries, pineapple, and peaches. Extending the shelf life of the products facilitates transportation, storage and processing for the manufacturer and the seller, as well as providing the consumer with the opportunity to use the product for a long time without spoiling. The ability of products to withstand transportation and processing can also be beneficial for farmers and consumers in developing countries where cooling systems are unsafe and expensive and the transportation network is inadequate (43).

Another application area of agricultural biotechnology is the production of food enzymes. With this application, it is tried to obtain rennet, which will enable the production of 60% harder cheese (50).

3.4. Effects on Human Health

3.4.1. Potential Toxicity

Toxic effects are an important problem in terms of health risks. Bt is a gram (+) bacterium that lives in the soil. The Cry toxin it produces kills insects, and its spores and crystal proteins have been used as pesticides since the 1920s. Biotechnology, on the other hand, has transferred the gene encoding this poison to the plant and made the GMO corn or potato contain the poison that will kill the insects that eat it. Fares and El-Sayed, in their study in 1998, observed proliferation in the small intestine of mice fed potato containing the Bt toxin gene.

Ewen and Pusztai's studies also concluded that GMO potatoes caused thickening of the intestinal wall of mice. It is stated that this situation is not caused by the end product, but by the new proteins that are revealed during the genetic change process and formed during the genetic coding process. It has not been determined what caused the effect. It has been emphasized that a number of proteins and enzymes such as starch, sugar polymers, lectin, trypsin inhibitor and chemotrypsin inhibitor of the new generation potato have been changed and that the changed proteins of the new potato may be responsible for these toxic effects (61).

Malatesta et al., on the other hand, added a different dimension to the process with their ultrastructural, microscopic and immunohistochemical examinations. While there was no apparent problem in the liver and pancreas of the mice fed with GTS Soy, they found that the shape of the liver cell nucleus was distorted, the metabolic rate of the cell increased, and the pores of the cell nucleus increased, thanks to special dyes. They found that there was an increase in the number and size of the enzyme-containing packets in pancreatic cells, and that traffic in the cell accelerated in line with all these findings (62, 63, 64). Findings regarding toxic effects have also led to questions regarding the effects of these toxic structures in excretory organs. In the study of Seralini in 2007, it was stated that the excretion of phosphorus and sodium in the urine of the mice fed with GMO Corn (MON863) for 90 days decreased, the fat in the liver increased, in short, the two excretion organs showed signs of damage. Moreover, this effect occurs in a dose-dependent manner, that is, the result is more severe in those who consume more (65).

Between 1988-1989, 37 people died and 1000 became ill due to the phenomenon known as Eosinophilia-Myalgia Syndrome (EMS). Researchers cited dietary supplements containing L-Tryptophan as the cause of these events. Studies have shown that the fermentation process is modified by Bacillus spp. using recombinant DNA technology. shown to be carried out by microorganisms. Changes in processing conditions, particularly reduced filtration, have been cited as the source of the problem

rather than the incorporation of L-Tryptophan itself or GMOs into the process. Despite this, GMOs were largely blamed for this event, but no proof could be made in this direction (66).

Lectins, which are plant proteins, act as insecticide in plants, and adding lectins to GM plant products increases insect resistance. The negative effects of lectin-rich GM potatoes on rats highlighted the suspicious approach in these products, but this situation could not be based on any scientific facts. However, as a result of the evaluations, it was agreed that sufficient in vivo tests should be performed on lectin-added GM products offered for consumption by animals or humans (66).

Numerous studies have been conducted on the toxic effects of GM plants, and these studies are mostly conducted on animals. In 2008, EFSA had 39 scientists from different EU member countries prepare a report on the safety of GM plants and the food and feed obtained from them. In this report, a large number of individual studies on food consumption, blood chemistry, organ weights, and histopathological findings of rats and mice fed on herbicide and insect resistant GM maize, potatoes, rice, soy and tomatoes are reviewed. In the majority of these studies, significant clinical findings and abnormalities in organs and tissues were not found. Although side effects were observed in some cases, it was stated that more detailed studies should be done. In addition, there was no significant difference in sheep, pigs, chickens, cattle and fish fed GM plants compared to those fed non-GM plants (EFSA 2008). Similar results have been confirmed by studies conducted in recent years (67).

3.4.2. Antibiotic Resistance

The possibility of transferring the antibiotic resistance characteristic of GM foods to microorganisms found in the human gut under normal conditions through marker genes was emphasized, and it was thought that the development of resistance to therapeutic antibiotics would cause significant health problems if this situation occurs. However, this has not been proven experimentally (66). Antibiotic resistance genes used as marker genes during GMO production are mostly of bacterial origin. The transfer of these antibiotic resistance genes to human intestinal microflora or pathogenic microorganisms by the consumption of GMO products may lead to an increase in the level of antibiotic resistance in microorganisms, which is already a common phenomenon in nature (43).

Gene transfer frequency to plant cells is extremely low in Agrobacterium tumefaciens and direct gene transfer methods. In addition, the rate of obtaining new plants from cells with gene transfer is extremely low. For this reason, marker genes are used to select the cells from which gene transfer is made and the plantlets that develop from these cells (67).

In the process of genetic change, marker genes are used to distinguish and identify genetically modified cells from unmarked ones. For this purpose, antibiotic resistance genes are used as marker genes. There is a concern that GMOs containing these antibiotic-resistant genes may cause resistance problems to oral antibiotics. Moreover, these antibiotics, which are at risk of possible resistance, have serious clinical importance and value in terms of human and veterinary drugs. For example, kanamycin and neomycin, which develop resistance with the NptII gene, are included in the "highly important antimicrobials" classification by the World Health Organization. Especially kanamycin is the drug of choice in cases of tuberculosis with multidrug resistance, and it is one of the important weapons in the increasing global tuberculosis resistance (61).

There are concerns that genes providing resistance to this type of antibiotic may have allergic effects and that they may pass from cultivated plants to bacteria in our digestive system and become antibiotic resistant. However, while studies have not found any allergic or toxic effects of these genes, there has been no evidence that they can be transmitted to bacteria in our digestive system (61). Although the probability of gene transfer is low, the World Health Organization and the United Nations Food and Agriculture Organization recommend the use of biotechnology without the use of antibiotic resistance genes (68).

In order to alleviate the concerns, the use of antibiotic-resistant marker genes in GM plants developed in recent years has been abandoned, and herbicide resistance genes have become used (67).

3.4.3. Allergen Feature

It has been shown that several genetic modification proteins may be allergens in assessments of the allergy risks of GM foods. Some GM foods, which have been shown to carry a potential risk of allergy, have raised serious concerns and debates about GM foods in both the USA and European countries. Starlink corn has been one of the most cited examples of claims about the allergy risks of GM foods. A gene transferred from a soil bacterium, Bacillus thuringiensis, to Starlink maize encodes a Bt toxin called Cry9C in maize, increasing the maize's resistance to insects. Bt toxin, which can be found naturally in hundreds of forms, is known to be harmless to humans, and has even been used in sprays and powders for years. In the safety evaluation, it was determined that the gene source of Cry9C was not allergic, the amino acid sequence was not similar to known allergen amino acid sequences, and protein-specific IgE did not occur in individuals who were thought to develop allergic reactions to corn containing this protein; however, the protein was found to be resistant to digestion. Therefore, it has been suggested to limit the use of these maize

in animals and humans.

On the other hand, it has been suggested that Cry9C is present in a corncob in such a low amount (only 0.0129%) that it cannot cause an allergic reaction. Another well-known example is soybean, a gene transferred from Brazil nut to enrich its sulphurous amino acid content. It has been determined that 2S albumin protein increases the allergenicity of soybean together with its sulfur amino acid content (69). It has been reported that no difference was found in the pathological examinations of the immune system organs, liver and kidneys, in rats fed with modified potato.

In a 2008 study, experimental mice were fed transgenic corn and soy diets for 30 and 90 days. Changes were seen in T and B type cells in these mice (70). In addition, the detection of a five-fold increase in the incidence of anaphylactic shock caused by food allergies in the last 10 years, when the production of GM foods has also increased, partially supported the suspicions about the allergy risks of GM foods (69).

Based on the results of the studies on this subject, the US FDA in 1992 and the Food and Agriculture Organization / World Health Organization (FAO / WHO) in 1996 stated that the probability of GM foods to cause allergic reactions in humans is not more likely than the products obtained by traditional methods to cause allergic reactions.

The ENTRANSFOOD Project, which lasted 3.5 years and was carried out with an expenditure of 11.5 million Euros, with the participation of 65 scientists from 13 EU member countries, in order to eliminate the intense public concerns that have arisen in the EU countries in the recent period, has been carried out with the aim of eliminating the GM products that are still being produced and consumed from the products produced by traditional methods in terms of human health. proved to be no more dangerous. In addition to the positive results of laboratory and clinical studies, no cases have been reported showing that GM food consumption negatively affects human health in the period from the 1990s when the first GM foods were produced to the present. These data show that the genetic modification method, which has the same possible allergy risk with other production methods, will not cause harm that threatens human health, but it is necessary to evaluate the production processes of GM foods and possible allergy risks with appropriate evaluation processes (69).

In addition to all that is known about the possible allergy risk of genetic modification, it has been suggested that this method can also be used to eliminate the allergenic properties of allergic foods. By using genetic modification methods, processes such as post-transcriptional gene truncation, changing the secondary and tertiary structures of the allergen,

or changing the primary amino acid sequence of the allergen are possible and thus the allergic protein in the allergen food can be inactivated (69).

3.4.4. Other Effects

The use of foods for vaccination purposes, many people die or become disabled due to preventable health problems around the world. Vaccination is the most effective method in preventing many of these diseases. Vaccines are expensive, the way they are administered, the need for trained personnel to administer them, the difficulty of transporting and storing them, and the socio-cultural structure of people. Many people cannot access the vaccine. Plants that synthesize various proteins of pathogenic microorganisms are obtained by means of genes that will be transferred to ordinary plants that we consume, and these plants are tried to be used as vaccines. The most important advantage of this method is that the vaccine can be taken orally. In this way, it facilitates transportation and contributes to the provision of mucosal immunity in the body (72). For example, with the gene transferred to potato, the immunological response against Hepatitis-B was increased by 60% (71). For this purpose, studies are continuing on bananas, tobacco and lettuce (72).

In the health sector, an important part of all insulin, test kits, cancer drugs, vaccines and antibiotics are produced by modern biotechnological methods, that is, from GMO organisms (66). Genetically modified animals can be used to produce large amounts of recombinant proteins such as fibrinogen in mammary milk. Transgenic proteins can be used as an alternative to blood proteins derived from human donor blood, which is feared as a potential source of HIV or mad cow (43).

Because cloned animals are models for most human diseases, scientists can effectively study human diseases such as cystic fibrosis, for which there is still no cure. Genetically modified animals can be used to produce coagulation factor pharmacological proteins used by patients with hemophilia (43). Some farm animals such as goats, sheep and pigs can be cloned and transplanted into human heart, liver, kidney and fetal cells, etc., which are suitable for transplantation. can be used to improve. An important cause of tissue rejection is the immune reaction of the carbohydrate α-1,3-galactose, which is absent in human cells but on the surface of porcine cells. Removal of the α - 1,3-galactosyl transferase gene using “knock out” technology can produce animals that do not carry this carbohydrate on their cell surfaces. Thus, long waiting periods for organ transplantation to patients can be eliminated (43).

Consumers also have real “unknown fears” that lethal microorganisms or superplants may be released during field trials and field tests, and that accidents in biotechnology laboratories may lead to the release of toxic

agents, poisons or biological toxins that threaten human and animal populations (73). At the same time, there are fears about cancer, which is the disease of our age, and it is thought that GMO products will trigger the formation of cancer. Research on cancer and GMOs continues. However, a definite conclusion has not been reached yet.

3.5. Effects on the Economy

Since 1996, when GM crops began to be widely cultivated, their spread over a wide geography and the continuous increase in cultivation area are interpreted as successful in terms of agricultural business. This shows that GMOs, at least, easily find a place in agricultural practice. Considering that the main purpose of use is to fight against weeds and pests, the success in this fight should provide an economic contribution. The theoretical calculations and experimental results revealed that GMO products significantly reduce the use of pesticides and herbicides, and therefore provide an economic advantage. Despite intense discussions about the methods and numbers used, the fact that many scientific reports and macroeconomic indicators claim to confirm this economic advantage has facilitated the adoption of GE plants by many countries.

This economic return is generally associated with an increase in the quantity of crop harvested and a decrease in financial input (74-75). However, recent studies show that the use of drugs in the production of transgenic products has increased. As a matter of fact, despite the 17% increase in soybean cultivation area in Argentina in 1999 (from 7 million hectares to 8.2 million hectares), pesticide use has doubled. The increase in the use of pesticides cannot be explained by the increase in soybean cultivation area and it is stated that the yield decreased in the same period. The result of 8 000 field trials established by American universities is quite striking, as "3-5 times more glyphosate is used in transgenic soybeans than in non-transgenic soybeans" (76).

Global market size of GMO seeds was 10.5 billion USD in 2009. The commercial market size of GMO products such as corn, soybean and cotton in the world was around 130 billion USD in 2008 and it is estimated to grow between 10-15% on an annual basis (24). The USA and Argentina, which are in the first two places in the list of countries where GMOs are most widely cultivated, have allowed the cultivation of GM crops since 1996. The USA has provided a significant financial return thanks to the GE crops covering an area of approximately 64 million hectares (77, 75).

The 11-year contribution of GE crops to the US economy, including 2006, has been calculated to be approximately 16 billion dollars. Unlike the USA, Argentina, which did not have the opportunity to produce agricultural biotechnology at the beginning, started the legal regulations

and practical studies on GMOs very early and allowed the cultivation of GMOs simultaneously with the USA. The 11-year economic return of Argentina, which still cultivates 21% of the total GM crops planted in the world, has been calculated as approximately 6.6 billion dollars. In addition, agricultural biotechnological research has started within the country and local seed companies have started to appear. Argentina, which produced approximately 26 million tons of grain towards the end of the 1980s, started to produce 75 million tons of grain in 2002. Even if this increase is not entirely due to GM crops, GMOs have a large share in this increase (77, 75).

One of the biggest economic drawbacks of GM products is that the patent rights of these products are in the hands of several multinational companies all over the world. Today, GMOs have been evaluated within the scope of patents, both technically and as a product, especially by emphasizing their technique. Since it is very difficult to find and identify the gene and requires large investments, a patent can be obtained on the condition that it demonstrates its function according to the European Patent Convention. When the farmer planting the patented GM seed reused the seeds left in his hands after the harvest, he had to pay a price to the patent owner, so it was forbidden to store the patented seed. Some farmers who hid the seeds were sued by the patent owner companies; To avoid court proceedings, they burned their products, paid compensation to the manufacturer and opened their bank accounts for scrutiny. For this reason, the seeds sterilized by many farmers, called “terminator”, have to be taken every year and they are dependent on multinational seed producer companies. This situation causes harm especially to small farmers and the prevention of traditional agriculture (78). 90% or 13 million of the 14 million producers using GMO products are small and poor producers. These producers already benefit from GMO products such as Bt cotton, and this benefit is expected to increase with products such as GMO rice in the near future (77).

The spread of GMO products can also increase the cost of traditional agricultural products. Farmers incur extra costs to make sure the seed they use is non-GMO. Due to gene escape, it becomes difficult to protect the products grown in the region from GMO contamination. As a matter of fact, although GMO flax is not grown in Canada, due to contamination, farmers have their flax seeds tested (for $100) to make sure they are non-GMO. If classical and transgenic varieties are planted together in the same region, the possibility of mixing with each other due to pollen will increase. In this case, it will be impossible for the producers to produce the kind of product they want. As a matter of fact, upon the determination of transgenic gene transmission in “Terra Prima”, an important organic corn variety in the

USA, the entire product was destroyed.

In this context, it is among the discussed risks that domestic varieties may decrease over time due to the restriction of agricultural production preferences of domestic producers in the case of the production of modified products in countries that are buyers of GMO products and gene technology (76).

4. DISCUSSION AND CONCLUSION

It is inevitable to use gene technology in line with people's needs and benefits. With this technology, it is expected that great progress will be made in terms of economic, health, social and ecological aspects. However, in the face of this technology, organic farming activities are tried to be popularized at the last speed, producers are encouraged to produce organic agricultural products.

The implementation of the rules of labeling GMO products within the framework of harmonization with the European Union (EU) will have an important place in domestic and foreign trade. Thus, the right of consumers to choose products will be protected. Determining the GMO threshold value for each product determined by the regulation that entered into force on September 26, 2010 is an important development. However, it will be difficult to check whether this threshold value is met. While the new regulation states that there should be no GMO products in baby foods, the import of 25 GMO products, especially sugar beet, soybean, potato, rice and corn, is allowed. In particular, GMO sugar is used in candies and chocolates that children often eat. This situation reveals once again the importance of supervision.

Before transgenic products are imported, they must be tested in well-equipped laboratories. How can we say that a genetically modified creature, although it may seem beneficial for humans, will not cause negative consequences in the future, will not interact with the environment or disrupt the balance of the environment in nature.

For transgenic crops, it is not correct to say that "we changed only one gene and gave some characteristics". Because with the addition of a gene, a new protein is added to the environment. This protein not only does its job, it can also affect other proteins and other enzymes of metabolism, either changing their concentration or somehow preventing them from working properly. In some cases, it may even cause unexpected metabolic intermediates to occur. It is thought that these intermediates may trigger many metabolic diseases and cancers, as well as have cytotoxic effects.

Another important issue is the possible effects of modified plants on the ecosystem and overall biodiversity. It is necessary to determine the

effect of the genetically modified plant on the environment in which it is released. As we focus on cultivated crops, the expected good effects will not only be to increase the cultivation and production of GM crops, but also to reduce the damage caused by potential pests and diseases.

Roundup-Ready soy is an example. This soybean variety has been genetically modified for resistance to the Roundup herbicide. Thus, the farmers will be able to spray the field without harming the soybean and kill the harmful weeds that prevent the development of their crops.

Another example is Bt corn. This GM plant has been genetically modified to kill the bacterium Bacillus thuringiensis, a toxin that kills corn-feeding cockroaches and other insects. However, some studies show that Bt plants can have toxic effects on monarch butterflies, even though they are not target insects and do not feed on corn. Therefore, it is important to know whether the toxin is effective on insects belonging to a certain species or a wider group and whether non-target insects will be affected. Another question to consider here is whether the toxin can spread or be limited to corn only. Because maize is pollinated foreign and its pollen is carried to other plants by the wind, these plants at long distances can also have a toxic effect on some insects. A study of monarch butterflies showed that corn pollen can be carried by wind to milkweed plants (a food source for monarch butterflies) in a field next to a GM corn field. In this case, monarch butterflies feeding on silk grass can also inhale this pollen (and toxin).

Since the inception of transgenic plants, people have been concerned about their potential harmful effects on humans and the environment. Where natural is often equated with the term trustworthy, unnatural plants undoubtedly carry an air of danger. In 2000, potato processing companies in the northwest stopped buying genetically modified potatoes, amid intense protest from activists. There was no indication that these potatoes, engineered to resist pesticides, were of poor quality or dangerous.

They looked and tasted just like non-genetically modified potatoes, and farmers also didn't need to use barrels of chemicals to grow them. These potatoes were resistant to aphids and potato beetles, but not to public opinion.

Today, most scientists agree that genetically modified foods will not cause common allergic reactions. Few proteins have the potential to cause allergic reactions, many of which are already well known to scientists, according to a recent report from the American Medical Association.

It's very unlikely that an unknown allergen could sneak up on a genetically modified food on the grocery shelves. Indeed, biotechnology

may one day help prevent allergy-related deaths. Researchers are currently trying to produce peanuts that do not contain proteins that can cause severe allergic reactions.

In addition, heavy metals such as copper, lead, cadmium, chromium and mercury cause serious damage to human health and natural life. Mercury is used in the manufacture of batteries, electrical switches, medical equipment and many other products. Mercury and a related compound methyl-mercury (MeHg) can accumulate in organisms through a process called bioaccumulation. Many of the mercury removal methods in use today cannot remove mercury from wetlands at sufficient levels and to acceptable standards, because even very low doses of mercury are toxic. As a result of their research, scientists have developed strains of GD E.coli that can be useful in removing mercury and other heavy metals. Some genetically modified bacteria can directly assimilate mercury, while others can grow them into biofilms whose mechanism of action is like a sponge and can absorb mercury from water. Similarly, single-celled algae and cyanobacteria containing GM metallothionein also show promise in the assimilation of cadmium, another very dangerous metal that causes serious health problems in humans.

In fact, this is what should be discussed and experimental studies should be done on it.

If we think that there are thousands of genes in a living thing, we understand that there are thousands of gene products. We should not forget that it is necessary to know what the concentrations of these products are and how these concentrations change with the effect of an additional gene should be put on the agenda. Our scientists should establish libraries by determining the gene maps of Turkey's plants and animals. Whether the structure of any animal or plant has changed should be determined by comparing it with the original gene maps. Genetically modified organism must be specified.

Turkey, which is an agriculture and livestock country, should not compete in the production and use of genetically modified organisms, even in the export of these products, due to the fact that there are many points that are waiting to be clarified on GMOs, on the contrary, it preserves very important natural gene pools in both plant products and animal products. Both domestic and foreign consumption and export should be given importance.

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Chapter 9

ATTACHMENT SYSTEMS USED IN IMPLANT SUPPORTED OVERDENTURE PROSTHESES

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Introduction

Total edentulism is a condition in which the patient has lost all of their natural teeth, often seen in patients aged 65 and over. Today, it is aimed to regain the lost function, phonation and aesthetics of the patient with the use of removable, implant-supported removable and implant-supported fixed prostheses in case of total edentulism. The factors affecting the treatments applied to eliminate total edentulism also determine the path that the physician should follow in treatment planning. The planning of prosthetic treatment is determined based on the patient's habits, systemic condition, physiological and mechanical structure of the oral environment, and quality of life satisfaction. The condition of the existing prostheses and their effects on the patient also play an important role in planning.

In time, undesirable results may develop in case of total edentulism or in the oral environment or systemic condition of the patient with the prosthesis usage period. The physician's goal is to predict and treat these complications and problems. The response of each patient to different prosthetic treatments may vary. Patients using removable total dentures may apply to the physician with complaints such as losing the stability of the prosthesis, the support of the oral surrounding tissues and retention, social and psychological concerns, and loss of nutritional value in dietary habits. Patients using fixed prosthesis can apply to the physician because of hygiene of the prosthesis, implant continuity, retention, stability and material continuity problems experienced in the attachment systems used.

The purpose of the physician should be to plan the application principles of the most appropriate prosthetic systems socially, economically, physically, systemically and psychologically for the patient. In this context, the use of overdenture prosthesis on implants has become widespread in order to maintain the stability and retention of the prosthesis.

In implant-supported overdenture prostheses, the connection between the implant and the prosthesis is provided by an attachment system with a precision attachment structure, and its purpose is to connect the removable prosthesis with the implant using mechanical or magnetic systems, and to be a tool for the fixation, stabilization and retention of the prosthesis.[1,2] The structure, shape, retention and flexibility of attachment systems vary. The attachments allow some movement of the prosthesis so that the forces could be transmitted to the edentulous crest with the implants. In order to ensure retention in implant supported overdenture prostheses, ball, bar, magnet, telescope, locator or ERA attachment systems can be used.[1]

While these precision attachment types can be used alone depending on the situation of the case, they can also be used in combination with each other. Dentists generally prefer bar or stud attachments in implant

supported overdenture prosthesis cases. Bar attachments can be used alone or in combination with stud attachments. These type attachments have a wide range of use in implant-supported overdenture cases, as they allow both flexible and rigid design. Alveolar bone, activity of muscle connections, retention, effectiveness of chewing force, and patient-related factors should be considered in the selection of the precision attachment to be used. In addition to the retention and stability properties of the preferred attachment system, its effect on stress distribution should also be considered.[3]

The factors to be considered in the selection of the attachment system in implants that support full dentures are important for the dentist and it is an issue that requires careful decision. Oral hygiene habits of the patient, expectations from the treatment, amount and shape of alveolar crests, interocclusal distance, the distance between implants, the amount of residual bone, the shape of the edentulous arch and the distance between the arches, and the patient's economic situation should be considered.[1,3] The personal preference and clinical experience of the dentist, as well as the technical knowledge and experience of the technician, should be considered together in the selection of the most appropriate attachment system.[1]

Classification of Attachment Systems Used for Implant Supported Complete Dentures

1. Ball Attachments

Ball attachments are a highly preferred attachment type because they are an economic system, do not require much processing in laboratory stages, and provide stability and retention that meet the satisfaction of the patient.[4]

The ball attachment system is seen as a successful system for splinted and non-splinted implants, providing better anchorage and retention, and reducing the load on the implants, as well as a corrective and successful system in its application to non-parallel implants. It consists of a metal or nylon female part and a male part in the form of a knob in the metal occlusal part of the abutment with a certain anchor, which is attached to the implant with this rubber.[1,2]

Ball attachments provide hinge and rotation flexibility. They are specially designed not to wear out the abutment, and each design affects the amount of rotation differently. The diameters of ball-head abutments are different for each company and vary between 2-3 mm. If retention is lost after the application, the retention of the plastic retainers can be increased with the activation appliance. When retention is high, it is also

possible to reduce retention with a deactivation appliance.1 The activation appliance increases the retention by bending the ends of the plastic holders in; the deactivation appliance reduces retention by pushing the ends of the holders outward.[1]

Implant-supported overdenture prostheses supported by a ball attachment system show high implant and prosthesis success and patient satisfaction in the short and medium term, and low number of mechanical and biological complications are encountered. It provides better retention and stability.[5] The low cost of the ball attachment system, having different retention degrees, easy oral hygiene continuity around the implant, no time loss and cost of making the prosthesis in a complicated way, and easy change of materials when necessary are advantages in the use of implant-supported overdenture prosthesis.[1,6]

However, there are some disadvantages of ball attachments. In the long term, as a result of the loss of retention in the female part during the usage period, it is necessary to replace these parts at certain intervals. They are not preferred as upper jaw implant attachments, they occupy too much space in the prosthesis in patients with limited interocclusal distance and the knob violates the vertical space.[1,4,6] If there is gingival hyperplasia around the attachment system because of plaque formation and inadequate oral hygiene, loss of retention may cause complications in the use of ball attachment system in cases where the implants are not parallel or are positioned at an angle of more than 15 degrees.[5,6] It has been determined that the main complications in prostheses in which ball attachment systems are applied, occur when the implants are inclined more than 6 degrees towards the lingual or buccal direction rather than the distance between the implants or the parallelism between the implants.[1]

In the presence of ball attachments, implants should be placed in parallel, thereby ensuring retention and reducing matrix wear. When parallelism cannot be achieved, the use of angled abutment or bar attachment systems should be preferred as an alternative.[7]

2. Bar Attachments

In the use of bar attachment systems, the implants are connected to each other with a bar and act as an anchor for the retention of the prosthesis. It provides a new entry way to the prosthesis regardless of the implant angles when the implants are non-parallel to each other. In bar attachment systems, the mechanism is adapted to the bar system on the implants with the help of a clip attached to the prosthesis.2,6 Retaining elements or clips can be changed and reactivated.[2]

In order to use a bar as a attachment system in implant-supported full

dentures in the upper jaw, at least 4 implants must be attached to each other with a bar.1 For the use of a bar as an attachment system in implant-supported full dentures in the lower jaw, it is sufficient to have 2 implants.1 However, in cases where the patient has a narrow mandible or implants are placed beyond canine region, there will not be sufficient distance for the tongue. In these cases, bar attachment systems with 3 or 4 implants should be preferred and the necessary distance for the tongue should be provided.[6] The vertical restorative space for the application of the bar holder system should be at least 12 mm according to Warreth et al. (2015), and it should be at least 15 mm according to Emami et al. (2014).[6,8]

The use of bar attachment systems is the most ideal choice for patients who need maximum retention. The distance between implants plays a critical role in the selection of attachment system. It is suggested that in cases where the length of the bar between the two implants is short, it may cause the clip to separate from the denture base. In the use of one bar positioned on two implants, the required gap for stabilization and retention is between 20 mm and 22 mm. The shortness of the bar leads to inability to provide stabilization and retention, and the long bar may cause bending, twisting, and thus incompatibilities and fractures due to the prosthesis putting a load on the bar.[7]

When four implants are used in bar prostheses made in the upper jaw, cantilever extension may or may not be performed on the bars. However, if cantilevering is to be performed, it has been reported in studies that distal cantilever extensions have a positive effect on the stability of the prosthesis in cases where it is possible to place the implants more mesially than the first premolar region. The bar should not extend more distally than the first premolars and should not be short enough to cover a small area only in the anterior region.[1]

Bar attachment systems vary in different designs and functions; Ackerman bar, Dolder bar and Hader bar. Ackerman bar has a round cross-section, is flexible, and reduces the horizontal and transverse forces on the implants. Dolder bar is indicated as U or Ovoid shaped. The U-shaped bar is rigid and suitable where four implant supports are available. It can be used in Kennedy class 3 partially edentulous cases. The egg section bar is advantageous in terms of both flexibility and indirect retention.[1,2]

Hader bar is a semi-flexible attachment type that allows hinge movement in the appearance of a keyhole. It can tolerate slight non-parallel situations.[1,2]

The shape of the dental arch is important in the selection of the system to be applied and the dentist should make pre-treatment planning by evaluating this. It is not recommended to make 2 implants in individuals

with narrow and V-shaped dental arch because the bar attachment system in these patients reduces the lingual area and disturbs the tongue, affecting function and phonation.[7] When the bar attachment is positioned labially, it overlaps the lower lip, affects the stability of the prosthesis and impairs aesthetics. In this case, the use of 3 fixed implants is recommended. In the presence of sufficient bone and a U shaped dental arch, 3 bars can be applied to 4 implants. If the dental arch is narrow and U-shaped or V-shaped, the distance between the implants should be in length that will not disrupt the harmony of the bar and clip.[7]

Bar attachments are used in maxillary prostheses and oval crests. They are indicated in prostheses where high retention and stability are desired in cases like excessively resorbed crest in the lower jaw, in the presence of partial resection of bone and/or soft tissue 1.

It has been stated that the use of bar attachment is also indicated in cases where the implant length is less than 10 mm.[2] In non-resorbable crests where the interocclusal distance is insufficient, in cases where the distance between the implants is long and the stress that will occur on the bone is high, in cases where oral hygiene is not enough bar attachments are contraindicated.[1,7]

In the upper jaws, where there is poor bone quality, the implant exhibits an angled exit profile, the presence of a short implant used due to proximity to the sinus the use of bar attachments improves retention and stability.[2] and so less bone resorption and screw loosening occure. Besides these advantages, it is not economical, requires a large prosthetic space, activation of deformed clips is needed over time and the production stages are complex.[2,3,4] The most common complications are abutment screw loosening, development of mucositis under the bar due to inadequate oral hygiene and prosthesis fractures or cracking.[2,3,6]

3. Telescopic Attachments

Telescopic-retained restorations are widely used in prosthetic dentistry because of their versatility and long-term success.[1] Telescopic attachment is a unit consisting of a primary structure called inner coping, which is screwed to the implant, and an outer crown attached to the base of the removable prosthesis.[2] Low inclination differences of implants are eliminated by the contours of the primary structure of the telescopic attachments. The telescopic approach has nowadays gained importance in supported removable prostheses. In non-parallel implants contours can be modified to provide a suitable access route to the prosthesis.

In telescope-retained prostheses, the screw gaps of the abutment are only on the primers and so the formation of screw holes on the outer structure is prevented.[1]

Retention is achieved by friction between the primary and secondary structures. Over time the retention of the telescopic attachments increases with increasing cohesion. This is an important advantage of telescopic attachments. The disadvantage of this system is telescopic with the use of a telescopic attachment, most of the chewing forces are accumulated in the implant rather than the alveolar crest, therefore, implant fractures and failures can be observed.[6]

In the study conducted by Heckmann et al.[9], it has been reported that at the end of the period of 10 years usage of implant-supported prostheses in which 2 implants are placed in the interforaminal region and telescopic attachments are used, clinical and radiographic results were positive.

4. Magnetic Attachments

Magnetic attachments are utilized in prosthetic dentistry practice in overdentures, maxillofacial prostheses, full or partial dentures, implant-supported prostheses, and in cases where the implants are planned independently of each other.[1,4] They provide an alternative retention mechanism for implant-supported overdenture prostheses and are preferred for application comfort and simplicity for patients. Magnetic attachments have two components: the magnetic part inside the prosthesis and the abutment or the part made of metal that is attracted by the magnet on the implant.[1,2] The abutment is attached to the implant, and the magnet is placed in the prosthesis.[6] Magnet-retained implant-supported prostheses can be applied independently of the prosthesis entry way. Due to these features, magnetic attachment systems can be used alone or they can be used in combination with other systems.[1] Tokuhisa et al.[10] stated that the transmission of lateral force sharing to the natural tooth or implant in magnet-retained prostheses is very low due to the feature of the retaining system that allows the prosthesis to move in all directions.[1,10] When compared with ball and bar attachments, retention and patient satisfaction values of magnetic attachment systems were found to be lower.1 It is stated that patient satisfaction is very high in the first 1 year in patients who use magnetic attachment systems, however, with the corrosion and wear that occurs over time, the retention begins to decrease and patients' complaints increase. They can be placed directly in the mouth in the clinic and indirectly in the laboratory.[1]

Although the implants are not parallel, the magnetic systems work quite naturally, they can be placed and removed easily.6 The use of magnetic attachments, especially in patients with physical limitations due to various reasons such as elderly, limited tolerance, stroke, Parkinson's disease, or in cases where it is not possible for the person to put on and remove their prosthesis alone while it is preferred to be used in patients with bruxism.[1,2,4,6]

Magnets are generally in a corrosion-resistant capsule, so that it is intended to protect them from the effect of corrosion that will weaken the magnetic connection.6 However, this system is not reliable enough, and it is an attachment system that is weakly connected and can easily rust and weaken due to saliva.[2,4,6,7]

5. Locator Attachments

Locator attachment systems are used in situations where the implants are parallel or have an angle of up to 40 degrees if retention is not affected. Splinting of the implants is not required in this system.[4,6] They are universal flexible retainers and enable the prosthesis to move in all directions.[6] Locator attachments do not require a large prosthesis gap, allowing rotational movement of the prosthesis and they are attached to the base of the prosthesis with acrylic resin.[2] The system has parts with different colors and double-sided retention and can be selected to suit different vertical heights.[4] A male part consisting of a metallic abutment that can be screwed to the implant and a female part with a unit consisting of metallic caps placed in different colors of nylon depending on its retention capacity. Patient satisfaction may be adversely affected when there are cracks or breaks, the separation of the holders from the prosthesis, cracks or breaks in the prosthesis.1 In such cases, systems with locator attachments may be preferred over ball attachments due to their low profile.[1]

Locator attachment system is consist of locator abutments suitable for all implant diameters and lengths, metal holder (locator processing cap) with black plastic inside, locator inserts (nylon retainers) in transparent, pink, blue, green and red colors and with different retention strengths.[1] The female part contains nylon attachments according to the implant angles. There are two types of nylon retainers available; internal and external retainers together and only external retainers. [2]

Internal and external retainers are in transparent, pink and blue colours and used in implants angled up to 20 degrees. Transparent nylons 5 lbs (2.27 g), pink nylons 3 lbs (1.362 g), blue nylons provide up to 1.5 lbs (0.68 g) retention.2 External retainers are in green, orange and red colour and used in non-parallel implants angled up to 40 degrees. Green nylons 4 lbs (1,816 g), orange nylons up to 2 lbs (0.908 g), red nylons provide up to 1 lbs (0.454 g).[2]

Locator attachment system is in a length of 2.5 mm including the locator, abutment and the male part on it. For the application of the locator attachment system, the manufacturer provides an apparatus called ‘Locator Core Tool’ consisting of three parts.

The abutment inserter (Locator abutment driver), is used for screwing

the abutments that are suitable for gingival height into the implants. The insert removal tool, is used to remove the black nylons inside the metallic caps. The insert seating tool is used to replace the removed black nylon with one of the five different colour nylon retainer that is suitable for the patient.[1]

There is a gap of 0.4 mm between the metal cap and nylon retainers when they are placed in the cap. Thus, locator attachments provide both hinge movement and vertical flexibility.[3] Locator attachments can be placed in the prosthesis, both directly in the mouth in the clinic and indirectly in the laboratory.[1] It is a highly flexible, retentive and wear-resistant system. They can be repaired and replaced easily and quickly.[2,4,6]

6. ERA Attachments

Era attachments are classified as flexible attachments and can be applied in almost all implant systems. The most important feature is that they are economic. There are plastic female parts with different retention properties. In addition, in ERA attachment systems, angled abutment options are also available.[1]

As a result of the 2-year study by Landa et al.[11] ERA attachment system increases patient satisfaction positively, and when compared to other attachment systems no difference was found in the health of the soft tissue and bone around the implants.

It is also stated that, ERA attachments provide the most appropriate load transfer to the bone around the implant.[1,11]

Angled abutments in the ERA system, can cause problems in patients with low interocclusal distance. In addition, it is stated that problems may be encountered while connecting the attachments to the inside of the prosthesis in the case of angled abutments. Therefore, it is reported that ERA attachments should be preferred for implants that are parallel to each other.[1]

Federick and Caputo (1996) compared ERA and bar-ERA attachment systems. They reported that ERA attachment systems have less stress on implants. They concluded that there is less stress in the area of the implants because the incoming loads were covered by the edentulous crest.[12,13]

Discussion

Defined as the McGill and York consensus; it was stated that for edentulous patients, overdenture using at least two implants in the lower jaw should be the standard treatment protocol.[1,2,14] Although Raghoebar et al.[14] stated that a definitive protocol has not yet been determined for the upper jaw, however at least four implants should be placed for the upper

jaw. It has been also stated that the retention of the removable prosthesis can be increased by placing four implants, if possible, six implants in case of a defect that may occur in the bone.[1]

While edentulous patients using full dentures provide effective chewing at high-normal alveolar ridge size, it has been reported that patients using implant-supported overdenture prosthesis have a more effective result.[15] However, Erakman et al. stated that the bite force increased up to two times in patients who switched from conventional full denture to implant-supported overdenture prosthesis, while there was no change in chewing performance. It is stated that the satisfaction of patients using implant-supported overdenture prosthesis is higher in terms of patient comfort.[15]

Today, the use of bar and ball attachment systems is most common in terms of retention, but ball attachment systems are not preferred in cases where the implants are too inclined towards the labial or buccal region, because retention is lost in a short time due to wear of the retainer. However, if the alveolar ridge is curved so much ball or locator attachments are advantageous.[3]

Tuna[2] stated that as the number of implants placed decreases, the attachments are more exposed to stress and wear, thus loss of retention. He stated that although the loss of retention will always be experienced due to environmental factors, there is a direct proportionality between the number of attachments and the retention, and the retention will increase as the number of attachments increases. It is stated that it is more conservative against both vertical and oblique forces.[2] The placed implants are rigidly connected with the bar attachment.[3]

Savabi et al.[16] reported that bar attachments were the highest attachment systems, but suffered a retention loss of 44%, ball attachment systems suffered a retention loss of 33% during this period, and magnetic systems experienced a retention loss of up to 70% in their 5-year studies. They stated that the weakest option is magnetic attachment systems.[16] In a study that the effect of bar attachments on retention was investigated, and it was stated that the retention of 1 metal clip was less than that of 3 metal clips, but the retention of 1 metal clip was not affected compared to 3 plastic clips.[16] Savabi et al.[16] stated that 3 metal clips are more grippy than 3 plastic clips, and in the comparison they made between plastic clips, they stated that the difference in retention between 1 plastic clip and 3 plastic clips is small, so increasing the number of plastic clips affects the retention very little. They concluded that the difference in attachments is due to the difference in material and design. They also stated that in ball attachment systems, the green cap is more conservative in the posteroanterior direction than the white and pink caps, but there is no difference between white and pink caps.[16]

Goodacre et al.[17] stated that in studies on the success of the prosthesis in implant-supported overdenture prostheses, the prosthesis showed a 100% success rate in one year. In a 10-year study, they stated that the success rate of 6-implanted bar attachments in the upper jaw in implant-supported overdenture prostheses is 94.7%, and the success rate of 4-implant Dolder bar in implant-supported overdenture prostheses is 87.5%. It was reported that the success rate 4-implanted Dolder bar in the lower jaw was 97.7% and in ball attachment implant-supported overdenture prostheses with 2 implants was 98.8%.[17]

Goodacre et al.[17] investigated how often patients visit for implant-supported overdenture prosthesis lining, repair and renewal. For the first visit, 4% of the patients returned for the repair of the prosthesis, 33% for its renewal, and 22% for its lining. For the second time, 4% of the patients returned for the repair of the prosthesis, 15% for its renewal, and 4% for its lining. For the third time prosthesis repair, 4% of the patients, for its renewal 4%, for lining data were not specified.[17]

Considering that bar holders are disadvantageous in terms of mechanical cleaning in individuals for whom oral hygiene may be doubtful, the importance of this situation in terms of the prognosis of implants should be explained to the patient.[3]

Although hyperplasia and mucositis are observed in the soft tissue around the implant, it was reported that bar and ball attachments do not affect soft tissue health and periimplantitis. The main reason for these complications is that the base of the prosthesis preventing the flow of saliva due to its covering, and therefore, bacterial accumulation may occur. It was stated that the prosthesis should be removed at night for a satisfactory oral hygiene. It was also reported that magnetic attachments had a higher plaque index.[2]

In the photo-elastic studies, when the implant length, diameter and geometry were determined under the same conditions, it is stated that ball attachments transmit more stress than bar attachments. Bar attachments have been the design with minimal stress around the implants.[2] The overdenture prosthesis with bar attachments have shown three times more success than prosthesis with ball attachments.[1] Today, in terms of retention, mostly bar and ball attachment systems are used. The retention provided by the systems with the bar attachment is the highest, while the systems with the magnet holder is known to be minimal. The attachment of ball and locator systems to the prosthesis directly in the mouth, may be advantageous as it provides a more precise connection.[1]

Besides the studies reporting that the stress formation in the bone around the implant with bar attachments is more than ball attachments,

there are in vivo studies indicating that the effect of retention amount and attachment type on stress formation is little, the mainly effective factors are the compatibility and occlusion of the dentures.[1]

When chewing forces are applied to implant-supported overdenture prostheses, the force is distributed between the mucosa and implants that support the denture base.[18] In the study by Özcivelek Mersin et al.,[18] the strain value of ball attachment systems on implants was found to be significantly higher when compared to bar attachment systems. The reason for this was stated that in the use of bar attachments, the strain on the implants may be lower than the ball attachment system, since the incoming force is shared on the two implants due to the splinting of the implants to each other.[18]

Özcivelek Mersin et al.[18] found that when ball attachments are used, the strain on the implants increases significantly compared to bar attachments, that there is no space that allows flexibility between the male and female parts of the attachment in prostheses with ball attachments and stated that the applied force is directly transmitted to the implants.

In cases where the edentulous ridge is very curved, the choice of ball or locator attachments is more advantageous than bar attachments.[1] It has been reported that mucositis and hyperplasia in the soft tissue supporting the prosthesis are more common in dentures where bar attachment systems are used, while discomfort or ulcers are observed more frequently in ball and magnetic attachments.[1]

When a bar attachment is used in implant-supported overdenture prostheses, the vertical deformation of the mucosa-imitating material is higher than that of a ball attachment.[18] Özcivelek Mersin et al.[18] stated that the reason for the deformation observed is that the stress in the use of bar attachments is more concentrated on the mucosa, while the stress on the implants is accumulated in the use of ball attachments. Thus, in ball attachments, more force is transmitted to the implants and less to the tissue.[18] It was reported that wear-related problems in ball attachments are less than in bar attachments prepared from gold alloy.[1] In patients with interocclusal distance problems, the use of locators is recommended instead of ball attachments.1 The stress values of the Bar-ERA design were also within the limits that could be used safely.[13]

When the alveolar crest is resorbed, bar and telescopic attachments provide better horizontal stability and occlusal forces are distributed over the implants. If enough number of implants of width and length are not placed, these loads cause mechanical failures in the implants. If bone resorption is minimal, single attachment systems; locator, ball or magnet attachment systems can be used. In this way, the prosthesis is actually supported by the

tissue and the attachment systems provide retention.[7] Therefore, alternative attachment systems should definitely be recommended in cases where the required amount of retention is desired or the patient expects a prosthesis with high retention.[1] Savabi et al.[16] stated that the most important factor affecting patient satisfaction in implant-supported overdenture prostheses is retention loss.

Many studies have been conducted comparing the effects of different attachment types on patient satisfaction. In these studies, patient satisfaction values of only magnetic attachments were found to be lower than other attachments.[1]

Due to bone loss in the area of tooth extraction, it is difficult to achieve aesthetics, function and phonation in a full denture applied to the lower jaw. In addition, it has been shown that chewing efficiency is significantly reduced, even down to a quarter compared to the natural tooth situation. In patients using full dentures, psychosocial complaints related to this issue are also frequently encountered.[4]

Conventional full dentures, which are frequently used in the prosthetic treatment of edentulous patients, have some disadvantages. These are lack of retention and stability, continuous bone loss, and insufficient chewing function. In addition, speech difficulties and lack of self-confidence in social environments are among the disadvantages of full dentures.[3] Compared to conventional full dentures, implant-supported overdenture prostheses have significant advantages such as greater retention and stability, superiority in chewing and phonation, and the ability to open the palate in the upper jaw in individuals with nausea reflex. However, studies have shown that patients using implant-supported overdenture prostheses have a high level of satisfaction with the cleanability of their prostheses. The long process of implant treatment should also be considered in the application of implant-supported overdenture prostheses.[19] The waiting period of 2-4 months for the osseointegration of the implants varies between 2-4 months depending on the case, and the patient may have to spend this process without using any prosthesis. Especially for the elderly patients, this situation may adversely affect nutrition.[20]

Important points in cases of maxillary implant-supported overdenture are the degree of atrophy in the jaws, bone density, the number and location of the implants prescribed.[3]

Conclusions

A significant increase in patient satisfaction has been observed in patients who have transitioned from full dentures to implant-supported dentures.1 Implant-supported overdenture prostheses have emerged

as a more preferred treatment method in order to meet the anatomical, psychological and functional expectations of patients due to total edentulism.[15] Compared to conventional full dentures, stabilization and retention of the prosthesis is more successful. When it comes to implant success, implant-supported overdenture prosthesis applications have come to the fore as a reliable treatment option, especially in the lower jaws, and it has been stated that implant-supported overdenture prostheses may be a useful alternative treatment for patients who are not prone to conventional full denture use or have limited use. The ease of application in patients with oral-maxillofacial and anatomical defects, the suitability of various treatment options in cases where functional and parafunctional movements occur, and the increase in patient motivation aesthetically, psychologically and phonatically, have made implant-supported overdenture prostheses more useful.[8]

Key Words: Implant supported overdentures, attachments, total edentualism

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Chapter 10

BISPHOSPHONATES AND MEDICATION-RELATED OSTEONECROSIS OF THE JAW

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1. MRONJ

First reported by Marx in 2003, bisphosphonate-related osteonecrosis of the jaw (BRONJ) is defined as a clinical manifestation of exposed bone lesions in the oral cavity that do not heal within eight weeks of onset in patients using bisphosphonates [1]. BRONJ, which was subsequently reported by Ruggiero et al. in 2004, was defined by the American Association of Oral and Maxillofacial Surgeons (AAOMS) in 2009 as "bone image exposed from the mucosa for more than 8 weeks in the jaws of patients who have not previously received radiation therapy for the head and neck, previously used or are currently using bisphosphonates" [2]. In 2014, AAOMS proposed the term medication-related osteonecrosis of the jaw (MRONJ) instead of BRONJ since not only bisphosphonates but also newly released chemotherapy drugs have the same effect [3].

According to the latest definition of MRONJ, patients should have previous or current treatment with antiresorptive or antiangiogenic agents, exposed bone or bone with an intraoral or extraoral fistula that has persisted for more than eight weeks, and no history of radiation therapy or metastatic disease of the jaws.

The characteristics of the jawbones listed below explain why BRONJ is seen in the jawbones.

- Being directly related to the external environment through teeth causes microorganisms to spread to the jawbone [4].
- Since the jawbones are covered by a thin periosteum and mucous layer, an infection may develop in the relevant bone secondary to trauma, even during normal physiological activities (chewing function) [4].
- Around 800 bacterial species in the oral environment increase the susceptibility to infection [4].
- It is known that bone turnover is higher compared to other bones in the body. Thus, the drug causes faster accumulation in this tissue [5].
- Direct contact of the bone with the external environment after dentoalveolar procedures and tooth extraction is a condition to facilitate contamination [6].

Considering MRONJ from a physiopathological point of view, it is seen that the bisphosphonate used has an inhibitory effect on osteoclasts, therefore suppression of bone remodeling and vascularization creates a suitable environment for necrosis [7, 8]. Moreover, it is believed that pH changes in the acidic direction are observed in the surrounding tissue as a result of the infiltration of inflammatory cells into the bone, thus breaking the bond between the bone and bisphosphonate. It has been

reported that the resulting free bisphosphonates with toxic effects cause mucosal fenestration and exposure of bone to the oral environment [9]. The histological examination of the resulting necrotic bone shows plasma cells, bone marrow fibrosis, inflammatory cells, and fungal and bacterial microorganisms. In microbiological evaluation, enterococci, streptococci, enterobacteria, lactobacilli, Candida albicans, and primarily actinomyces have been isolated [7, 8, 10].

1.2. Risk Factors

When assessing the likelihood of developing MRONJ, a major factor is the cumulative accumulation of bisphosphonates or denosumab or antiangiogenic in the patient. The factors that play an important role in such accumulation are medication administration route, dose, and duration. Low-dose, short-term (less than 3 years), and oral administrations pose a lower risk, while high-dose, long-term (more than 3 years), and IV administrations increase the risk of MRONJ. The type of bisphosphonates used is also important for the development of MRONJ. Especially in patients treated with intravenous bisphosphonates, the risk of developing osteonecrosis is much higher than the use of oral bisphosphonates; however, there are also reported cases of osteonecrosis in patients using oral bisphosphonates. It is known that zoledronic acid has a fifteen times higher risk of causing MRONJ compared to other bisphosphonate types. Other high-risk bisphosphonates include risedronate, ibandronate, alendronate, and pamidronate, respectively [11]. In addition to these, factors such as cancer diagnosis, trauma or surgery history, poor oral hygiene, dental infections, incompatible dental prostheses, long-term corticosteroid therapy, immunosuppression, vascular insufficiency, and advanced age increase the likelihood of this complication [12].

The duration of bisphosphonate use is also an independent factor for the development of MRONJ. While the rate of MRONJ development after 1 year of treatment is 1% among all patients exposed to IV bisphosphonates, this rate increases to 13% at the end of 4 years [13]. Likewise, increasing the cumulative dose also increases the risk of MRONJ development. In the literature, the time to MRONJ development has been reported as 12-24 months for zoledronic acid, 19-30 months for pamidronate, and 13-21.5 months for ibandronate [13-15]. Moreover, combined administrations have a high effect on the development of MRONJ [14]. Since zoledronic acid can remain in the bone for more than 10 years, drug holiday is not very significant, but there is not enough evidence for its positive effects. Concomitant chemotherapy, erythropoietin therapy, renal dialysis, and medical problems such as hypothyroidism and diabetes are also risk factors for the development of MRONJ. Moreover, there is a study suggesting that corticosteroid treatment also increases the risk of developing MRONJ [16, 17].

One of the important ways to identify risk factors is the serum CTX test. The serum CTX level is the most important marker of resorption and provides a reference by identifying changes in bone for up to some 2 weeks. It measures the serum level of the C-terminal telopeptide in the opposing chain of type 1 collagen and is useful in assessing the ratio of resorption to the amount of regenerated bone [18, 19].

Invasive dental procedures such as tooth extractions are one of the important risk factors for the development of MRONJ. Dental surgical interventions increase the incidence of MRONJ by 5.3 to 18 times. Conditions such as increasing age, smoking, and obesity are also factors that increase the risk of MRONJ. Gender does not have a significant effect on the risk of MRONJ [16, 20, 21].

The risk of MRONJ has been found to be 0.2% in cancer patients receiving bevacizumab, an antiangiogenic agent. The risk has been shown to be even higher (0.9%) in patients receiving both bevacizumab and zoledronic combination therapy [22]. Chiu et al. reported that osteonecrosis might be severer as the use of steroids in combination with bisphosphonates induces osteonecrosis earlier [23].

1.3. Clinical Findings

MRONJ is a clinical condition for which patients present to the dentist with typical symptoms. Symptoms can develop spontaneously as well as after dentoalveolar intervention. In general, patients may have many non-specific findings such as pain, tooth mobility, swollen mucosa, erythema, ulceration, paresthesia, anesthesia, necrotic grayish-yellow, and irregularly exposed bone surface, acute abscess, osteomyelitis, sequestration formation, trismus, halitosis, skin fistula, Actinomyces infection, lymphadenopathy, maxillary sinusitis or oroantral fistula, and pathological fracture of the mandible [5, 24-26]. In case of progression of maxillary infection, it may spread from the sinus and adjacent tissues to the eye, meninges, and brain, leading to life-threatening complications [27]. According to the report published by AAOMS in 2009, MRONJ is divided into 4 stages depending on its clinical findings.

- Stage 0: No clinical evidence of necrotic bone, but the presence of non-specific clinical findings and symptoms
- Stage 1: Asymptomatic patients have exposed and necrotic bone, with no clinical evidence of infection.
- Stage 2: Infected exposed necrotic bone with signs of erythema and pain in the exposed bone site without purulent drainage.

- Stage 3: Patients with pain have necrotic exposed bone, as well as one or more of the following: pathological fracture, extraoral fistula or oroantral connection, and enlargement of osteolysis areas [2].

1.4. Radiologic Findings

Orthopanoramicradiographs are the main method to evaluate the radiological findings of osteonecrosis. Typical features include the presence of necrotic bone, dense trabeculae, and irregular radiolucent areas with radiopaque sequestrum. Additional findings may be incomplete healing of the extraction socket, sinus wall cortication, and periosteal bone formation [28]. If a border image is visualized between normal bone and necrotic bone, this radiograph is sufficient for diagnosis and additional imaging is not required. However, in the early stages of the disease, orthopanoramic radiographs may be insufficient for diagnosis and additional imaging may be required [29].

Computed tomography (CT) is very sensitive for diagnosing osteonecrosis of the jaws secondary to MRONJ. On CT images, osteonecrosis is characterized by irregularities in the margins of cortical bones and destruction of cortical bone. Periosteal thickening is obviously visualized. Moreover, osteolysis and osteosclerotic areas can be noted. In advanced cases, fistulas, incomplete extraction socket wounds, and altered trabeculae can be visualized [28, 30]. Furthermore, the study of Stockman et al. showed the efficacy of orthopanoramic radiographs for the diagnosis of MRONJ as 54%, while this rate was shown to be 96% for CT [31].

Magnetic resonance imaging (MRI) also comes to the fore as an imaging technique that demonstrates abnormalities of the bone marrow and surrounding soft tissues in MRONJ. With this technique, the edematous structure formed in the bone can be visualized in the early stages. However, its efficacy for the diagnosis of MRONJ is a little low compared to CT, with a rate of 92% [31].

In MRONJ, cone-beam computed tomography should be considered as the standard imaging modality to measure the extent of disease and surgical planning. Although panoramic imaging is mandatory, it is recommended to be completed with a CT scan.

1.5. Differential Diagnoses

The clinical presentation can be confused with MRONJ in osteoradionecrosis, osteomyelitis, trauma, osteonecrosis related to herpes zoster infection, benign sequestration of the lingual cortex, necrotizing ulcerative periodontitis, excessive absorption of heavy metals, neuralgia-induced cavitational osteonecrosis, multiple myeloma, and jaw bone metastases. A clinical presentation considered to be MRONJ may hide

the metastasis in the bone or both osteonecrosis and metastasis may be seen simultaneously. At this point, the history taken from the patient gains importance. Previous or current use of oral or IV antiresorptive and antiangiogenic drugs should absolutely be questioned [32].

1.6. MRONJ Treatment

In general, there are two types of treatment approaches for MRONJ. While the first of these is a conservative approach, the other includes a more aggressive approach for severe cases, which is the closure of the tissue to prevent microbial contamination after removal of necrotic and exposed bone. According to the MRONJ treatment protocols updated by AAOMS in 2014, conservative treatment is recommended for stages 0 and 1, superficial debridement for stage 2, and surgical debridement and resection for stage 3 [3]. In addition to these basic approaches, hyperbaric oxygen therapy, bone stimulation with growth factors, tissue engineering applications, low-dose laser therapy are also used as supportive care. Moreover, there are ongoing studies on the role of tocopherol and pentoxifylline in the treatment of MRONJ.

Given the treatment strategies, debridement is a logical treatment option for stages 1, 2, and 3, but treatment should be focused on restoring functions and controlling pain. For this purpose, 15 ml of 0.12% chlorhexidine mouthwash at least 3 times a day\ and oral 1gr penicillin 2x1 should be used. In cases of a penicillin allergy, doxycycline 100 mg daily, levofloxacin 500 mg daily, or azithromycin 500 mg daily can be used, provided that their use is limited to 14 days. Patients who do not respond positively to these antibiotics can mostly be given 500 mg metronidazole 3x1 for 10 days. Since clindamycin is not very effective against Eikenalla and Moraxella bacterial species, it is not preferred for the treatment of MRONJ.

According to Ristow et al., surgical approaches should be preferred at all stages, provided that fluorescent lamps are used. These fluorescent lamps allow for the differentiation of viable bones from necrotic bones [33]. At the same time, the sharp bone edges around the extraction socket are important in surgical interventions to be performed together with tooth extraction. These sharp edges must be removed as it is necessary to cut the connection between the bone and the oral environment [34]. Moreover, attention should be paid to ensuring a tension-free soft tissue closure with mucosal incisions to be made. Studies have also stated that the soft tissues close to the MRONJ region should also be excised since these tissues are affected by chronic infection, and therefore are not suitable for reconstruction [35].

Today, there are many studies effectively using autologous substances

with tissue repair factors such as platelet-rich plasma (PRP), platelet-rich fibrin (PRF), recombinant human bone morphogenetic protein 2 (rhBMP-2), and stem cells for the treatment of MRONJ. The use of these substances is increasing day by day as they contain growth factors and stimulate healing. Among these substances, leukocyte-rich PRF inhibits oral bacteria, especially Actinomyces and its species, thus significantly improving the prognosis. There is no significant difference between the use of stem cell cultures obtained from blood, bone marrow, or adipose tissue in terms of healing, and it has been reported that stem cells inhibit the progression of MRONJ and significantly heal it when they are used in combination with other healing factors besides epithelialization. rhBMP-2 is more effective in bone healing, and a study using it together with PRP showed significant vascularity increase, fibrin, and collagen restoration [36-40]. In the literature, there are also reported cases where hyperbaric oxygen (HBO) and ozone therapy are used for the treatment of MRONJ. As an adjunct to surgery and antibiotics, HBO and ozone therapy may be useful for the treatment of MRONJ as they produce reactive oxygen and nitrogen species that positively modulate redox-sensitive intracellular signaling molecules involved in bone turnover [41]. The use of vitamin and hormone supplementation for the treatment of MRONJ is increasing. Teriparatide is one such supplement. Teriparatide is a synthetic polypeptide hormone that contains 1-34 amino acid fragments of recombinant human parathyroid hormone and is the only osteoporosis treatment that stimulates bone formation. It has been shown to directly stimulate bone formation, with a positive effect on bone strength [42]. Pentoxifylline is a vasodilator agent used for diseases of peripheral and cerebral vessels. Tocopherol, on the other hand, is a derivative of vitamin E. There are studies investigating the combined use of these two substances for the treatment of MRONJ. Significant coverage of the bone with mucous membranes, improvement in bone healing, and improvement in symptoms have been reported in the treatment of MRONJ in patients with multiple myeloma or metastatic bone disease [43, 44].

2. Drugs That Cause MRONJ

2.1 Antiresorptive Drugs

Bisphosphonates: The discovery of bisphosphonates, one of the drugs frequently used for the treatment of bone diseases, has a history of approximately one hundred years. Bisphosphonates, produced by the German chemists von Baeyer and Hofman in the mid-19th century, were first used in industry as anti-scaling agents to inhibit calcium salt formation on oil pipes. However, their commercial use was in the 1960s when they began to be used in detergents. As the biological properties of bisphosphonates were reported in 1968, their clinical use came to the fore,

and they were first used to prevent kidney stone formation. Later, they were used for the first time for Paget's disease in 1971 [5].

Chemically, bisphosphonates are analogs of pyrophosphates. Pyrophosphates are composed of two phosphoric acids bound to oxygen and are the simplest form of polyphosphates. Bisphosphonates contain carbon instead of the oxygen in the pyrophosphate and become resistant to chemical or enzymatic degradation with their phosphorus-carbon-phosphorus structure.

Bisphosphonates have two side chains. The R1 side chain affects the binding affinity of the compound to the bone; as it is a hydroxyl group, the binding ability of the compound to the bone is enhanced. The R2 side chain, on the other hand, is responsible for the antiresorptive potency and side effect profile of the compound (Figure 2.2-b). Etidronate and clodronate are the first compounds to come into clinical use and have a methyl in the R2 position in etidronate and a halogen in clodronate. Newer bisphosphonates such as alendronate, pamidronate, and risedronate carry nitrogen in the R2 position.

When the bone resorption inhibitory effect of etidronate in rats is accepted as 1, it is known that alendronate has a potency of 100-1000 and zoledronic acid has a potency of 10000. The antiresorptive effects of bisphosphonates from the lowest to the highest are as follows: etidronate, clodronate, tiludronate, pamidronate, alendronate sodium, risedronate, ibandronate, and zoledronate. Etidronate is a bisphosphonate derivative drug that was first discovered but is currently banned. Following its discovery, it was used for the treatment of osteoporosis. However, its inhibition of mineralization at doses that suppress bone resorption emerged as a major problem. It may cause focal defects in bone mineralization at doses of 5 mg/kg/day. At higher doses (≥10 mg/kg/day), it completely inhibits mineralization. In contrast, the dose at which alendronate sodium suppresses bone mineralization is approximately 1000 times the dose at which it inhibits resorption. Therefore, there is no risk of developing osteomalacia in chronic treatment with alendronate sodium [45].

Bisphosphonate-based drugs are also divided into two groups as those containing nitrogen (alendronate, risedronate, pamidronate, ibandronate, zoledronic acid) and those that do not contain nitrogen (etidronate, clodronate) [46].

Although the exact effects of bisphosphonates on bone resorption are unknown, they are mostly explained by cell mechanisms [47]. At first, it was thought that the effect of bisphosphonates to inhibit bone resorption was due to their physicochemical effect on crystal solubility and reduction of hydroxyapatite dissolution. Today, in the light of the studies, their

mechanisms of action have been revealed in detail.

The mechanism of action of bisphosphonates can be summarized as "reducing bone resorption by acting on osteoclasts". When administered at physiological doses, bisphosphonates readily bind to the bone matrix due to their high affinity to the bone. They remain in the inorganic structure for a long time by binding to hydroxyapatite crystals and accumulate on the bone surfaces in the form of layers at repeated doses [48, 49].

Osteoclasts, which are the principal cells involved in bone resorption, also resorb the bone after binding to the bone surface and take the bisphosphonate molecules present there into the cell. The bisphosphonate compound taken into the cell disrupts the indented structure on the surface of the active osteoclast as a result of a series of enzymatic reactions, causing the active osteoclast bound to the bone surface to detach from here and eventually to be unable to function. Osteoclasts detached from the bone surface undergo apoptosis over time, inhibiting bone resorption. They act on bone metabolism by reducing both the activity and number of osteoclasts. Bisphosphonates also indirectly inhibit the activity of osteoclasts by altering the signals sent by osteoblasts to osteoclasts. They also inhibit the release of the factor with which osteoblasts recruit osteoclasts to the site of resorption and prolong their lifespan [9, 50].

The main effect at the tissue level is the reduction in bone turnover secondary to the inhibition of bone resorption. As part of their action to inhibit bone resorption, bisphosphonates reduce the activation frequency, which is the birth rate of new bone remodeling units [47].

At the cellular level, bisphosphonates act through five mechanisms:

1. Inhibition of the differentiation of precursor cells into mature osteoclasts by activation of osteoclast precursor cells,

2. Inhibition of chemotaxis and attachment of osteoclasts to the bone,

3. Shortening the lifespan of osteoclasts due to early apoptosis,

4. Inhibition of osteoclast activity,

5. Reducing the rate of dissolution of bone minerals [47, 51].

At the molecular level, the nitrogen atom in the alkyl chains creates a toxic effect on osteoclasts or damages specific intracellular mechanisms in osteoclasts [52]. Non-nitrogen-containing bisphosphonates such as clodronate and etidronate are metabolized by osteoclasts and incorporated into non-hydrolyzable analogs of ATP. Osteoclast function is inhibited by intracellular accumulation of these cytotoxic metabolites, causing osteoclast apoptosis. Unlike non-nitrogen-containing bisphosphonates, nitrogen-containing bisphosphonates such as pamidronate, alendronate,

and risedronate cannot be metabolized. They act by inhibiting the mevalonate mechanism, which is responsible for the synthesis of isoprenoid lipids such as cholesterol and isopentenyl diphosphate, farnesyl diphosphate (FPP), geranylgeranyl diphosphate (GGPP), and therefore is important in osteoclast function.

FPP and GGPP are required for the prenylation of proteins (guanosine triphosphate-binding proteins) such as Ras, Rho, and Rac, which are regulatory proteins of osteoclast function. Since the prenylation of these signal molecules, which are responsible for cell proliferation, survival time, and skeletonization, does not occur as a result of the inhibition of nitrogen-containing bisphosphonates in the mevalonate mechanism, osteoclast function is inhibited, causing apoptosis of osteoclasts [53].

Bisphosphonates can be used both orally and intravenously. They are absorbed from the small intestine. When taken orally, only 1% of a dose of bisphosphonates is effective, with very little being absorbed. Therefore, it is considered more appropriate to take them 2 hours before meals. They are distributed within the body by the blood, stored in the bones, and excreted by the kidneys without any degradation. The pharmacodynamics of these compounds, which have not been observed to interact with other pharmacological agents, are determined by the gastrointestinal tract, blood, bone, and kidneys.

Intestinal absorption of orally taken bisphosphonates is relatively small, ranging from 0.6% to 5% of the dose. This amount is further reduced when the drug is consumed together with calcium and iron-containing foods. The half-life of bisphosphonates in plasma is as short as 0.3 - 2 hours. About 20-60% of the drug is absorbed by the bone, which can be explained by the affinity of bisphosphonates to hydroxyapatite crystals. The remaining portion is excreted by the kidneys without degradation. Bisphosphonates are stored in osteoclasts when given at low doses, while they are stored in the resorption and apposition areas when administered at high doses [9].

Elimination of this drug from the skeletal system occurs very slowly during active bone resorption [54]. Therefore, the half-lives of bisphosphonates in bone are quite long, ranging from 1 to 10 years, depending on the bone turnover rate. When bone resorption begins, bisphosphonates are released into the environment from here. It has also been shown that some bisphosphonates can remain in bone for life [55].

Denosumab: Denosumab is a genetically engineered human monoclonal immunoglobulin G2 (IgG2) antibody developed from Chinese hamster ovary cells. It chemically consists of 2 heavy and 2 light chains. Each light chain consists of 215 amino acids. Each heavy chain consists of

448 amino acids containing 4 intramolecular disulfides. Its mechanism of action has been described as disrupting the RANK-RANKL interaction via the "receptor activator of nuclear factor kappa-B ligand" (RANKL). Thus, osteoclast formation from osteoclast precursor cells is inhibited. As a result, bone resorption and bone turnover decrease, while bone mass in the cortical and trabecular layers increases. Denosumab, approved by the FDA in 2010, is used for the treatment of bone metastases (Xgeva) of solid tumors such as osteoporosis (Prolia) and breast and prostate cancer [56].

Subcutaneous administration of denosumab to osteoporotic patients every 6 months has shown a decrease in vertebral and hip bone fractures and monthly administrations have shown a decrease in complaints of bone disease secondary to metastasis of solid tumors. The half-life of denosumab, which is not indicated for the treatment of multiple myeloma, is around 25-32 days. In contrast to bisphosphonates, RANKL inhibitors do not bind to bone and their effects on bone remodeling decline 6 months after discontinuation of treatment [57]. Unlike bisphosphonates, it is not nephrotoxic, therefore zoledronic acid may be an alternative for bone metastasis patients with kidney dysfunction. The advantages of denosumab over zoledronic acid are that it is administered subcutaneously and does not require renal monitoring and dose adjustment [58]. Osteonecrosis caused by denosumab is still not fully understood, and its incidence is similar to that of zoledronate (zoledronate 1.3%, denosumab 1.8%) [59]. In the literature, the incidence of MRONJ caused by denosumab has been reported as 0%-4.7% [60-62]. The most common side effects are low phosphate level, back pain, hand and foot pain, skeletal muscle pain, and anemia. Although more serious side effects have been identified in 9% of patients, no vital risk has been associated with this treatment [63].

2.2 Antiangiogenic Drugs

Angiogenesis inhibitors affect the formation of new vascularized structures by binding the angiogenesis signaling cascade to various signaling molecules. These new drugs are used for the treatment of gastrointestinal tumors, kidney cancer, endocrine tumors, and others [3].

Bevacizumab: It is a recombinant, G1-humanized monoclonal antibody used to inhibit vascular endothelial growth factor (VEGF) function in vascular endothelial cells. By binding to VEGF, it inhibits the binding of VEGF to VEGFR-1 (Flt-1) and VEGFR-2 (KDR) on the surface of endothelial cells. Thus, it inhibits tumor angiogenesis, which is necessary for the growth and metastasis of solid tumors. Bevacizumab is indicated for patients with metastatic carcinoma of the colon or rectum, glioblastoma, lung cancer, and neoplastic neurovascular diseases. It has other non-neoplastic uses, including the treatment of certain ocular

diseases [64].

MRONJ caused by bevacizumab occurs due to VEGF inhibition and consequent reduction in angiogenesis. VEGF is essential for the formation, self-regulation, and survival of osteoclasts. Disruption of this factor causes a decrease in bone restoration ability. The decreased effect of VEGF by microtrauma (caused by tooth brushing and chewing) or invasive dental treatments can lead to mandibular lesions and the development of MRONJ [22, 65].

Although the first case of MRONJ caused by bevacizumab was described in 2008 [66], clinical cases of MRONJ where bevacizumab use alone is a risk factor have since been described [65, 67, 68]. To date, only three studies have directly evaluated MRONJ in patients treated with bevacizumab alone or in combination with bisphosphonates. Aragon-Ching et al. detected MRONJ in 11 patients (18.3%) of 60 patients, 55 of whom were treated with bevacizumab in combination with bisphosphonates and 5 of whom were treated with bevacizumab alone, but they found no evidence of osteonecrosis in patients treated with bevacizumab alone [69]. A study by Guarneri et al. on 1309 patients treated with bevacizumab (233 received bevacizumab in combination with bisphosphonates, and 1076 received bevacizumab only) showed that 4 patients developed osteonecrosis, 2 with combined therapy and 2 with bevacizumab monotherapy [22]. On the other hand, the study by Francini et al. on 59 patients did not find any signs of MRONJ during a 20-month follow-up [70].

Whether bevacizumab is directly involved in the onset of MRONJ is controversial; however, in most cases of MRONJ, this has been attributed to the use of the drug in combination with bisphosphonates. With the knowledge of the pathogenic mechanisms and clinical outcomes, it will be possible to administer the appropriate treatment to these patients.

Sunitinib: The tyrosine kinase receptor family plays an active role in tumor growth, pathological angiogenesis, and cancer progression (metastasis). Sunitinib inhibits members of the tyrosine kinase receptor family, including platelet-derived growth receptors (PDGFRD and PDGFRE), vascular endothelial growth factors (VEGFR1, VEGFR2, and VEGFR3), stem cell factor receptor (KIT), colony-stimulating factor 1R (CSF-1R), glial cell line-derived neurotrophic factor receptor (RET), FMS-like tyrosine kinase 3 (FLT3). In this way, sunitinib inhibits angiogenesis and tumor progression [71, 72]. It was first used in the USA in 2006 to treat renal cell carcinoma as well as gastrointestinal, pulmonary, thyroid, and hematological tumors.

Although it is not clear whether sunitinib is a medication that causes MRONJ, it is believed to cause osteonecrosis as it slows bone remodeling

and antagonizes the mucosal healing process by inhibiting surrounding fibroblasts and endothelial cells. On the other hand, the decrease in angiogenesis caused by sunitinib will increase the risk of osteonecrosis by impairing the host's defense system against infections.

Albeit very rarely, MRONJ cases associated with other antiangiogenic agents such as sorafenib, pazopanib, and axitinib have been reported in the literature.

There are also a few cases of MRONJ associated with the mTOR inhibitors everolimus and temsirolimus.

3. Conclusion

Tooth extraction is the most important risk factor for the development of MRONJ. Maintaining good oral hygiene, thorough dental examination, and surgical treatments including extraction of teeth with a poor prognosis should be completed before initiating any medication likely to cause osteonecrosis. Patients with removable dentures should be examined for areas of mucosal irritation. Procedures that may cause direct bone trauma should not be preferred. In addition to prescribing antibiotics before tooth extraction, primary closure of the wound will reduce the risk of developing medication-related osteonecrosis of the jaw. Furthermore, a detailed anamnesis taken by the physician before starting dental treatments is very important for the prevention of MRONJ.

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Chapter 11

IDIOPATIC GRANULOMATOUS LOBULAR MASTITIS

Nazlı Sena ŞEKER

Although idiopathic granulomatous lobular mastitis (IGLM) is a benign disease, it is an entity that can mimic malignancy clinically and radiologically. It was first described by Kessler et al. in 1972 as a rare, benign lesion that can mimic cancer [1]. There are different terms used for this disease in the literature, such as granulomatous mastitis, idiopathic granulomatous mastitis, granulomatous lobular mastitis and idiopathic granulomatous lobular mastitis [1-4]. In this article, idiopathic granulomatous lobular mastitis terminology will be used.

Trauma or breastfeeding can be found in the clinical history of idiopathic granulomatous mastitis cases. However, sometimes no history or etiology is found [5]. The factors in the etiology in the literature can be summarized as oral contraceptive use, autoimmune processes, an infectious agent that cannot be eliminated with today's technology, and an enhanced inflammatory response against the extravasated secretion from the lobules [1, 6-10].

Approximately 24% of chronic inflammatory diseases of the breast are granulomatous inflammation [11]. In order to define it as idiopathic, other granulomatous reaction factors must be ruled out first. These are diseases such as tuberculosis, sarcoidosis, Wegener's granulomatosis, ductal ectasia. Idiopathic GLM can be diagnosed after no other clinical or histopathological cause is found [12-14]. If there is a factor that may cause granulomatous inflammation, its removal is the first step for treatment. The treatment method in idiopathic granulomatous lobular mastitis is a combination of surgery and/or anti-inflammatory therapy. However, these patients can often undergo repetitive surgical procedures [15].

Clinical features

Idiopathic GLM is a disease of young-middle-aged women. Often there is a history of recent birth or breastfeeding [16]. It occurs on average two years after birth, so it is thought that it is not closely related to breastfeeding. Some studies suggest that it is linked to the use of oral contraceptives [17, 18]. In some studies, hyperprolactinemia is blamed, and it is stated that reducing the prolactin level during the treatment phase also helps the treatment [19].

Patients usually present with a large hard mass. Other clinical presentations are pain, erythema, hyperpigmentation, induration, nodule, abscess, sinus tract, abscess, and nipple retraction, in order of frequency [20]. Nipple discharge is usually not accompanied. Rarely, enlargement of the axillary lymph nodes may accompany. In addition, when IGLM cases progress, abscess formation and fistulization can occur at the first admission or as a complication in recurrences [17, 19].

After the admission, patients are referred to radiology in terms of imaging in order to rule out malignancy. The most common ultrasonographic finding is a hypoechoic mass with tubular extension. In mammography, it can be observed as asymmetrical density or multiple small masses that do not contain calcification or spiculation. They can also be seen on mammography as a poorly circumscribed mass with spiculated contours or bilateral multiple poorly circumscribed nodular lesions [21, 22]. Dynamic magnetic resonance (MR) imaging applied to a case revealed focal homogeneous masses with irregular borders. This finding can also be seen in malignant processes [22].

It is seen that imaging methods are not sufficient for definitive distinction in cases presenting with a mass radiologically. Tissue sampling is performed from patients in order to definitively rule out malignant processes. The definitive diagnosis of idiopathic granulomatous lobular mastitis can be made by histopathological examination.

Pathological features

Needle biopsy is usually performed for diagnosis in cases. The main lesion on histopathological examination is lobulocentric granulomatous reaction (Figure 1a-b). Granulomas consist of epithelioid histiocytes, some Langhans type multinuclear giant cells, lymphocytes and plasma cells. Inflammatory cells are found in or around the lobule. In addition, a few eosinophil leukocytes may be found in the inflammation [23]. Granulomas are noncaseating and there is no necrosis. Occasionally, the granulomatous reaction within the lobules may coalesce. Fat necrosis and abscess formations may accompany the progressive cases. In cases where excisional biopsy was performed, abscess formation, which is a sign of progression, can be seen macroscopically. Areas of fat necrosis and fibrotic changes are observed in the surrounding breast parenchyma (Figure 2). In this case, the normal duct architecture of the breast may disappear [9]. Microorganism should not be detected to give the diagnosis of idiopathic. For specific microorganisms, histochemical examinations such as PAS, Ziehl Neelsen and GMS should be performed.

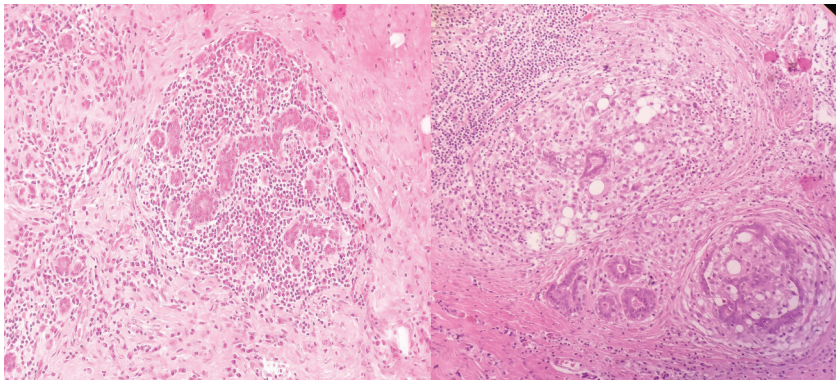

Figure 1a-b: Lymphohistiocytic inflammation is seen within the lobule (H&E, 200x)

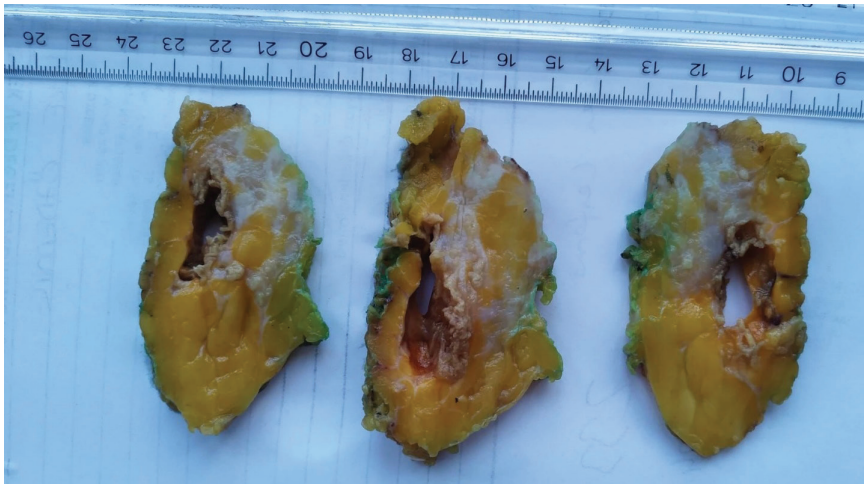

Figure 2: Abscess formation, fat necrosis and fibrotic changes in the surrounding tissue are observed in the breast excision material.

For cytological diagnosis, smears from fine needle aspirations from the lesion include multinuclear giant cells, lymphocytes, epithelioid histiocytes [24, 25]. However, in progressive cases, a large number of neutrophil leukocytes can be seen. In this case, abscess is included in the differential diagnosis, so the definitive diagnosis may not be given cytologically. Focal lactational changes can be seen in the surrounding breast parenchyma, as the cases usually have a recent birth history [26].

Differantial diagnosis

As mentioned earlier, the differential diagnosis of idiopathic granulomatous lobular mastitis includes infectious and inflammatory diseases that cause other granulomatous reactions. These are primarily diseases such as tuberculosis, cat-scratch disease, and sarcoidosis.

Clinical and microbiological findings are also important in the differential diagnosis of these diseases. The causative agent of tuberculosis infection is Mycobacterium tuberculosis, an acid-resistant bacillus. It mostly causes necrotizing granulomatous inflammation. According to one study, tuberculosis has more eosinophil leukocytes, while IGLM has more plasma cells. In addition, cases with tuberculous mastitis occur in younger patients compared to IGLM and present with larger lesions [27].

It is very important to make the differential diagnosis of tuberculous mastitis and IGLM. Because IGLM treatment is steroid-based anti-inflammatory treatments. This treatment can exacerbate tuberculosis [28].

Sarcoidosis is a multisystemic granulomatous chronic disease. It often involves the lung, and very rarely the breast can be involved extrapulmonary. It may appear as an irregular, poorly circumscribed and/or spiculated mass on mammography. Ultrasonographically, it is detected as an irregular hypoechoic mass [29]. It has radiologically overlapping features. While breast involvement of sarcoidosis is usually detected in young-middle-aged women, IGLM is frequently seen in the postpartum period. Idiopathic GLM manifests histopathologically as poorly formed granuloma structures within intense chronic inflammation involving the lobule. Sarcoidosis, on the other hand, is observed as a nonnecrotizing granulomatous reaction with well-formed smooth granuloma structures. There is no underlying chronic inflammation in sarcoidosis [30]. In addition, the absence of systemic involvement of idiopathic granulomatous lobular mastitis helps in the differentiation.

Since sarcoidosis, tuberculosis and IGLM have overlapping findings, a clear distinction should be made by evaluating them in the light of pathological, microbiological, biochemical and clinical findings with a multidisciplinary approach.

Cat-scratch disease usually presents with localized lymphadenopathy and a history of contact with a cat. Very rarely, they may present clinically with breast mass and mastitis findings. However, there is also concomitant lymphadenopathy. Histopathologically, necrotizing granuloma structures are observed both in the breast and in the lymph node. In the middle of necrosis, pleomorphic gram-negative bacteria can be detected by Steiner histochemistry. In addition, Bartonella hensela can be detected serologically. Although rare, cat-scratch disease must be ruled out before diagnosing IGLM [31].

One of the other entities included in the differential diagnosis is xanthogranulomatous mastitis, which is a rare condition. In this disease, the inflammation of foamy histiocytes, which shows a more diffuse pattern in the breast histopathologically, is predominant. A small number

of lymphocytes accompanies this inflammation. Often accompanied by a ruptured ductal ectasia or fat necrosis [32].

Another entity in the differential diagnosis is invasive breast carcinoma, which can be considered both clinically and sometimes histopathologically. Clinically, they may present with a rapidly growing mass, and there may also be nipple retraction and orange peel appearance on the breast skin, suggesting malignancy [33]. In cases presenting with a mass, imaging methods cannot help in the definitive distinction. In this case, tissue biopsy is applied for definitive diagnosis.

Invasive breast carcinoma may be accompanied by a granulomatous reaction. In invasive carcinoma, granuloma structures consisting of epithelioid histiocytes and Langans type giant cells are usually seen only within the invasive carcinoma area in the stroma. Usually, there is no concomitant granulomatous reaction in the lymph node. This granulomatous reaction is thought to be a component of the stromal response of the tumor [34, 35]. Before making a pathological diagnosis of granulomatous mastitis, care should be taken in terms of an occult invasive carcinoma.

Another entity considered by some authors to constitute a part of IGLM cases is cystic neutrophilic granulomatous mastitis. Cornybacterium group gram-positive bacteria are involved in the etiology of this disease, which has been detected recently. Although clinical and radiological findings are similar, suppurative lipogranuloma structures are observed histopathologically. These structures are a predominant inflammation of the neutrophil leukocytes lining the cavity and epithelioid histiocytes. Gram-positive bacillus can be shown histochemically in the cavities. The treatment of cystic neutrophilic granulomatous mastitis, on the other hand, is not a definitive consensus, but long-term use of antibiotics. Therefore, it should be differentiated from IGLM [36].

Treatment

Idiopathic GLM cases are usually treated with antibiotics at the first admission because they present with breast redness and pain. In these cases, a definite response is not obtained with antibiotics alone. However, antibiotic therapy may be beneficial in cases with secondary fistula and abscess.

The basis of treatment is usually immunosuppressive treatments such as steroids. Steroids, azathioprine, and methotrexate are generally used as immunosuppressive therapy. In some centers, treatment is started with high-dose steroids. Then, the steroid dose is reduced and methotrexate is added to the treatment. In this way, it is thought to be beneficial in

reducing the systemic side effects of steroid therapy [37, 38].

In another study, topical steroid application was added to the breast skin in addition to systemic steroids. In these cases, a decrease in the redness observed in the breast, closure of the erosions and a reduction in the fistula openings were detected. In addition, systemic side effects of steroids were not observed due to topical use [39].

Resistant cases that do not respond to drug therapy are usually treated with surgery. There are different results about surgical treatment options in the literature. One study found that surgical excision was the treatment associated with the best remission and less recurrence for IGLM. However, only oral steroid treatment is applied to patients who do not accept surgery and do not want surgical scars [3]. In another study, recurrence was found in all cases treated with drainage and incisional biopsy, and it has been shown that recurrence can also be seen, rarely, in cases with wide excision [40]. It has been reported that wider excisions compared to limited excision and total mastectomy in some cases minimize the recurrence [40-43]. Although it is reported in the literature that surgical wide excision is generally more successful, there are recurrence rates of up to 50%. In addition, complications such as delayed wound healing and fistula formation can be seen [44]. Patients with mild symptoms and small painless lesions can be followed only by observation. Up to 50% of these cases were in remission [45].

As a result, IGLM treatment can range from observation alone to extensive surgical resections such as total mastectomy. Although there is no definitive treatment scheme for idiopathic GLM, the combination of antibiotics, steroid and surgical treatment is the most preferred treatment method. Despite all treatments, IGLM cases have a risk of recurrence. For this reason, the cases should be followed clinically for a long time. Although idiopathic granulomatous lobular mastitis is rare, it is a disease that enters the differential diagnosis with many infectious and inflammatory diseases. For this reason, these patients can apply to many clinics in hospitals. Close clinical follow-up with accurate diagnosis is very important for these patients.

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Chapter 12

NEURONAL DIFFERENTIATION AND RELATED FACTORS[1]

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1. Introduction

The creation of induced pluripotent stem cells (iPSCs), which are a valuable cell source for disease modeling, drug screening, and regenerative medicine by somatic cells by epigenetically reprogramming by Takahashi and Yamanaka in 2006 showed that somatic cells could undergo impressive cell fate changes. Pluripotent stem cells can provide an unlimited repository for various somatic cell subtypes and appear as an intriguing approach for various *in vitro* studies (Ebrahimi et al., 2019; Wichterle et al., 2002). In addition to pluripotent stem cells, embryonic stem cells (ESC) especially can provide neuronal lineage in a manner appropriate for neural development, in patients with the neurodegenerative disorder without a mitigating or neuroprotective treatment. These properties of stem cells make them a promising approach for regenerative medicine. However, this stem cell strategy faces several security concerns such as tumor formation and ethical issues. In addition to the stem cell strategy, direct reprogramming of somatic cells into various neuronal subtypes can be accomplished without going through a pluripotent intermediate stage using the temporary expression of certain factor combinations (Vierbuchen et al., 2010). This process is a very suitable strategy in terms of cellular therapy and disease modeling in neurodegenerative diseases (NDs), which is a disorder that results in a gradual loss of cognitive and physical function in the patient. With this strategy, somatic cells can be transformed into neurons specific to ND patients to create an appropriate neurological model, thus developing alternative strategies for personalized medicine (Mollinari et al., 2018). In the transdifferentiation strategy, somatic cells can be directly induced into functional neurons by various methods from different cell sources including the expression of pioneer transcription factors (TFs), microRNAs (miRNAs), and small chemical compounds. The first study on transdifferentiation shows that overexpression of myogenic differentiations (MyoD), which plays an important role in regulating muscle differentiation, can lead to the transformation of the fate of fibroblasts into myoblasts through Direct Neuronal Differentiation (DND) (Davis et al., 1987). Following this study, many cells were directly conversed by the transdifferentiation of various somatic cells (Figure 1).

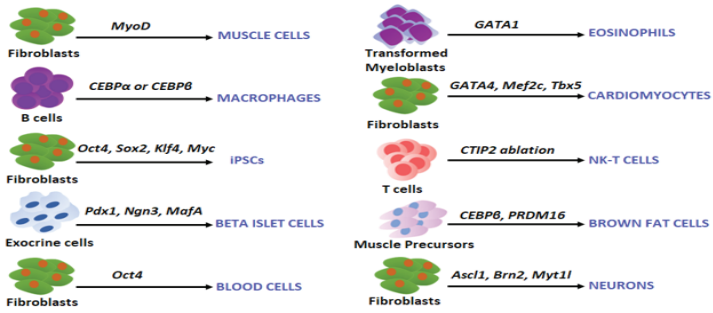

Figure 1: Examples of transdifferentation and reprogramming with specific factors.

But to clarify the ongoing events here we need some basic knowledge about Neural system development. Neuroembryology is a branch of sciences deal with developmental biology of nervous system, essentially from neural tube (Neuroglial blast) and neural crest cells. A brief introduction of neuroembrology before detail discussion is necessary here. Several critical moleculs/factors are involved in formation, growth, and differentiation of neural tissue. There are four accepted groups involve in growth and differentiation processes: Fibroblast Growth Factor (FGF), Transforming Growth Factor β (TGFβ), Wingless/Int-1 (Wnt) and Hedgehog proteins. Cholinergic neuron differentiation from neuroglial progenitor cells is induced by bone morphogenetic protein 2 (BMP2) as a member of TGFβ family or Neural crest cells migration happened with down-regulation of N-cadherin expression. Signaling transduction pathways this molecules are not totally understood. As pointed above many aspects of central nervous system (CNS) neuroembryology such migration and polarity, are orchestrated by paired homeobox genes and proliferation of neuroblast is regulated mainly by protein kinase. On the other hand another approach such as Isoformal development, Microenvironment of cells and interaction between them probably play an important role in this process through inductive factors.

Neurulation in 3rd week of intrauterine life or early segmentation in neuroepithelium of the neural tube constitute the essential parts of the CNS including the prosencephalon (forebrain), Mesencephalon (Midbrain) and Rhombencephalon (hindbrain). In the next weeks, Telencephalon (cerebral hemispheres), diencephalon (thalamus and hypothalamus) is derived from forebrain; Hindbrain forms Metencephalon (pons and cerebellum), and myelencephalon (medulla); and mesencephalon (midbrain) Remains undeveloped.

2. Transdifferentiation Strategies in Inducing Neuronal Cells from Fibroblasts

After the discovery of the direct differentiation of fibroblasts into induced neurons (iNs), various strategies have been developed to converse different somatic cells into iNs. There are various pioneer and auxiliary factors that increase the efficiency of transdifferentiation or give cells neuronal properties required in the production of iNs. In the direct differentiation strategy, fibroblasts can be efficiently converse directly into various subtypes of neurons as described below with different combinations of precursor and auxiliary factors such as TFs, miRNAs, and small molecules. Additionally, activation of Long Interspersed Element-1 (LINE-1 or L1), which is known to be important in neural development, also plays an important role in neuronal transdifferentiation. In this way, with the development of various strategies for direct neuronal differentiation and the rapid advancement of this technology, patient-specific neurons can now be produced from various somatic cells. With various studies carried out in this way, the mechanisms underlying various diseases can be analyzed by producing neuronal subtypes specific to different NDs, and in this direction, the drugs associated with the disease can be identified and therapy opportunities can be offered with alternative approaches.

2.1. Transcription Factors

Transcriptional transdifferentiation is started and stabilized by specific TFs and chromatin modifications, and these processes are believed to protect cellular identity (Vierbuchen & Wernig, 2012). In various experimental approaches, changes made by transcriptional and epigenetic networks have been shown to go beyond epigenetic boundaries and direct the cells to various somatic lineages or pluripotency (Ladewig et al., 2013; Vierbuchen & Wernig, 2011). Both iPSC and some somatic cells, including fibroblasts, can be differentiate directly into iNs with lineage-specific TFs. The first published DND strategy was demonstrated in a study based on overexpression of Achaete-scute like 1 (Ascl1), also known as mammalian achaete scute homolog-1 (Mash1), Brain 2 (Brn2) and Myelin transcription factor 1 like (Myt1l) TFs, also known as BAM factors, in mouse fibroblasts (Vierbuchen et al*,* 2010). Ascl1, a powerful pioneer TF and activator of neuronal reprogramming, can differentiate fibroblasts alone into functional iN cells under optimized conditions. Nevertheless, less efficiency is obtained from DND with BAM (Chanda et al., 2014). Directing somatic cells into the neuronal fate by the Ascl1 factor, which is very important for neuronal differentiation, depends on the Rat Sarcoma/Extracellular signal-regulated kinases (RAS/ERK) signaling pathway. The level of ERK activation determines which cellular identity Ascl1 will promote. The high ERK signal causes Ascl1 to direct the fate of cells towards glial identity

rather than γ-aminobutyric acid (GABA)ergic neurons (S. Li et al., 2014). Myt1l, another enhancer TFs in neuronal direct differentiation, such as Ascl1, is a repressor of non-neuronal genes. Excessive expression of Myt1l with pioneer TFs increases the efficiency of iN and improves those that do not yield sufficient neuronal features (Vierbuchen et al., 2010). The depletion of Myt1l in primary postmitotic neurons suppresses neuronal gene expression and impairs its function (Mall et al., 2017). Furthermore, in non-neuronal cells, Myt1l can be considered an antagonistic of the RE1-Silencing Transcription factor (REST) complex which plays an important role in the suppression of neuronal genes and consequently greatly suppresses neuronal destiny (Treutlein et al., 2016). Brn2 also known as POU class 3 homeobox 2 (Pou3f2), another TF that plays an important role in neuronal differentiation, participates in neurogenesis and DND with overexpression along with the other TFs. Brn2, which supports neuronal maturation in human cells, binds to regions that are opened in the genome in response to pioneer neuronal TFs. In this way, Brn2 regulates a large set of genes critical for neurogenesis and neuronal maturation (Yuanchao Xue et al., 2016). Beyond the expression of Brn2 in subventricular zone (SVZ) progenitor cells, this protein also plays an important role in the development of paraventricular nuclei and supraoptic nuclei neuronal lineages in the hypothalamus, and Brn2 deficiency in neonates leads to differentiation failure in hypothalamic paraventricular nuclei and supraoptic nuclei neurons, which subsequently results in neuron loss (Nakai et al., 1995; Urban et al., 2012).

Besides BAM factors, DND can also be induced with Neuronal differentiation 1 (NeuroD1), which is necessary for the development of the CNS. After it has been found that iNs can be generated by transduction of human fibroblasts with NeuroD1, it has been shown that iNs can be formed by culturing fibroblasts with a combination of NeuroD1 and BAM factors (Vierbuchen et al., 2010). Transcriptional activation of NeuroD1 required for neuronal differentiation depends on the activation of the canonical Wnt/β-catenin signaling pathway. Wnt signaling, which has a regulatory role at various levels especially in hippocampal neurogenesis, induces NeuroD1 activation, and differentiation into hippocampal granule neurons in Dentate Gyrus (DG). β-catenin and NeuroD1 are required for adult neurogenesis and their deficiency causes a decrease in neural progenitor cell (NPC)s and newborn neurons in DG (Kuwabara et al., 2009; Yamaguchi et al., 2007). The Wnt/β-catenin signaling pathway also induces the differentiation of cortical NPCs into neurons by expressing Neurogenin (Ngn) 1 (Ngn1) and Ngn2, which are transcriptional regulators responsible for the differentiation of progenitor cells to neurons in neurogenesis (Adnani et al., 2018). In addition to neural induction, Wnt signaling also plays a role

in the inhibition of NPC's differentiation into neurons in the early stages of neural development (Hirabayashi et al., 2004). In addition to all these factors, Sry-related-HMG box (Sox) B1 (SoxB1), SoxB2, SoxC, SoxD and SoxE protein groups from nine Sox protein groups play a role in neuronal induction. Among these protein groups, especially Sox1, Sox2, and Sox3 in the SoxB1 family are very important for CNS development and are expressed by NPCs in this developmental process. The lack of Sox2 in the CNS can be compensated by Sox3 and thus functional equivalence can be achieved (Miyagi et al., 2008). NPCs, in which the SoxB1 group is expressed, differentiate into neurons as well as astrocytes and oligodendrocytes. However, for oligodendrocyte specification, in addition to SoxB1, Sox9 TF from the SoxE group are also required (Hoffmann et al., 2014; Stolt et al., 2003). Like Sox TFs, Zic family member 1 (Zic1), Zic2, and Zic3 factors, which are members of the zinc finger TF family, are important in neural induction and development. Expression of the these three Zic genes allows the neural plate and neural crest to expand (Brewster et al., 1998). In early neurogenesis, of these genes expressed in the ectoderm, Zic1 enables more effective Noggin-mediated neuronal differentiation, which is a BMP signal suppressor in the ectoderm (Kuo et al., 1998). However, Zic2 does not direct cells to neuronal differentiation in neural plate formation and keeps them as an immature progenitor. To sum up, as an example of transcriptional transdifferentiation, in the study in 2015, it has been shown that human fibroblasts can be directly converted into iNs (GABAergic and glutamatergic neurons) efficiently by using Ascl1 from BAM factors, Sox2 and Ngn2 (Zhao et al., 2015).

2.2. MicroRNAs

Additional factors such as miRNAs may be required to induce the neuronal state with TFs or other DND methods to efficiently generate mature neurons from adult fibroblasts. For example, miR-9/9* and miR-124 regulate the expression of anti-neuronal genes, playing a key role in differentiation into mature neurons (Packer et al., 2008; V. Makeyev et al., 2007; Y Xue et al., 2013). In direct differentiation from human fibroblasts into neurons, over-expression of miR-9/9* and miR-124 (miR-9/9*-124) can induce neuron-like cells expressing microtubule-associated protein 2 (Map2), a neuronal marker (J. Xu et al., 2015; Yoo et al., 2011). Also, the direct conversion of human fibroblasts into neurons can be stimulated by the expression of miR-9/9*-124, and a process facilitated by the NeuroD2 transcription factor (Yoo et al., 2011). Neuronal cells could be produced by overexpressing miR-9/9*-124 with NeuroD2, Ascl1, and Myt1l (abbreviated as DAM), which are positive for expression of Map2, a transcriptional regulator involved in determining dendritic shape during neuronal development (Yoo et al., 2011). Besides, a population of neurons

similar to striatal medium spiny neurons (MSNs), a subtype of neurons, can be formed directly from human postnatal and adult fibroblasts by the co-expression of miR-9/9*-124 with four TFs including distal-less homeobox 1/2 (DLX1/DLX2), which play a role in the differentiation of neurons in the forebrain, Myt1l and Coup-interacting protein 2 (CTIP2) (CDM). However, CDM factors alone are ineffective for neuronal transformation. As a result, this reprogramming is based on the activities of miR-9/9*-124 (Victor et al., 2014). In addition to miR-9/9*-124, miR34b/c also enables reprogramming of fibroblasts into neuronal subtypes when combined with certain TFs. miR34b/c, when combined with Ascl1 and Nuclear receptor related 1 (Nurr1), a transcription factor required for midbrain dopaminergic development and functional maintenance, modifies Wnt family member 1 (Wnt1) expression, leading to cell cycle exit and induces transdifferentiation of fibroblasts into dopaminergic neurons (DAN) (De Gregorio et al., 2018; Le et al., 2008). Alternatively, transdifferentiation of fibroblasts into functional neurons can be induced by suppression of a single RNA binding protein, polypyrimidine tract-binding protein (PTB) under the action of miR-124, during normal brain development. MiR-124 and many other neuronal-specific miRNAs are known for targeting the REST complex that suppresses many neuronal genes in non-neuronal cells. If PTBs are not suppressed by miR-124 during conversion from fibroblasts to iNs, the inhibition process of the REST complex by neuronal-specific miRNAs will be inefficient in the initial stages of direct conversion (Y Xue et al., 2013). Thus, blocking the PTB-mediated miRNA effect on components of the REST complex during neuronal induction causes neuron-specific TFs and de-representation of many neuronal genes in non-neuronal cells (Y Xue et al., 2013).

2.3. Long Interspersed Element-1

While half of the human genome consists of transposable elements that have affects on our genome; 17% consists of LINE-1 retrotransposons, which are transposable elements that can move on their own throughout the human genome (Lander et al., 2001). Most of the L1s, which are 6-7 kbp long and contain two open reading frames (ORF1p and ORF2p) that encode the proteins necessary for retrotransposition, can be rearranged, mutated or cut, and can be considered a molecular fossil (Brouha et al., 2003).

In addition to the human germline, some somatic cell types have significant retrotransposition levels. The activity of LINE-1s can significantly affect the human brain genome among these somatic cells. Studies such as sequence analysis performed in this direction show that somatic retrotransposition occurs during neural development and may increase in neurons (Erwin et al., 2016; Evrony et al., 2012). Engineered

human L1s can move in non-dividing neuronal cells' genome (MacIa et al., 2017). Engineered L1's can also perform retrotransposition in NPCs of adult rats *in-vitro* and *in-vivo* (Muotri et al., 2005). L1 elements are re-expressed and activated in the early stages of reprogramming. In this process, L1 activation plays a role in induced dopaminergic neuron (iDAN) maturation, while inhibition stops transdifferentiation of mouse embryonic fibroblasts (MEFs). Della Valle and his colleagues has shown that with L1 activity, the conversion of MEFs to iDANs can be achieved by using a reverse transcriptase inhibitor, Lamivudine, three specific TFs; Nurr1, Ascl1, and LIM homeobox transcription factor 1 (Lmx1) alpha (Lmx1A) which play a role in the development of DANs in embryogenesis, and LINE-1 anti-sensory oligonucleotides (Della Valle et al., 2020). It has also been shown that L1 inhibition prevents the upregulation of pathways required for iDAN functionality, thereby impairing iDAN cell maturation during the reprogramming of somatic cells with L1 (Della Valle et al., 2020).

2.4. Small Molecules

Several studies have proven that the addition of small molecules increases the effectiveness of cell fate changes in transdifferentiation as well as the differentiation of iPSCs into various cell types (Ebrahimi et al., 2019; Huangfu et al., 2008; Ichida et al., 2009; Sayed et al., 2015). Also, in some transdifferentiation cases, the small molecule strategy does not require the need for transgene expression (Hou et al., 2013) and the use of small molecule compounds in this process is an important way to avoid the risks that can result from genetic manipulation. Another importance of this method is the control of its effects in the experimental process by optimizing the period of effect, combinations and concentrations of these compounds. As a result, the small molecule-based transdifferentiation strategy, one of the most promising methods, has great potential for disease modeling and therapeutic strategies.

In the study conducted by Ladewig et al. in 2012, it was shown that by using small molecules and TFs, fibroblasts can be efficiently differentiated into neuronal cells. Ladewig et al. provided DND of fibroblasts by suppressing the Glycogen synthase kinase 3β (GSK3β) and TGFβ/Caenorhabditis elegans Sma genes and the Drosophila Mothers against decapentaplegic (SMAD) signal pathway with the small molecule combination SB-431542 and CHIR99021, in addition to Ascl1 and Ngn2 TFs (Ladewig et al., 2012). Subsequently, in 2015, Li et al. demonstrated that using 4 small molecules, including Forskolin (cAMP activator and inhibitor of BMP signal involved in neural inhibition), ISX9 (a chemical compound that induces the NeuroD1 gene expression and differentiation of SVZ progenitors to neurons), CHIR99021 (a chemical compound that

acts as an inhibitor of the GSK-3 enzyme), and SB431542 (Potent inhibitor of TGFβ type 1 receptor), direct conversion of MEFs into neurons can be achieved (X. Li et al., 2015). Also, same year, Dai and his colleagues showed that human fibroblasts can be converted into glutamatergic or GABAergic neurons by using small chemical molecules CHIR99021(C), activin-like kinase 5 (ALK5) inhibitor SB431542 (S), MEK/ERK inhibitor PD0325901 (M), BMP type I receptor inhibitor LDN193189 (L), Pifithrin-α (P), a p53-dependent gene transcription and apoptosis suppressor and Forskolin (F) (CSMLPF) (Dai et al., 2015). The fact that small molecules are effective in this combination indicates that the relationship between molecules is very important in diret differentiation of fibroblasts into neuronal cells.

3. Neurogenesis and Neuronal Subtypes

Neurogenesis is known as the process in which various neuronal cells are produced by the proliferation and differentiation of progenitor and stem cells in the embryonic and adult brain. Many neuronal cells such as GABAergic neurons, motor neuron (MN), DANs are generated with this highly regulated process in the mammalian nervous system. Adult neurogenesis, preserved throughout evolution, consists a series of important developmental events in the production of various neurons and is an active process involving maturation, fate specification, and differentiation of neural progenitors. Adult neurogenesis, an important biological process, takes place in two specific neurogenic brain regions: the SVZ of the lateral ventricles that provide interneuron to the olfactory bulb (OB), and the subgranular zone (SGZ) of the DG in the hippocampus. In addition, a limited level of neurogenesis normally occurs in other parts of the CNS.

Transiently proliferating cells, which are generated from radial glia-like cells in the adult SVZ, form neuroblasts that migrate to the OB by rostral migration stream (RMS). These neuroblasts mature into 2 different interneurons, granule cells (94%) and periglomerular cells (4%) (Lledo et al., 2006), after migrating through a tube formed by astrocytes (Lois et al., 1996). Apart from that, they also developed <2% astrocytes. Periglomerular interneurons contain glutamatergic and GABAergic neurons involved in interactions between coactive glomeruli. These Periglomerular neurons receive cholinergic, noradrenergic, and serotonergic axonal signals. On the other hand, granule cells, can receive signals from mitral and tasseled neurons and secrete GABA mutually from the same or neighboring synapses. As a result, after neuroblasts reach OB, immature neurons migrate from RMS to glomeruli and the majority forms GABAergic granule neurons, while some form GABAergic periglomerular neurons (Ming & Song, 2011).

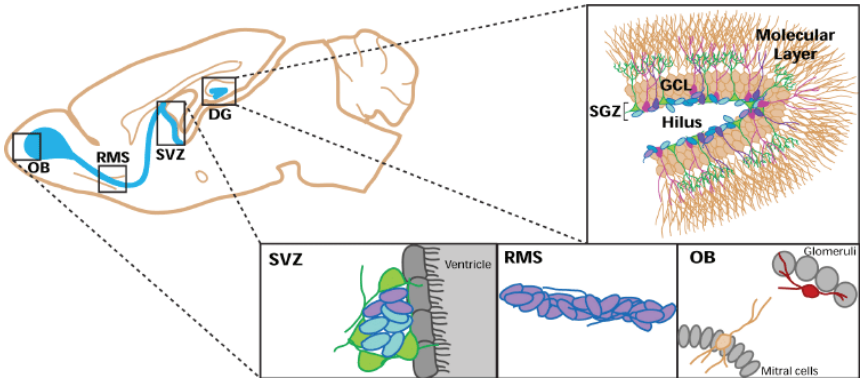

Figure 3. SVZ and SGZ are the main neurogenesis source in the adult mouse brain. *In this schematic view, the neurogenic area showed in blue and stem cells in green near to paranchymal layer of Lateral ventricle (gray). From here neuroblasts (purple) migrate the RMS to reach the OB to differentiated to periglomeruli cells (red). In comparison the stem cells in the SGZ of the hippocampal DG, move barely into the granule cell layer (GCL) to give rise to mature neurons. Progenitors (light blue), Newborn neurons (purple), Immature (magenta) and Mature (peach) granule cell neurons (Johnson et al., 2009).*

The intermediate progenitor cells of the DG consisting of radial and non-radial precursors in the SGZ of the adult hippocampus develop neuroblasts. Immature neurons make axonal protrusions to Cornu Ammonis 3 (CA3), another part of the hippocampus, and their complex dendrites extend into the molecular layer, thereby maturing into glutamatergic granule neurons of the DG receiving GABAergic inputs. While depolarizing GABA-mediated inputs are taken slowly in developing and immature granule cells, rapid GABA-mediated inhibition occurs in mature granule cells, and then glutamatergic inputs that act as neurotransmitters are taken. Additionally, various inputs, including dopaminergic and serotonergic inputs, are received in this process, thereby regulating emotional state, neural activity, and adult neurogenesis directly or indirectly (Amaral et al., 2007).

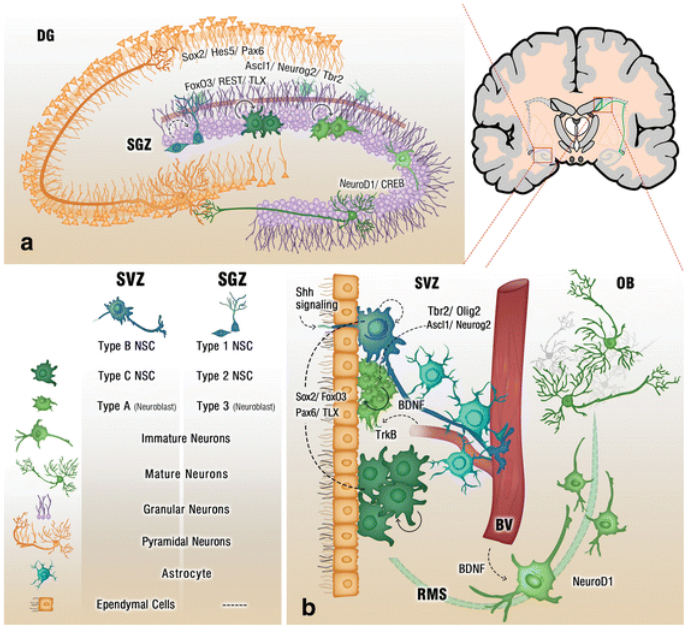

Figure 4. In coronal section of the human brain molecules that alter adult neurogenesis in SVZ and SGZ (Shohayeb et al., 2018).

3.1. Motor Neurons

Motor neurons are a group of cells responsible for both voluntary and involuntary movements in the body by combining signals from the brain and sensory systems. Dividing into spinal and cranial subsets, MNs form two-neuron circuit as upper motor neurons originating from the cerebral cortex and lower motor neurons commencing from the spinal cord (Zayia & Tadi, 2021). The production of MNs depends on the 2 periods of sonic hedgehog (Shh) signaling, which occurs in the early stage when neural plate cells turn into ventral progenitors and the late stage which Shh enables these progenitors to differentiate into motor neurons. In the late stage, depending on the concentration of the Shh that determines the neuronal cell model produced in the neural tube, it is determined whether motor neurons or interneurons will be formed from ventral progenitors (Johan Ericson et al., 1996).

The ventral spinal cord contains five progenitor domains, p0, p1, p2, p3, and pMN, where progenitor cells differentiating into motor neurons and V0-V3 interneurons subtypes are distributed (Davis-Dusenbery et al., 2014). pMN progenitor cells can differentiate into both motor neurons and oligodendrocytes by selectively expressing Oligodendrocyte transcription factor 2 (Olig2) (Ben-Shushan et al., 2015). NK6 Homeobox Protein (Nkx) 6 (Nkx6) proteins trigger Olig2 expression and this expression of Olig2 in

the pMN domain triggers expression of Ngn2, which is very important for cell cycle output, induction of terminal MN TFs and providing neuronal properties (Davis-Dusenbery et al., 2014; Novitch et al., 2001). Expression of Olig2 together with Ngn2 basic helix loop helix (bHLH) protein in this way enables pMN progenitor cells to differentiate into motor neurons (Ben-Shushan et al., 2015; Davis-Dusenbery et al., 2014). However, the low expression of Ngn2 in pMN progenitor cells causes them to remain proliferative pMN progenitors directed to oligodendrocyte fate (Ben-Shushan et al., 2015). In addition to Nkx6, Olig2 and Ngn2 TFs, expression of another TF Paired box 6 (Pax6) expressed in pMN progenitors also controls MN identity (J. Ericson et al., 1997).

In 2011, Son et al. showed that by overexpression of LIM homeobox protein (Lhx) 3 (Lhx3), Ngn2, ISL LIM homeobox 1 (Isl1), which are necessary for the specification and development of motor neurons, Homeobox Protein Hb9 also known as motor neuron and pancreas homeobox 1 (Mnx1), and BAM factors, mouse fibroblasts can differentiate directly into the motor neuron lineage (Son et al., 2011). Also, in 2013, Meng Lu-Liu demonstrated that two small molecule compounds (Dorsomorphin and Forskolin) used in combination with the Ngn2 transcription factor, can directly differentiate human fetal lung fibroblasts into motor neuron-like cholinergic neurons (M. Liu et al., 2013) And also, in another study which conducted by Abernathy et al. in 2017, it was shown that spinal cord MNs can be created from human adult fibroblasts by using the factors Isl1 and Lhx3 together with miR-9/9* and miR-124 (Abernathy et al., 2017).

3.2. Dopaminergic Neurons

DANs, the main source of dopamine of the mammalian CNS, are found throughout the CNS, particularly in the midbrain. DANs, constituting 3-5% of the neurons in Substantia Nigra, are a heterogeneous cell group both functionally and anatomically. DANs that can secrete dopamine, a catecholaminergic neurotransmitter, play an important role in the control of many behavioral processes such as voluntary movement, addiction, and stress, and their loss leads to Parkinson's Disease (PD), one of the most critical NDs.

Midbrain DAN (mDAN) neurogenesis occurs in the ventricular region of the midbrain floor plate and is regulated by the *Mash1* and *Ngn2* proneural genes regulated by Shh-forkhead box (Fox) A2 (Foxa2) pathway that induces ventral phenotype and Lmx1A/B-Wnt1-orthodenticle homeobox 2 (Otx2) which regulates the expression of functional DAN genes (eg. Tyrosine Hydroxylase and dopamine transporter) (Arenas et al., 2015; Chung et al., 2009). DANs consist of progenitors expressing Shh or Wnt1, and the identity of dopaminergic progenitors causing these DANs

is determined by the presence of Shh and FGF8 (Chinta & Andersen, 2005). Also, ventral midbrain progenitors cannot easily generate DAN by expressing the Foxa2, engrailed homeobox 1 (EN1), Otx2, and Lmx1A markers while efficiently differentiate into DANs in the presence of FGF8, Pax5, EN2, and canopy FGF signaling regulator 1 (CNPY1), an FGF signaling regulator (Tao & Zhang, 2016; Xi et al., 2012). FGF8 increases the expression of Nurr1 and TH, which are necessary for DAN synthesis in the late stages of neuronal differentiation, and directs progenitors to DANs, retaining Foxa2 and Lmx1A/B expression (Kirkeby et al., 2017; Xi et al., 2012). Additionally, DAN with midbrain characteristics can be produced with overexpression of paired like homeodomain 3 (*Pitx3*) and *Nurr* genes in murine and human ESCs (Martinat et al., 2006).

In 2011, Pfisterer et al. reprogrammed human embryonic fibroblasts into midbrain mDANs with ectopic expression of the Foxa2 and Lmx1A, and BAM TFs (Pfisterer et al., 2011). Furthermore, in 2011, Caiazzo et al. showed that iDANs can be generated directly from mouse or human fibroblasts with Mash1 and mDAN-specific TFs Nurr1 and Lmx1A (Caiazzo et al., 2011). Also, in 2013, it was shown that human fibroblasts can be transformed into TH-expressing DANs with the Lmx1A, Foxa2, Lmx1B, and Otx2 transcription factors (Torper et al., 2013). In another study with TF-based DND, Oh et al. demonstrated that MEFs can be reprogrammed into TH^{+} DANs by using Ascl1 and Nurrl transctiption factors together with Shh and FGF8b neurotrophic factors (Oh et al., 2014).

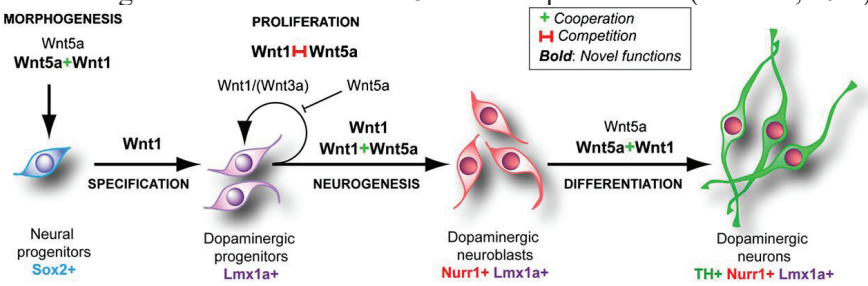

Figure 5. Signaling pathwyas of Dopaminergic Neurons development (Andersson et al., 2013).

3.3. GABAergic Neurons

GABAergic neurons are found in the adult brain as a primary source of neurotransmitter inhibitor by producing γ-aminobutyric acid. GABAergic neurons occur in the ventral telencephalon and then migrate to the cerebral cortex, hippocampus, olfactory bulb, and piriform(Anderson, Eisenstat, et al., 1997; Pleasure et al., 2000; Poitras et al., 2007). GABAergic interneurons, a heterogeneous group of cells, have a great variety. It can form various functional groups according to its morphological and

molecular properties and activities, as well as subclassified according to calcium-binding proteins and neuropeptide content (Kawaguchi et al., 1995; McBain & Fisahn, 2001). GABAergic interneurons regulate the activity of basic neurons as well as the organization of pyramidal neurons in the mammalian cerebral cortex. This is why GABAergic interneurons are vital to normal brain function, making them one of the important neuronal networks in the brain.

Dlx family genes with 6 members in mammals (*Dlx1*, *Dlx2*, *Dlx3*, *Dlx4*, *Dlx5*, and *Dlx6*) are very important in forebrain development. *Dlx1*, *Dlx2*, *Dlx5* and *Dlx6* genes expressed in telencephalon and diencephalon are expressed in differentiating GABAergic projection neurons and interneurons, as well as in neurons migrating from the basal telencephalon to the cerebral cortex (Anderson, Qiu, et al., 1997; Eisenstat et al., 1999; Stühmer et al., 2002). Inactivation of Dlx1 and Dlx2 causes a reduction of GABAergic interneurons in the cerebral cortex. The main reason for this situation is the deficiency in the migration of immature interneurons from the ventricular zone and SVZ of the telencephalon (Anderson, Qiu, et al., 1997; Poitras et al., 2007). Like Dlx1 and Dlx2, Ascl1, GS homeobox (Gsx) 1 (Gsx1), Gsx2, and Lhx6 transcription factors also support GABAergic neurons fate (Fode et al., 2000; Long et al., 2009; Miyoshi et al., 2010; Wang et al., 2013). The collaboration of forkhead box protein G1 (Foxg1) and Sox2 with Ascl1 is necessary for the progression of the GABAergic neuronal lineage in embryonic development (Colasante et al., 2015). In addition, the deficiency of the Nkx2.1, which is necessary to induce *Shh* expression and plays a role in the regionalization of cells, causes a decrease in cortical GABAergic neurons in mice (Horton et al., 1999; Sussel et al., 1999).

In the study based on these factors involved in neurogenesis, MEFs were directly conversed into GABAergic neurons using the factors Foxg1, Sox2, Ascl1, Dlx5, and Lhx6 (Colasante et al., 2015). In another direct differentiation study with MEFs, Vierbuchen et al. showed that these fibroblasts can be directly differentiated into neurons expressing GABAergic markers by using Zic1 and Olig2 in addition to BAM factors (Vierbuchen et al., 2010). In addition to fibroblasts, in the study conducted by Heinrich et al. in 2010, astroglias from the cerebral cortex were transdifferentiated into functional GABAergic neurons using the Ascl1 and Dlx2 (Heinrich et al., 2010).

3.4. Glutamatergic Neurons

Glutamatergic neurons that produce glutamate, the main stimulating neurotransmitter in the CNS, are produced in the forebrain dorsal telencephalon neocortical ventricular zone, then migrate to form the

cortical plate (Nadarajah & Parnavelas, 2002). Glutamatergic neurons, also exist in midbrain dopamine regions containing GABAergic and dopaminergic neurons, are mainly located in the rostro-medial ventral tegmental area (Nair-Roberts et al., 2008; Yamaguchi et al., 2007, 2013). In CNS development, most of the ventral progenitors in the forebrain differentiate into GABAergic neurons, while glutamatergic neurons are composed of dorsal forebrain progenitors. Extrinsic signaling pathways affect the expression of bHLH genes in telencephalic progenitors and direct their fate. For instance, the Wnt signaling that actives in progenitor cells, induces *Ngn2* expression, which leads to the production of glutamatergic projection neurons. Like Ngn2, Ngn1 is a very important factor in telencephalic development and is necessary for the formation of glutamatergic neurons. FGF provides Ascl1 expression and progenitors expressing Ascl1 also differentiate into either oligodendrocyte progenitor cells or inhibitory GABAergic neurons (Adnani et al., 2018). In addition, Notch signaling prevents the differentiation of NPCs by suppressing the expression of genes such as Ascl1, Nscl1, NeuroD1, and Ngn1/2 through Hes and Hey genes, which protect NPCs in the telencephalon (De La Pompa et al., 1997; Imayoshi & Kageyama, 2014). In 2011, in a DND study using TFs necessary for the formation of functional neurons such as Brn2 and Myt1l, overexpression of miR-124 was shown to induce the formation of glutamatergic, GABAergic and dopaminergic neurons from human postnatal fibroblasts (Ambasudhan et al., 2011). Also, in the study conducted in 2013, Xue et al. showed that fibroblasts can directly differentiate into glutamatergic neurons as well as GABAergic neurons by suppressing PTBs via miR-124 (Y Xue et al., 2013). In addition to *in-vitro* transdifferentiation studies, Pereira et al. demonstrated that mouse NG2 glia can be directly conversed into glutamatergic and GABAergic neurons by using of Ascl1, Lmx1A and Nurr1 factors *in-vivo* (Pereira et al., 2017).

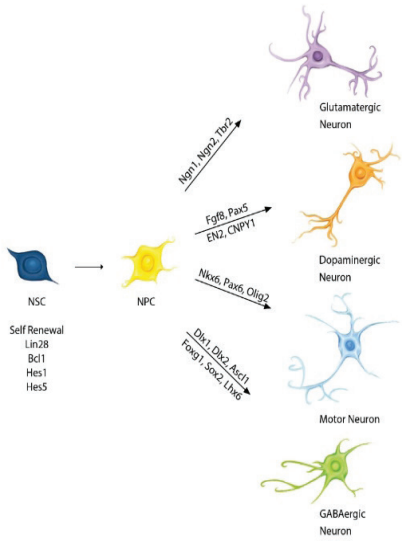

Figure 6. Transcription factors involved in neurogenesis of specific neuronal subtypes

Tablo 1. DND Strategies into Various Neuronal Subtypes

Initial cells	Species	DND factors	Target neuronal subtype	**References**
Fibroblast	Human	miR-9/9*-124, Isl1, Lhx3	Motor Neuron	(Abernathy et al., 2017)
Fibroblast	Mouse	Forskolin, ISX9, I-BET151, CHIR99021	Motor Neuron	(X. Li et al., 2015)
Fibroblast	Mouse	Brn2, Ascl1, Myt1l, Lhx3, Hb9, Isl1, Ngn2	Motor Neuron	(Son et al., 2011)
Fibroblast	Human	Ascl1, Ngn2, Sox2, Nurr1, Pitx3	Dopaminergic Neuron	(X. Liu et al., 2012)
Fibroblast	Mouse	Lmx1A, Foxa2, Ascl1, Brn2 or Lmx1B, Otx2, Nurr1, Ascl1, Brn2	Dopaminergic Neuron	(Sheng et al., 2012)
Fibroblast	Mouse	Ascl1, Nurr1, Foxa2, Pitx3, Lmx1A, EN1	Dopaminergic Neuron	(Kim et al., 2011)
Astrocyte	Mouse	Ascl1, Lmx1B, Nurr1	Dopaminergic Neuron (in vivo)	(Addis et al., 2011)
Fibroblast	Mouse	Ascl1, Foxg1, Sox2, Dlx5, Lhx6	GABAergic Neuron	(Colasante et al., 2015)
NG2	Mouse	Sox2	GABAergic Neuron (in vivo)	(Heinrich et al., 2014)
Fibroblast	Human	Brn2, Ascl1, Myt1l, NeuroD1	Glutamatergic Neuron	(Pang et al., 2011)

Astrocyte	Mouse	Ngn2	Glutamatergic Neuron	(Heinrich et al., 2010)
Astrocyte	Mouse	Ngn2, Bcl2	Glutamatergic Neuron (in vivo)	(Gascón et al., 2016)
Fibroblast	Human	miR-9/9*-124, NeuroD2, Ascl1, Myt1l	Glutamatergic and GABAergic Neurons	(Yoo et al., 2011)
Fibroblast	Mouse	Brn2, Ascl1, Myt1l, Zic1, Olig2	Glutamatergic and GABAergic Neurons	(Vierbuchen et al., 2010)
Fibroblast	Human	miR-124, Myt1l, Brn2	Glutamatergic, Dopaminergic and GABAergic Neurons	(Ambasudhan et al., 2011)
Fibroblast	Mouse	PTB knockdown by miR-124	Glutamatergic and GABAergic Neurons	(Y Xue et al., 2013)
Astrocyte	Mouse	Ascl1	Glutamatergic and GABAergic Neurons (in vivo)	(Y. Liu et al., 2015)
Fibroblast	Human	Ascl1, Foxa2, Lmx1B, FEV	Serotonergic Neuron	(Z. Xu et al., 2016)
Fibroblast	Human	Ascl1, Ngn2, Nkx2.2, FEV, GATA2, Lmx1B	Serotonergic Neuron	(Vadodaria et al., 2016)
Fibroblast	Human	miR-9/9*-124, Dlx1, Dlx2, Myt1l, CTIP2	Striatal Medium Spiny Neurons	(Victor et al., 2014, 2018)
Fibroblast	Human, Mouse	Ngn1, Brn3a or Ngn2, Brn3a	Sensory Neurons	(Blanchard et al., 2015)
Fibroblast	Human	Brn2, Myt1l, FEZF2	Cortical Neurons	(Miskinyte et al., 2017)

4. Neurodegenerative Diseases

NDs are characterized by progressive loss of neuronal populations due to high toxicity, metabolic disorders, oxidative stress, and abnormalities in neuroinflammation. NDs can be classified according to primary clinical features such as dementia, parkinsonism, or motor neuron disease (MND), the distribution of degeneration that neurodegeneration creates in various regions of the nervous system, or basic molecular abnormalities in the nervous system (Dugger & Dickson, 2017). In general, it is very difficult to obtain patient-derived neuronal cells associated with neurological diseases, which means that the source of cell replacement therapy for NDs is very scarce. However, the DND strategy facilitates the generation of patient-specific neuron types associated with these diseases *in-vitro*. We will later in this study look at the modeling of some neurodegenerative diseases through DND.

4.1. Huntington's Disease

Huntington's disease (HD) is a progressive ND with a different phenotype such as coordination disorder, motor, behavioral and cognitive decline. There is no developed treatment for HD, which is an autosomal dominant inheritance pattern, and success in defining the treatments studied on HD to date is very low. In HD, the excessive repetition of CAG codons in the huntingtin gene (HTT) leads to the formation of excessive protein clusters and progressive degeneration of striatal MSNs. The mechanism underlying the disease can be understood by evaluating the pathogenesis of HD with the transdifferentiation strategy, which provides easy access to these HD-related target neuronal cells. In this way, a patient-specific neuronal resource is provided for a possible cell replacement therapy after genomic manipulation and correction in HTT gene.

In 2018, Matheus B. Victor generated a specific MSN-based model for HD patients with the transdifferentiation strategy. In the study conducted by Victor et al, fibroblasts containing 40, 43 and 44 CAG repeats taken from HD patients were directly conversed into HD patient-derived MSNs by miRNA and TF-mediated transdifferentiation based on the study conducted by the same group in 2014 (Victor et al., 2014, 2018). As a result of the analysis of patient-specific MSNs obtained, it was observed that HD-MSNs contain mutant HTT aggregates. In the results observed in the study with transdifferentiation, it was indicated that in addition to the formation of HTT aggregates, HD-MSNs also showed basic HD-related phenotypes such as spontaneous neuron death in culture and a decrease in mitochondrial function (Victor et al., 2018). Analysis of patient-specific neuronal cells obtained from studies with such DND strategy at the cellular and molecular level, followed by genetic corrections in the next step, can make them suitable for cell therapy in HD and many other NDs.

4.2. Alzheimer's Disease

Alzheimer's Disease (AD), the most common cause of cerebral cortex dementia, is another ND characterized by degeneration of neurons and synapses. This irreversible ND, in which memory, behavioral, and social ability are gradually diminished, exhibits neurofibrillary tangles composed of β-amyloid plaque deposition and hyperphosphorylated tau protein (Keske et al., 2019). Loss of cholinergic neurons and functional degeneration in AD, which develops with progressive and chronic cognitive decline, is one of the main neural changes of the disease (Moghadam et al., 2009).

The strategy of direct conversion of patient-specific differentiated cells into neurons enables the evaluation of the cellular and molecular mechanisms of these changes underlying AD pathology. In addition, current treatments for AD, which is a very serious neurodegenerative

disease, are only seen as symptomatic, though DND strategy to these neurons has been seen as a promising therapeutic strategy for AD (Qiang et al., 2011; Yavarpour-Bali et al., 2020). In this regard, Ziyuan Guo showed that glial cells can be differentiated into functional neurons in the AD mouse brain model with the *in-vivo* DND study he conducted in 2014. With the study by Guo et al., AD mouse astrocytes were directed into glutamatergic neurons with the *in-vivo* DND strategy by retroviral expression of the neural TF NeuroD1; AD mouse NG2 cells have also been differentiated into glutamatergic and GABAergic neurons (Guo et al., 2014). Also, Wenxiang Hu showed in the study conducted in 2015 that fibroblasts obtained from AD patients can be turned into neurons by skipping the path to NPCs. Hu et al. transdifferentiated AD fibroblasts into neurons without going through the NPC stage, using Forskolin (F), SP600125 (S), a JNK inhibitor, GO6983 (G), a protein kinase C inhibitor, Y-27632 (Y), a Rho kinase (ROCK) inhibitor molecules together with Valproic Acid-V, CHIR99021-C and Repsox-R (VCR) small chemical molecules that direct the fate of mouse and human fibroblasts to NPCs (Hu et al., 2015). Thus, these studies offer an important strategy that enables the production of AD patients-specific neuronal cells, and this can provides the neural population required for cell replacement therapy.

4.3. Parkinson's Disease

PD is an ND characterized by progressive mDAN degeneration in the Substantia Nigra. This ND, whose prevalence increases with age, causes the death of neurons and then permanent movement disorders. PD patients-specific DAN generation with transdifferentiation approch enables them to characterize cell changes *in-vitro* by creating the possibility to mimic pathological processes of the disease as a model. DANs expressing midbrain-specific TFs for PD and rescuing some motor behavior can be directly differentiate from somatic cells by adopting human or animal neuro-morphology by transdifferentiation. And these obtained healthy DANs can replace degenerated and lost DANs in PD patients. This strategy can be developed as a treatment for PD disease by increasing efficiency, developing systems to target somatic cells to directly differentiate *in-vivo*, and ensuring safety in humans.

In this regard, in 2011, Jongpil Kim showed that tail-type mouse fibroblasts can transdifferentiation to mDANs with 6 TFs (Pitx3, Ascl1, Nurr1, Lmx1A, EN1, and Foxa2), which have important roles in neuronal differentiation (Kim et al., 2011). In addition to this study, Pia Cervo, in the study she conducted in 2017, demonstrated that human and mouse astrocytes can be differentiated directly into DANs by applying miR-and TFs-based DND strategy. With this alternative strategy, di Val Cervo et al. directly differentiated human astrocytes *in-vitro* and murine astrocytes *in-*

vivo with miR-218 and three TFs including NeuroD1, Ascl1, and Lmx1A into iDANs which allow correction of some aspects of spontaneous motor behavior (Rivetti Di Val Cervo et al., 2017). Consequently, in addition to the *in-vitro* transdifferentiation strategies, the *in-vivo* DND strategies may also be useful for cell replacement therapy, thus, the use of immunosuppression and the application of cell transplantation steps are no longer required.

4.4. Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is an ND that causes MN dysfunction and affects other cells as well, including astrocytes, oligodendrocytes, and microglia, causing muscle weakness and eventually death from ventilation failure. The progressive degeneration of MN, resulting in dysphagia, motor speech impairment, respiratory failure, and fatal paralysis, occurs in ALS, which is mostly sporadic and a small part is familial. The most common known cause of ALS, which is an autosomal dominant inheritance pattern, is defects in the 9th chromosome open reading frame 72 of the gene (C9orf72) seen in approximately 25-40% of all familial cases (Oskarsson et al., 2018). In addition, mutations in the Cu/Zn superoxide dismutase 1 (SOD1) gene on chromosome 21 are seen in more than 1-4% of sporadic ALS, and more than 20% of familial ALS (J. Liu & Wang, 2017; Rosen et al., 1993). Currently, there is no effective treatment for ALS, which is a very serious ND. DND strategy in this regard preserves age-related characteristics in MND and holds promise for ALS and many other MNDs, as it has great potential for therapeutic strategies (Tang et al., 2017).

In this regard, it was showed that MN cells can be efficiently produced from ALS patient-specific fibroblasts with mutations in the *FUS* gene. In this study conducted by Liu et al., the cellular mechanism underlying ALS patient-specific MN (ALS-MN) cells obtained with DND was examined and it was determined that these cells showed disease-specific degeneration. It has been shown in the study that degenerative features such as poor survival, hypoactivity, and inability to form functional neuromuscular junctions observed in ALS-MN can be saved by using small molecule (kenpaullone). In conclusion, Liu et al. describe the DND strategy as an effective and promising strategy for drug discovery by modeling the disease-related neuronal cells obtained in this study (M.-L. Liu et al., 2016).

4.6. Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is an ND characterized by motor neuron degeneration and progressive muscle weakness in the spinal cord. SMA, which is an autosomal recessive inheritance pattern, is caused by deletions or mutations in the survival of motor neuron (SMN) 1 (SMN1)

gene located on human chromosome 5q13, which encodes SMN proteins that play a very important role in the living of motor neurons. These variations in the SMN1 gene, seen in more than 95% of SMA cases, prevent neurons from sending signals to the muscles. This causes muscle weakness, the most common symptom of SMA (Fan & Simard, 2002; Kolb & Kissel, 2015). Besides the SMN1 gene, the copy number of the SMN2 gene, which produces a relatively small amount of functional SMN mRNA, changes the severity of the disease and thus helps determine which type of SMA develops (Arnold & Fischbeck, 2018; Kolb & Kissel, 2015). SMA, which is much seen especially in infants and children, and causes death, does not have an effective treatment available. Therefore, it is very important to define MN specific to SMA patients. In this regard, in the study conducted by Qi-Jie Zhang et al. in 2016, it was shown that fibroblasts can be directly reprogrammed into SMA patient-specific MN cells (SMA-MN) and modeled the SMA phenotype *in-vitro* on the SMA-MN cells produced. Zhang et al. generated SMA-MN with exhibiting reduced neurite growth rate and showing neuronal degeneration by forcing the expression of 8 defined TFs including Ascl1, Isl1, NeuroD1, Brn2, Hb9, Lhx3, Myt1l and Ngn2 in fibroblasts in their DND strategy (Zhang et al., 2017).

To sum up, this method is seen as an attractive approach for neural diseases-based personalized medicine, disease modeling, drug discovery, and RM. DND offers alternative cellular therapy strategies by change the somatic cells' fate into patient-specific neural cells. In this way, cell replacement therapy with the induction of functional neurons patient-specific from somatic cells with transdifferentiation is a promising approach which we discussed in this chapter. After all, NDs can be alleviated by direct differentiation of somatic cells to neurons using various transdifferentiation methods such as TFs-, miR- and small molecule compounds-based DND.

Abbreviations

AD	Alzheimer's Disease
ALS	Amyotrophic Lateral Sclerosis
ALS-MN	ALS patient-specific MN
Ascl1	Achaete-scute like 1
BAM	Brn2, Ascl1 and Myt1l factors
bHLH	Basic Helix Loop Helix
BMP	Bone Morphogenetic Protein
Brn2	Brain 2
CNS	Central Nervous System
CTIP2	Coup-interacting protein 2
DAN	Dopaminergic Neuron
DG	Dentate Gyrus
Dlx1	Distal-less homeobox 1
DND	Direct Neuronal Differentiation
EN1	Engrailed Homeobox 1
ESC	Embryonic Stem Cells
FGF	Fibroblast Growth Factor

Foxa2	Forkhead box A2
Foxg1	Forkhead box protein G1
GABA	γ-aminobutyric acid
GSK3β	Glycogen Synthase Kinase 3β
Gsx	GS homeobox
HD	Huntington's disease
HTT	Huntingtin
Isl1	ISL LIM homeobox 1
iDAN	Induced Dopaminergic Neuron
iNs	Induced Neurons
iPSC	Induced Pluripotent Stem Cell
Lhx3	LIM homeobox protein 3
LINE-1 or L1	Long Interspersed Element-1
Lmx1a	*LIM homeobox transcription factor 1 alpha*
Map2	Microtubule-associated protein 2
Mash1	Mammalian Achaete Scute Homolog-1
mDAN	midbrain Dopaminergic Neuron
MEFs	Mouse Embryonic Fibroblasts
miRNA	microRNA
MN	Motor Neuron
MND	Motor Neuron Disease
MSNs	Medium Spiny Neurons
Myt1l	Myelin transcription factor 1 like
NDs	Neurodegenerative Diseases
NeuroD1	Neuronal differentiation 1
Ngn1	Neurogenin 1
Nkx6	NK6 homeobox
NPC	Neural Progenitor Cell
Nurr1	Nuclear receptor related 1
OB	Olfactory bulb
Olig2	Oligodendrocyte transcription factor 2
Otx2	Orthodenticle homeobox 2
Pax6	Paired box 6
PD	Parkinson's Disease
Pitx3	Paired like homeodomain 3
PTB	Polypyrimidine Tract-Binding
RAS/ERK	Rat Sarcoma/Extracellular signal-regulated kinases
REST	RE1-Silencing Transcription
RMS	Rostral migration stream
SGZ	Subgranular Zone
Shh	Sonic hedgehog
SMA	Spinal muscular atrophy
SMA-MN	SMA patient-specific MN
SMN1	Survival of motor neuron 1
Sox	Sry-related-HMG box
SVZ	Subventricular Zone
TFs	Transcription Factors
TGFβ	Transforming Growth Factor β
Wnt1	Wnt family member 1
Zic1	Zic family member 1

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Chapter 13

EFFECTS OF TESTICULAR TORSION ON MALE REPRODUCTION

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INTRODUCTION

Testicular torsion is a urological problem that is associated with ischemia and may cause testicular dysfunction and requires urgent treatment (Pogorelić et al., 2013). Rotation of the testis blocks blood flow to the testis, interrupting venous drainage in the testis. If this situation continues, edema and ischemia develop (Turkmen et al., 2012). Since germ cells are exposed to hypoxia, especially in testicular torsion, necrosis may occur in germ cells depending on the duration of torsion. Continuation of this condition can cause subfertility and infertility (Gezici et al., 2006; Wei et al., 2011).

Testicular torsion is manifested by sudden onset scrotal pain. This pain can spread to the groin and lower abdomen from time to time. In some cases, there may be nausea and vomiting as well as pain (Boettcher et al., 2012). Causes of torsion include trauma, excessive exercise, and contraction of the cremaster or dartos muscles. It has been reported that torsion is more common in cases where the ambient temperature decreases (<2°C) (Shukla et al., 1982). Because the spermatic cord of the left testis is longer, left testicular torsion is more common than right testicular torsion (Fonkalsrud, 1987). Testicular torsion can be seen in two different ways, extravaginally or intravaginally. Extravaginal torsion as a result of rotation of the tunica vaginalis occurs during the descent of the testis into the scrotum (Vasdev et al., 2012). May occur prenatally or early postnatally (Nandi and Murphy, 2011). Intravaginal torsion caused by the rotation of the spermatic cord in the tunica vaginalis is seen in older children and adults (Witherington and Jarrell, 1990). Two peak incidences in intravaginal torsion are 1 to 5 and 11 to 15 years of age (Mizrahi and Shtamler, 1992). It has been reported that the age at which torsion occurs affects the last pregnancy rate and the time to pregnancy (Zhang et al., 2021).

In testicular torsion, inappropriate or delayed treatment will lead to male infertility, therefore early diagnosis and appropriate treatment are required to protect the testicles (Gielchinsky et al., 2016). Blood flow to the ischemic testis should be restored as soon as possible (Bartsch et al., 1980; Romeo et al., 2010; Thomas et al., 1984). In clinical practice, the decision to preserve or remove the torted testis is determined by the duration of ischemia and the intraoperative appearance of the testis at the time of surgical examination. If the testis appears salvageable, an orchiopexy may be performed, in which the testis is fixed to the scrotum. Completely black and necrotic testicles should usually be removed by performing an orchiectomy (Castañeda-Sánchez et al., 2017; Romeo et al., 2010). Survival of the torted testis depends on the degree and duration of torsion (Jacobsen et al., 2020). If treatment is started within the first 6 hours of the onset of symptoms in testicular torsion, the rate of saving

the testicles is 90% to 100%. It is 20% to 59% in the first 6-12 hours. If the intervention is done between 12-24 hours, this rate falls below 10% (Pogorelić et al., 2016). However, despite successful surgery, testicular atrophy and infertility may develop in 40-60% of patients in the following years (Cummings et al., 2002).

Sperm and all cells in the body produce reactive oxygen species (ROS) during their normal metabolism (Dokmeci et al., 2007). Produced ROS have very important roles in normal physiological processes in the body (cell differentiation, sperm capacitation, acrosome reaction, fertilization, etc.). However, in situations that cause stress in the body, when ROS rise above physiological limits, they cause oxidative stress (Du Plessis et al., 2015; Ichikawa et al., 1999). Excessive production of ROS under oxidative stress causes organ dysfunction, DNA damage and increased local inflammatory response. Inflammatory cascades and oxidative stress can then cause cytokine storms, causing damage to cellular structures (Ornellas et al., 2017). In addition, overproduced ROS damage mitochondrial membranes, cell membrane lipids, proteins and DNA, leading to apoptosis of germ cells in the testicles (Kostakis et al., 2017). Apoptosis occurs in both physiological and pathological stages (Elmore, 2007). In the process of spermatogenesis, apoptosis plays an important role in maintaining homeostasis. However, apoptosis occurring in spermatogenic cells during testicular ischemia/reperfusion injury may damage spermatogenesis, resulting in impaired testicular function and infertility (Lysiak et al., 2003).

Testicular tissue is very sensitive to free radicals as cell division is active and spermatogenesis is constantly ongoing (Agarwal et al., 2008). Enzymatic antioxidants [(superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)] and non-enzymatic antioxidants (zinc, vitamin C, vitamin E, melatonin and cytochrome C) naturally found in the body effectively protect the testicles (Aitken and Roman, 2008). However, when excessive free radicals are produced in ischemia/reperfusion injury, the available enzymes are insufficient to scavenge these free radicals (Dogan et al., 2016).

It has been reported that hemorrhagic infarction occurs 2 hours after the onset of testicular torsion, irreversible changes occur in the parenchyma after 6 hours, and complete necrosis is observed after 24 hours (Yang et al., 2011). Studies have reported that testicular necrosis develops within 2 hours in arterial occlusion and within 6 hours in venous occlusion (Melekos et al., 1988). In testicular torsion studies, when the testicles of rats were examined during detorsion, it was noted that the testicles were purple in color, edematous, and the presence of serohemorrhagic fluid around them (Samy et al., 2020). In experimental studies, it has been determined that ischemia in testicular torsion causes loss of spermatogenesis in 4-6 hours

and hormonal function loss in 10-12 hours (Bartsch et al., 1980; Smith, 1955). In the case of hypoxia developing during torsion and reperfusion injury after detorsion, the testis is damaged in both cases (Turner and Brown, 1993). It has been reported that testicular ischemia/reperfusion injury causes a significant increase in oxidative stress and inflammation, causing germ cell apoptosis and damaging spermatogenesis (Kohsaka et al., 2021).

While studies claimed that the contralateral testis is not affected in case of unilateral testicular torsion (Madgar et al., 1987), other studies have suggested that the contralateral testis is affected by this condition **(**Aydıner et al., 2012; Cankorkmaz et al., 2009; Jacobsen et al., 2020; Kaplan, 2000; Samy et al., 2020). There is also a claim that the integrity and function of the testis is impaired if the damaged testis is not removed by orchiectomy in long-term torsion (Filho et al., 2004).

EFFECTS OF TESTİCULAR TORSİON

1-The effect of testicular torsion on sperm parameters

Ischemic damage occurs in testicular torsion, and reperfusion damage occurs after detorsion (Akgür et al., 1993). Especially during reperfusion injury, large amounts of ROS are formed. These ROS play a role in sperm damage (Agarwal and Said, 2005). Sperm are not resistant to these free radicals produced during reperfusion because they contain large amounts of polyunsaturated fats and the amount of antioxidant enzymes in their cytoplasm is low (Al-Maghrebi and Renno, 2016). Testicular tissue is a very sensitive tissue against free radicals, as cell division is very active and spermatogenesis continues continuously. It therefore consumes a lot of oxygen and is extremely vulnerable to oxygen depletion (Agarwal et al., 2008). It has been reported that apoptosis caused by ischemia/reperfusion injury in the germ cell leads to loss of spermatogenesis (Turner et al., 1997). During ischemia, the amount of oxygen falls below levels that can meet metabolic needs. In addition, the amount of oxygen in cellular energy stores decreases and toxic metabolites begin to accumulate and germ cells die (Mogilner et al., 2006). In particular, ROS formed during reperfusion stimulate the formation of inflammatory cytokines. This causes germ cell apoptosis and loss of spermatogenesis (Altavilla et al., 2012; Li et al., 2019; Turner et al., 2004).

In a study conducted on 67 patients with testicular torsion; The patients were followed up for 4 years after torsion and the semen quality was evaluated. It was found that sperm motility and morphology were abnormally low in 64% of these patients, and sperm count was less than 20 million in 39%. Semen parameters were within the normal range in only 14% of patients (Mellick et al., 2019).

Ghasemnejad-Berenji et al. (2017), in their testicular torsion study on 72 rats, they performed the torsion by bending the right testis 720° clockwise for 1 hour. They gave the metformin they administered in their studies at a dose of 300 mg/kg half an hour before the detorsion. They found that metformin significantly increased the sperm count and sperm motility, which decreased with torsion/detorsion. Kheirollahi et al. (2018), in their study on 40 rats, divided the rats into 4 groups and applied torsion/detorsion on 3 groups. In the torsion groups, they created the torsion by keeping the relevant testis bent 720° for 90 minutes. Then, detorsion was created and sperm evaluation was made after 50 days of detorsion. Researchers administered 150 mg/kg troxerutin to one of the 3 groups in which they created torsion/detorsion, and 20 mg/kg vitamin C to the other group. They found that the sperm count in the group treated with troxerutin was higher than the control group, and the sperm count in the group treated with vitamin C did not show significant differences compared to the control group. Davoodi et al. (2020), divided the rats into 3 groups in their study on 18 Wistar albino rats. They applied 720° torsion to the left testicles of the rats in the torsion group and performed the detorsion 2 hours later. They administered salvia miltiorrhiza hydroalcoholic extract intraperitoneally 30 minutes before detorsion to rats in the same group. It was determined that progressive motility was significantly lower in the torsion/detorsion group compared to the sham group, and non-progressive and immotile motility was significantly higher than the sham group. They determined that the progressive motility of the group to which they administered the salvia miltiorrhiza hydroalcoholic extract was significantly higher than the torsion/detorsion group. It was observed that sperm viability was significantly lower in the group to which they applied torsion/detorsion and extract compared to the sham group. However, there was no difference in sperm viability between the torsion/detorsion group and the extract group. It was also reported that the sperm count and normal sperm morphology of the group in which they administered the extract were significantly higher than the torsion/detorsion group and the sham group.

In a study investigating the effect of L-carnitine and betamethasone on ischemia-reperfusion injury, 4 groups were formed. Betamethasone was administered to one of the groups and L-carnitine to the other. The ischemia time was determined as 6 hours and the reperfusion time as 12 hours. Sperm count and motility were found to be significantly higher in the betamethasone group compared to the control group. Again, it was determined that sperm viability was higher in betamethasone and L-carnitine groups compared to the control group. As a result of their findings, the researchers reported that betamethasone and L-carnitine may be beneficial in ischemia-reperfusion injury (Kazemi-Darabadi et al.,

2019). In another experimental study, 96 rats were divided into 6 groups in equal numbers. Torsion was created by bending the right testis 720° clockwise for 1 hour. It was then allowed to remain in detorsion for 4 hours. The effect of Cyclosporine A has been investigated (Yazdani et al., 2019).

Raisi et al. (2020), divided 24 rats into 3 groups and determined the torsion/detorsion times as 2 hours. They found that nitroglycerin administration significantly increased sperm motility and the percentage of live sperm. They reported that there was no significant difference in sperm concentration between the torsion/detorsion group and the sham group, but there was a significant difference in terms of motility and sperm viability. In a study investigating the effects of verapamil and heparin on sperm parameters in testicular torsion, it was reported that co-administration of verapamil and heparin gave significantly better results than verapamil injection alone (Davoodi et al., 2020).

2-The effect of testicular torsion on biochemical parameters

Testicular tissue contains some antioxidant enzymes [malondialdehyde (MDA), SOD, CAT, GPx] that neutralize free radicals produced during physiological events under normal conditions. However, the excessive amount of ROS generated during ischemia/reperfusion injury causes these enzymes to be insufficient (to scavenge all free radicals) (Agarwal and Said, 2004). Excessive oxygen delivery to the tissues after detorsion causes excessive production of ROS. Increased ROS causes tissue damage by damaging cell membranes. This process, called "ischemia/reperfusion injury", results from an imbalance between ROS and antioxidant defense mechanisms (Caglayan et al., 2014).

In an experimental study, 720° torsion was applied to the left testicles of rats and chrysin was administered 30 minutes before detorsion. In the torsion/detorsion groups, MDA levels, tumor necrosis factor-α (TNF-α), interleukin-4 (IL-4), interleukin-6 (IL-6) and interleukin-10 (IL-10) levels were increased. In the study, chrysin administered for treatment decreased MDA and TNF-α levels (Belhan et al., 2020). In another study, glutathione (GSH) levels, SOD and GPx activities decreased, while MDA levels increased in testicular tissue of torsion/detorsion groups. It has been determined that the silymarin administration gives an opposite result to the values in the specified parameters (Belhan et al., 2021). In other studies, it was reported that the tissue MDA level increased and CAT, SOD and GPx activities decreased compared to the sham operation group. However, SOD, CAT, and GPx activity were significantly have increased with metformin injection and minocycline administration (Azarabadi et al., 2020; Ghasemnejad-Berenji et al., 2018). In addition,

it was reported that the increase in the expression levels of interleukin-1β (IL-1β) and TNF-α genes observed with torsion/detorsion application decreased significantly with minocycline administration (Azarabadi et al., 2020). Raafat et al (2021), in their experimental study, reported that obestatin reduced testicular damage after testicular ischemia reperfusion by reducing oxidative stress and weakening the inflammatory response. In another study, it was determined that the increased MDA, protein carbonyl (PC), nitric oxide (NO), TNF-α, IL-1β and IL-6 levels in torsion groups were reversed with sinapic acid administration (Unsal et al., 2021). Kohsaka et al (2021), in their experimental testicular torsion study, reported that the relaxin, significantly reduced myeloperoxidase (MPO) activity by decreasing IL-6 and TNF-α expression. It was determined that salvia miltiorrhiza hydroalcoholic extract and nitroglycerin decreased MDA levels and increased GPx and CAT levels in testicular torsion studies (Davoodi et al., 2020; Raisi et al., 2020). Samy et al. (2020) found that the platelet-rich plasma they administered in their study greatly improved testicular degeneration by reducing IL-1β, TNF-α levels and increasing GSH levels. In other testicular torsion studies, it was reported that rapamycin and metformin decreased MDA levels and increased SOD, CAT and GPx activities (Ghasemnejad-Berenji et al., 2017; Ghasemnejad-Berenji et al., 2018).

3-The effect of testicular torsion on histopathological and immunohistochemical parameters

In the literature research, it was understood that the histological structure of the testis was damaged in the case of ischemia / reperfusion, and the damage was alleviated by the administration of some chemical agents. Belhan et al. (2020) found that caspase-3 and caspase-8 expression increased in torsion/detorsion groups in their testicular torsion study in rats. They reported that caspase-3 and caspase-8 expression levels decreased with Chrysin administration. In another study, a significant increase in the number of TUNEL positive cells was observed in the ischemia/reperfusion group. In addition, an increase in the expression levels of Bcl-2-associated x (Bax) and caspase-3 genes, and a decrease in B-cell lymphoma (Bcl-2) were detected. After applying minocycline 30 minutes before detorsion and continuing for 8 weeks, Johnson score, height of seminiferous tubule epithelium, mean seminiferous tubule diameter increased significantly. There was also a significant decrease in caspase-3 and Bax expression levels. Minocycline Bcl-2 also increased the expression levels of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) genes. Researchers reported that minocycline reduces the rate of germ cell apoptosis (Azarabadi et al., 2020).

Raafat et al. (2021), in their study on 30 adult rats, they performed the

torsion by bending the right testicles 720°. The researchers observed that the levels of Bax, caspase-8 and caspase-3 increased, while the expression level of Bcl-2 decreased in the group to which they applied torsion. They reported that obestatin administered before detorsion decreased Bax, caspase-8 and caspase-3 levels and increased Bcl-2 expression levels. In different studies, it has been determined that sinapic acid and relaxin decrease the increased caspase 3 level during ischemia/reperfusion and increase the decreased Bcl-2 expression level (Kohsaka et al., 2021; Unsal et al., 2021). Raisi et al. (2020) and Kazaz et al. (2020) reported that testicular torsion caused a decrease in the Johnson score, germinal epithelial cell thickness and seminiferous tubule diameter, and nitroglycerin and berberine corrected these negativities. Also, Davoodi et al. (2020) reported that germinal epithelial cell thickness and mean seminiferous tubule diameter decreased in torsion groups, but these parameters increased in groups where salvia miltiorrhiza hydroalcoholic extract was administered. Samy et al. (2020) detected necrosis in spermatogenic cells, separations in the basement membranes of the seminiferous tubules, and deterioration in the interstitial tissue of the testicles in the torsion group. They also reported that there was an increase in caspase-3 expression and a decrease in Bcl-2 expression, and these values improved with platelet-rich plasma administration.

4-The effect of testicular torsion on reproductive hormones

Sangodele et al. (2021), in their study evaluating the effects of testicular torsion on reproductive hormones; They found that there were significant decreases in follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels in the ischemia/reperfusion group. Researchers have observed that there is an increase in the related hormone levels after proxeed plus application. In another study, it was reported that there was no change in reproductive hormones in testicular torsion (Unsal et al., 2021). Kheirollahi et al. (2018) used vitamin C and troxerutin for treatment in their experimental study to investigate the effect of testicular torsion on the pituitary-gonadal axis. The researchers found that FSH, LH and testosterone levels were higher in the troxerutin administered group than in the control group. They only observed an increase in testosterone levels in the vitamin C administered group. In another study, 4-hour torsion was applied to the left testicles and hormonal parameters were evaluated. It was determined that the FSH and LH values of the ischemia/reperfusion group were higher than the group in which platelet-rich plasma was administered. However, it has been reported that the testosterone level of the platelet-rich plasma group was significantly higher than the Ischemia/reperfusion group (Kutluhan et al., 2021).

CONCLUSION

Based on the reported study results, it is understood that testicular torsion is a urological problem that requires urgent treatment. The degree and duration of torsion are key to the survival of the torted testis. The detorsion procedure performed for therapeutic purposes alone is insufficient to correct the testicular damage due to the reperfusion injury. For this purpose, many chemical agents with antioxidant, anti-inflammatory and anti-apoptotic properties have been used in addition to surgical intervention. However, the desired success in this field has not been achieved yet. Therefore, more experimental and large-scale prospective clinical studies are needed to preserve fertility in testicular torsion.

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Chapter 14

RADIOGRAPHIC EXAMINATION IN PAEDIATRIC PATIENTS

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INTRODUCTION

Radiographic images should not be used unless they contribute to the diagnosis in children. The age of the child is not a criterion for taking radiography, the necessity of radiological examination in addition to the clinical examination should be determined for each patient after the medical and dental history (Büyük 2018). It is known that x-rays used in dental radiology have ionizing effects. The sensitivity of cells to ionizing radiation is related to the rate of mitotic activity, cells with a high rate of mitosis are more sensitive to radiation. Mitotic activity in children is about 10 times faster than in adults, and therefore children are more vulnerable to ionizing radiation. The rate of sensitivity to radiation is highest in newborns and decreases with increasing age (Stutzki et al. 2015).

The radiology department and the technical equipment in the department, the sounds of the devices are foreign for the children. During the acquiring images, children may need to be separated from their parents or favourite objects. The methods and verbal commands used to prevent movement during the procedure may be perceived as frightening and may cause discomfort. These factors of stress can lead to paediatric responses such as fear, anxiety, and crying. For this reason, cooperation should be made with paediatric patients who can contacted, they should be approached positively and support should be provided (Linder 2017).

Caries diagnosis is the primary reason for referral to radiographic examinations in paediatric patients. This is followed by the evaluation of traumas to the dento-alveolar region, tooth development and pathological conditions (Madan et al. 2015). Bite radiographs (figure 1) are used in the determination of aproximal caries in cooperative children without fear of the dentist, and periapical radiographs (Figure 2) are used in the evaluation of the apical region (Scarfe et al. 2009). In addition to the purpose of evaluating the jaws, primary and permanent dentition as a whole, panoramic radiographs (Figure 3) are used in children who cannot cooperate adequately, have a nausea reflex, and cannot effectively apply intraoral radiographs due to the fear of swallowing the film. Before orthodontic treatments, cephalometric radiographs in which maxillofacial anomalies and the compatibility of the jaws are evaluated are other two-dimensional radiographic examinations that are frequently applied (Scarfe et al. 2017).

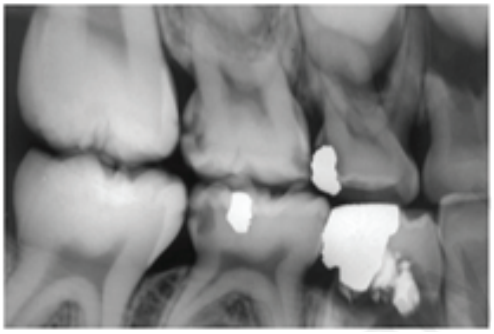

Figure 1: *Bite-wing radiographs used in the diagnosis of interface caries in compatible paediatric patient*

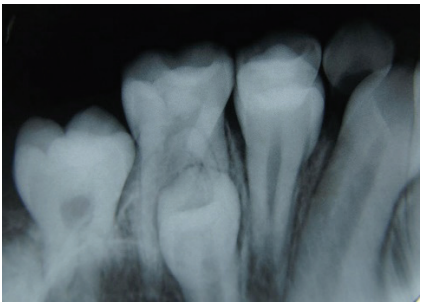

Figure 2: *Periapical radiographs used in the diagnosis of apical lesions in compatible paediatric patient*

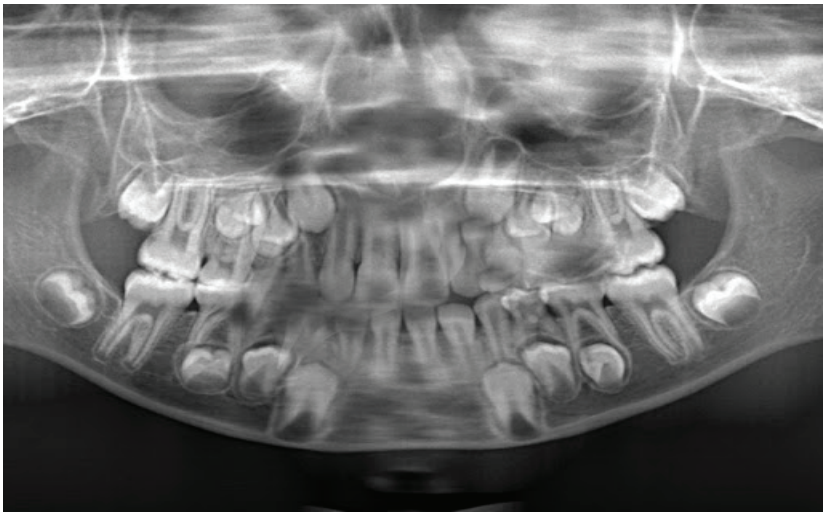

Figure 3: *Panoramic radiographs used in the evaluation of the whole dentition*

It is known that panoramic and cephalometric radiographs have disadvantages such as magnification and superposition. In cases of trauma of craniofacial region, extensive pathologies, the evaluation of relations

with tooth germs and adjacent anatomical structures two-dimensional radiographs are insufficient. Cone-beam computed tomography (CBCT), computed tomography (CT) and magnetic resonance imaging (MRI) which allow cross-sectional examination instead of these methods, offer a more detailed diagnostic examination. In children, CBCT is preferred as an alternative to CT in the examination of dento-alveolar morphology, craniofacial anomalies and pathologies due to its low radiation dose(Scarfe et al. 2017; Stutzki et al. 2015). CBCT offers good spatial resolution and detailed reconstruction with low radiation dose compared to CT. Although the noise increases due to the low dose, it is known that the image quality is sufficient in terms of diagnosis. One of its other important advantages over CT is its use in patients with claustrophobia due to it is an open device and shooting can be done in a short time like 6-36 seconds thanks to the cone-beam geometry (Stutzki et al. 2015; Keriş 2017). On the other hand, when deciding on CBCT in paediatric patients, the diagnostic benefit should be considered. An appropriate size imaging area should be used, and the dose should be limited by adjusting the dose according to the age and body weight of the child. Also, repetition of imaging should be avoided.

It is known that the patient's movement while acquiring CBCT images adversely affects the image quality. When the patient moves during the acquisition of image, the signal cannot be received from the scanning area; accordingly, black and white lines, double contour formation and loss of clarity may occur in the reconstruction image. Especially when it is desired to increase the geometric resolution by choosing a small voxel size, the sensitivity of the detector to motion also increases (Schulze et al. 2011). Although the shooting time is short, it is difficult to prevent minimal movements such as breathing, heartbeat and swallowing, and it is not always possible to make a child patient who is afraid of the device stand still. In one study, they stated that the main reason for patient movement during CBCT scan was that the patient was younger than 16 years of age (Spin-Neto et al. 2015). Proper fixation of the patient's head with the device's apparatus during the scanning process helps limit patient movement. It is foreseen that the production of faster detectors in the future will shorten the scanning time and decrease the sensitivity to motion (Schulze et al. 2011). However, these measures may be insufficient in paediatric patients with dental phobia (Büyük 2018).

In paediatric patients with dental anxiety or fear, communication can be achieved with correct behaviour guidance techniques and dental procedures can be performed (Baier et al. 2004; Büyük 2018). Even in children with dental fear, compliance during the treatment shows that the physician can communicate with the child with the right behaviour management techniques (Baier et al. 2004). In dentistry radiology, anxiety

or fear may develop in paediatric patients, either because they do not know how the device works, or because of the fear that they will get hurt. In order to prevent this situation, behaviour management techniques applied to paediatric patients should be used.

Analogies such as a camera for an x-ray device used in radiology and a photograph/picture for radiography can be made. With the tell-show-apply technique, verbal information can be given about the radiological examination to be performed according to the understanding level of the child patient. By using the direct observation technique, the child can be watched through the lead-protected glass while the device is running empty or while radiography is being taken from another patient (Büyük 2018). Afterwards, radiographs can be taken from the child patient with the same verbal explanations (Figure 4). In the child patient who is worried about being alone in the room where the device is located, the parent may be with the child by wearing a lead apron. It may be necessary for the physician to change the tone of voice, to call out to the child in an authoritative tone, in order for the child to adapt to commands such as staying still during the radiographs to be taken. Non-verbal communication methods such as body language, posture and facial expression of the person who will perform the examination also affect the cooperation with the child patient (Büyük 2018; Abanto et al. 2017). In addition, as with the positive encouragement technique, if the child patient fulfils all the duties that fall on him during the radiograph, thanks to the appropriate motivation, small rewards determined according to the age and needs of the child such as a sticker, pen, notebook or courage diploma can be presented (Xia and Song 2016). Protective stabilization or sedation techniques can be applied in a controlled manner in cases where the patient's age is too young to cooperate or when behaviours such as severe pain cannot be controlled in the patient.

Figure 4: *Panoramic scanning from a cooperative pediatric patient*

Images can be obtained from CBCT devices in a sitting, standing or supine position depending on the production method (Figure 5) (Doğan et al 2015). Although images can be obtained easily with devices in a sitting position, the image quality may deteriorate significantly since the risk of head movement is high in this position (Scarfe et al. 2009). As it is known, undesirable image disorders such as magnification, distortion and superposition occur in traditional radiographic imaging in children, especially in the mixed dentition period (Kamburoğlu et al. 2009). This is not the case with images obtained with CBCT systems.

Figure 5: *CBCT devices that can obtain images in the sitting or supine position*

One of the most important advantages of CBCT is the ability to

organize and view data in three dimensions on personal computers and archive them (Howerton Jr et al. 2008). After the obtained data is edited on the computer, two- and three-dimensional images can be obtained from any angle and it can be examined in axial, sagittal, coronal or different planes with the help of computer manipulation (Hashimoto et al. 2003; Iwai et al. 2000).

However, there are some disadvantages of the CBCT device such as the device is expensive, at least 4-5 hours of training are required to examine the image data on the CD, a fast computer is required for the analysis of the images, time consuming to the examination and analysis of the images, radiation dose higher than conventional dental radiographs, requirement of monthly and annual calibrations of CBCT devices to maintain image quality, the environment in which the device is located must be at room temperature (Doğan et al. 2015).

It is a fact that new horizons are opened in diagnosis and treatment planning in dentistry with the widespread use of CBCT systems day by day, but it is thought that radiation dose and device cost should be reduced in order for CBCT to replace traditional radiography in the future(Doğan et al 2015; Büyük 2018).

As a result, CBCT is a diagnostic method that has many indications, obtains images with a lower dose than CT, and is widely used in paediatric dentistry radiology. Anxiety and fear caused by the device and the shooting conditions in the child can generally be controlled with behaviour management techniques. Making use of colourful, illustrated decoration options or objects that may attract the attention of children in radiology clinics, as in Pedodontics clinics, can facilitate effective communication with the child.

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Chapter 15

NEUROMUSCULAR DISEASE AND SOCIAL WORK

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Introduction

Social work works in different social work environments with many different client groups such as women, children, elderly, disabled, immigrants, adolescents, patients and families. Accordingly, generalist social work practice has been adopted in the social work trainings given in Turkey. Students who receive education in this field are trained to serve clients from all groups by learning basic knowledge and practice skills from the field of social work.

Disability is one of the important working areas of social work. Micro, mezzo and macro level services are offered in this area. While the trainings received in the field of social work provide the ability to evaluate disability in many aspects and to develop practices, there are deficiencies in the evaluation and application of the disability according to the types of disability and rare diseases.This situation requires social workers to receive training on social work practice with special groups. Neuromuscular diseases are one of these special areas. There is no specific training in this field in social work education. In order to increase the effect of the service to be given to this group of patients, studies in this area are very valuable.

In this study, it is aimed to reveal the need for social work in neuromuscular diseases. In this context, first of all, social problems that arise in children and families due to neuromuscular diseases will be addressed, and social work intervention proposals will be presented together with the biopsychosocial model.

Children

Neuromuscular diseases are often struggled with physical and motor problems. However, the disease is often accompanied by some specific psychological and social problems. Hendriksen, Vles (2006) et al. (2009) stated that two types of problems have a significant influence on the general functioning of children with Duchenne. These are children's problems with learning potential and with psychosocial adjustment.

Children with chronic diseases are three times more likely to have psychiatric disorders and are also at risk for social and school adjustment problems (Cadman et al. 1987, Davis 1993).

It is accepted that children with disabilities are limited in their activities in society due to environmental restrictions and their access is limited due to wheelchair use, as a result of which their daily activity patterns are less varied and they experience more social isolation (Law & Dunn 2001).

Older children with disabilities tend to spend more time watching television and are five times more likely to be socially isolated than children without chronic illness (Cadman et al. 1987). However, family life plays an extremely important role in the lives of people with disabilities (Bach et al. 1991).

Family

Caregivers experience a heavy emotional and physical burden (Boyer, Drame, Morrone, & Novella, 2006). Thomas, Rajaram and Nailini (2014) concluded in their study that caregiver parents are worried about the future of their children. In addition, parents saw that they needed more information about the disease, had problems in interpersonal and family functioning, and were worried about child care.

In a study of parents of children with DMD, Chen, Chen, Jong, Yang, and Lue (2003) identified the following as their main concerns and problems:

• obtaining information about the progression of the disease,

• coping with the disease,

• receiving health services,

• find support groups.

• supporting the child,

• maintaining a close couple relationship and

• coping with fatigue.

In high-risk diseases, it is necessary to evaluate how prepared the family is for a bad course. Thus, the family can be given the opportunity to express their feelings; the present situation deviates from the future situation. It is generally stated that this process increases families' sense of control (Rolland, 1994).

Problems for people with disabilities and their families have been identified elsewhere, many of them social; for example, relationships, family roles, isolation, boredom, loneliness, lack of social interaction and lack of recreation (Toombs et al. 1995). Physical impairment can be seen as a social stigma and can lead to social feelings. isolation (Emery 1993) because society attributes many negative connotations to a visible physical disorder.

Cultural beliefs about disability are also very important. It is important for families' perceptions of disability and affects how families behave in terms of treatment, prevention and rehabilitation (Sen, 1988).

Social Work's Biopsychosocial Approach

Today, the importance of the biopsychosocial approach in the delivery of health services is increasing. The biopsychosocial model considers the biological, social, environmental, psychological and behavioral aspects of illness. This considers the social model along with the traditional model of medical health care, which focuses primarily on the biological causes of disease.

For example, a biopsychosocial model of health care considers patients' ability to purchase recommended medications when creating a treatment plan for patients, rather than focusing solely on laboratory results and physical condition, as a medical model approach would. Because socioeconomic opportunities and problems also contribute to a treatment that is aimed to be effective.

In this section, evaluation and intervention in neuromuscular diseases will be discussed in terms of biopsychosocial model.

Assessment

A comprehensive social history taking and evaluation is necessary for an effective social work intervention. This is an important component that will also determine the effect of medical intervention. As children, adolescents or adults with neuromuscular disease may have different social needs and problems, they should be taken into account in the evaluation.

Birnkrant et al. (2018) emphasized the importance of working together with professionals working in this field in the evaluation of psychosocial care and creating an intervention plan in working with those with neuromuscular diseases and their families. They also explained how to make an assessment and develop interventions in terms of outpatient care, early and late stages, children, adolescents and adults.

The importance of performing a basic assessment during the first year of diagnosis during the ambulatory or childhood stage was emphasized. Again in the same stage;

• Conducting a developmental or neuropsychological assessment when there are social or emotional concerns or cognitive delays,

• Evaluation by a speech language therapist for children with suspected speech or language development delays,

• Evaluation by a social worker at or after diagnosis is recommended.

In this stage, the social worker should use approaches such as the ecological system, the perspective of powers, the developmental model, and make an assessment at micro, mezzo and macro levels. Taking a good

social history will facilitate the solution of social problems that may arise during the treatment and care process, and will increase the effectiveness of medical care and treatment.

Birnkrant et al. (2018) in early non-ambulatory stage, or adolescence, or young adulthood;

• Conducting an assessment to identify cognitive or learning problems when there are concerns about school performance,

• Recommends an assessment during the transition to adulthood to assess whether public assistance is required.

The social worker should evaluate the social environment factors that affect school performance while making evaluations in this stage. In addition, it should determine the services provided by public and local governments and evaluate the needs of those with neuromuscular diseases and their families.

Birnkrant et al. (2018) in late non- ambulatory stage or adulthood;

• An assessment is made when there are concerns about a change in functionality or ability to manage day-to-day affairs,

• Evaluation by a speech language therapist for patients with loss of functional communication ability and impairment, chewing difficulties or dysphagia,

• He suggested that the needs of the patient and his family be evaluated by the social worker.

During this stage, the social worker should evaluate the social support systems, economic conditions, and the necessary conditions for the uninterrupted continuation of medical care. In terms of vocational rehabilitation, the possibilities of the patient and their families should be investigated.

Invervention

The following issues should be considered while developing an intervention plan as a result of the problems and resources that emerged after the evaluation. According to this;

• When mental health problems are detected for the patient and his family, he should be referred to a psychiatrist, child and adolescent mental health specialist or a psychologist,

• Necessary arrangements should be made at the school for the safety, health and accessibility of the patient in the school environment, and measures should be taken to prevent the interruption of education

regarding the absence of school attendance,

• Targets for the future of education and profession should be determined,

• Plan to educate parents, teachers and all school staff about neuromuscular diseases,

• Resources should be provided to patients and their families and school administration to educate peer groups on neuromuscular diseases,

• Social skills training should be given when necessary,

• Encourage patients and their families to be active and assertive,

• The patient and his family should be supported to defend their own rights and independence,

• It should be supported for the transition to continuing education, vocational education and generalized education with individualized education programs,

• Arrangements should be made to meet the business needs of the patient and his family,

• If the patient's health is at risk because adequate care is not provided in the living environment, information and guidance should be provided for home health services,

• Inform the patient and his family about palliative care,

• Plan support services (such as day care, home care services) for family members of caregivers,

• Hospice care should be provided for patients at the end of life (Colvin et al., 2018).

Teamwork in Health

Teamwork in health services; consisting of different health professions and members, whose common goal is to meet the patient's demand for health care at the highest level by providing the most comprehensive and quality health care to the individual, who have come together to share their expertise, different skills, knowledge and experience to achieve this goal; It is a union where each of them fulfills their own duties, information and experiences are constantly shared, joint decisions are taken and decisions are implemented together (Öğüt and Kaya, 2011).

Cowles (2003) listed the specific goals necessary for maximum team collaboration:

• role clarity and flexibility;

- mutual respect and trust;
- consensus on group norms, values, commitment and purpose;
- an egalitarian attitude; sense of equal importance;
- a sense of group bond and interdependence rather than autonomy;
- open communication and sharing;
- flexible leadership and decision making; shared power;
- flexible membership structure based on case needs;
- a stable core membership;
- a sense of both group and professional identity;
- the ability to negotiate and reach consensus;
- target focus and target clarity;
- record keeping of meetings;
- attention to both the task and maintenance functions of the team;
- a systems perspective.

Conclusion and Recommendations

Health social work has a dual focus on increasing the responsiveness of social institutions to human needs and strengthening the social functioning of individuals (Dhooper, 1994). Health social workers use their clinical skills to help patients and their families cope with illness and treatment recommendations. Many diagnoses such as amyotrophic lateral sclerosis (ALS) are very difficult for patients to accept. ALS is a progressive neuromuscular disease that is very debilitating and ultimately fatal. A person diagnosed with ALS may be depressed, angry, and afraid. A health social worker is trained to provide counseling to help the patient cope with his diagnosis, to provide grief counseling for losses due to his illness, and to encourage follow-up with medical care to maximize quality of life (Browne, 2019).

As can be seen, different social and psychological symptoms develop due to neuromuscular diseases and these affect the course of treatment and prognosis. A holistic approach that considers these together will minimize the problems of patients and their families or eliminate the poor prognosis. For this purpose, teamwork in neuromuscular diseases and the work of social workers in this field as an important member of the team are important.

For those with neuromuscular disease and working with their families, it is recommended to consider the following;

- Empower the Families of Patients through NGOs,
- Organize periodic briefing programs and meetings to empower NGOs,
- Increase the quality of life of the patient and his family,
- Help families become self-sufficient by improving their coping skills,
- Inform about your legal rights,
- Inform about public services,
- Plan and implement group work with families with similar problems,

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Chapter 16

HUMAN ADENOVIRUSES AND OBESITY (INFECTOBESITY)

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Obesity

Obesity, defined as a metabolic disorder caused by excessive caloric intake, is considered a major nutritional burden in both high-income and low-income countries. Obesity is also a serious health problem that has an impact on individual and public health, affects economic and social development, and increases health-care supply and demand (Berberoğlu & Hocaoglu, 2021). Body Mass Index (BMI) is widely used for diagnosing obesity. BMI is calculated by dividing body weight in kilograms by the square of height in meters (kg/m^2). If the BMI is below 18.5, it is classified as underweight, between 18.5-24.9 as normal weight, between 25-29.9 as overweight, and above 30 as obesity (Cole, Bellizzi, Flegal, & Dietz, 2000). According to current researches, The World Health Organization (WHO) reported that more than 300 million people suffer from obesity all over the world. Turkey is the country that has the most obese population in Europe (Yavunç Yeşilkaya, 2021). According to Turkish Statistical Institute (TUIK) data, while the rate of obese individuals aged 15 and over was 19.6% in 2016, it became 21.1% in 2019 (TUIK, 2021).

The etiology of obesity includes genetic factors, gender, age, eating habits, physical activities, socio-economic status and psychological effects (Sahoo et al., 2015). Obesity also causes diseases such as non-insulin dependent diabetes mellitus, coronary heart disease, hypertension, cerebrovascular diseases, respiratory difficulty in breathing, some types of cancer, deep vein thrombosis, gastrointestinal system diseases, fatty liver and cirrhosis, dyslipidemia, polycystic ovary syndrome, stress incontinence, lymphedema, obstructive sleep apnea and osteoarthritis (Shen, Sambamoorthi, & Rust, 2008). It has been shown that obese people are more likely to be affected by Coronavirus disease (COVID-19). Recent studies indicated that people with a BMI above 30 increase the risk of being hospitalized with COVID-19 by 113%, the risk of being admitted to the intensive care unit by 74% and the risk of death by 48%. If the BMI is over 40, the risk of death increases by 90% (Senthilingam, 2021).

The first evidence of the genetic background of obesity was uncovered by Davenport in 1923. Children whose parents were obese could be normal weight, overweight or obese, but not underweight. Thus, the argument that obesity may be genetic was revealed (Bouchard, 2021; Davenport, 1923). Considering the gender factor, when the concept of obesity is examined, the phenomenon of obesity is seen in both genders. However, it is more common in females than males (Bae & Choi, 2021). The increase in this rate in women; It is attributed to the changing hormone ratios in postpartum, menopausal and postmenopausal periods and the associated changing eating habits (Racette, Deusinger, & Deusinger, 2003). Although it is seen in almost all age groups, the incidence of obesity may increase

in some periods when physiologically adipose tissue increases rapidly. These periods are the first 5 years of life and adolescence. Especially in adolescent girls, the increase in adipose tissue is higher than in boys.

Changing dietary habits, the prevalence of fast-food products and the increase in production of foods with high energy and carbohydrate content increase the prevalence of obesity. Obese individuals are more likely to consume foods high in fat and energy than normal-weight individuals (Jebeile, Lister, Baur, Garnett, & Paxton, 2021). Insufficient physical activity and sedentary lifestyle are among the reasons that increase the occurrence of obesity in all age ranges. Lack of physical activity is the most important cause of obesity. In modern societies, the possibility of carrying out work by consuming less energy paves the way for the body to accumulate the energy that cannot be used as fat (Wright & Aronne, 2012). While the children of families with high socioeconomic level become obese due to excessive nutrition, children of families with low socioeconomic level become obese due to unbalanced nutrition. On the other hand, it has been reported that the phenomenon is associated with obesity, since low socioeconomic level makes it difficult to reach healthy foods (Devaux & Sassi, 2013).

There is a connection between the psychological state and the eating behavior. It has been reported that there is an increase in the amount of eating during depression, distress and fatigue, and a decrease in the amount of eating in cases of fear, tension and pain. Emotional eating behavior has also been shown to increase in negative mood states such as anger, depression, anxiety, and loneliness (Monda et al., 2017).

In addition to all these, there are evidences that obesity can also occur through infectious agents that calls “infectobesity”.

Infectobesity

Obesity appears to be a major health problem, especially in western countries (van Ginneken, Sitnyakowsky, & Jeffery, 2009). It has an increasing prevalence all over the world since 1980, and it increased approximately 10 times in the United States between 1980 and 2000. Considering this 20-year period, obesity has increased 2 times in adults and 3 times in children. There are strong opinions that this emerging issue is not only due to changes in diet and lifestyle, but also to infectious causes, especially viruses. Animal (Canine distemper virus, Rous-associated virus-7, Borna disease virus, and SMAM-1) and human viruses (Adenovirus-36, Adenovirus-37, and Adenovirus-5) that cause obesity have been found since 1982 (Karamese, Altoparlak, Turgut, Aydogdu, & Karamese, 2015).

After the determination the relationship between obesity and animal viruses, obesity studies are focused on human adenoviruses (Murat & Ulku, 2016). One of those animal viruses, SMAM-1, was on the target for indicating the obesity–virus relationship (Dhurandhar, Kulkarni, Ajinkya, Sherikar, & Atkinson, 1997; Dhurandlar, Ajinkya, & Sherikar, 1990). In the mid-1970s, a dramatic increase in mortality was seen in commercial poultry farms in India, and it was determined that the agent was a poultry adenovirus called SMAM-1. SMAM-1 is a virus that suppresses the immune system and causes an increase in body fat. In a subsequent study, chickens were intraperitoneally inoculated with SMAM-1 virus, and after about three weeks, an increase in the amount of visceral fat and a decrease in serum cholesterol and triglyceride levels were observed (Dhurandlar et al., 1990). After this invention, in order to determine whether the SMAM-1 agent can cause obesity in humans, the sera of 52 obese people in India were screened for antibody positivity in the agar gel precipitation test. The results were amazing. The body mass index (BMI) of the people in the SMAM-1 positive group was higher and the serum cholesterol and triglyceride levels were lower than SMAM-1 negative group. According to these data, SMAM-1 took its place in the literature as the first factor that was revealed to be associated with human obesity (R. L. Atkinson, 2007; Dhurandhar et al., 1997). Some evidence obtained from animal and in vitro studies suggest that three human adenoviruses (Adv5, Adv36, Adv37) have a relationship with human obesity (Aldhoon-Hainerova et al., 2014; Almgren et al., 2014; R.L. Atkinson et al., 2005; Cakmakliogullari et al., 2014; Na et al., 2012; Trovato et al., 2012).

Adenoviruses were first discovered in 1953 by Wallace Rowe and his colleagues. Since the first isolation was made from human adenoid cell culture, the family name of this virus was determined as *Adenoviridae*. In 1962, a study showed that human adenovirus type 12 causes cancer in newborn hamsters and this study was the first to demonstrate the oncogenic activity of a human virus (Robinson, Seto, Jones, Dyer, & Chodosh, 2011). Adenoviruses are viruses that usually involve the respiratory tract, gastrointestinal tract, and the eye. This virus, which can be transmitted by respiratory droplets, direct contact and digestive tract, infects epithelial cells, multiplies and can easily spread to lymphoid tissue. They usually do not spread beyond the regional lymph nodes, but if the immune system is weak, they can cause viremia and systemic infection. After the disease has healed, latent persistent infection may persist for years in tonsils, adenoids, and other lymphoid tissues (such as Peyer's patches, leukocytes) and can be easily reactivated when the immune system is suppressed (L. Wang et al., 2020). In 2000, it was found that some adenovirus serotypes cause obesity in humans. And today, they have started to be used as vectors in gene therapy studies (Wold & Toth, 2013).

Human Adenovirus 5 (Adv5) is a double-stranded DNA virus with general characteristics of the *Adenoviridae* family. Infection begins when fiber antigens bind to CAR receptors on the cell surface with high affinity (Majhen et al., 2014). In a study conducted in 2005, a group of researchers found that mice were intraperitoneally inoculated with Adv5 and a 300% increase was detected in body fat in the virus inoculated group, although there had no difference in food intake. The body fat of the mice was measured by proton magnetic resonance spectroscopy, and fat storage was seen in the liver. After this study, strong evidence emerged that the Adv5 species can cause obesity in humans, and studies have focused in this direction (So, Herlihy, & Bell, 2005).

On the other hand, Atkinson et al. investigated antibodies against Adv2, Adv31, and Adv37 in a study they conducted on twin humans in 2005; however they could not detect any difference between experimental groups concerning BMI and serum lipids (R.L. Atkinson et al., 2005). A study by Whigham et al. in 2006 revealed another adenovirus serotype that may be a factor in obesity in humans (Whigham, Israel, & Atkinson, 2006). The researchers wanted to investigate whether Adv37, Adv31 and Adv2 could be a factor in obesity in chickens and it was determined that Adv2 and Adv31 did not cause increased fat storage. However, they found that chickens infected with Adv37 increased body fat by 111% and visceral fat by 262% compared to controls. There was no significant difference in food intake between the groups infected with different viruses and the control groups. For this reason, the researchers in this study attributed the obesity caused by Adv37 in chickens to changes in energy expenditure, not energy intake.

Human Adenovirus 36 (Adv36) was first isolated in 1980 and belongs to the group of 56 known human adenovirus serotypes and seven subgroups based on their immunochemical responses, nucleic acid characteristics, hexon and fibre protein characteristics, biological properties and phylogenetic analysis. Adv36 is a non-enveloped icosahedral virus comprising double-stranded DNA (Karamese et al., 2015). The obesity effect of human Adv36 virus was first investigated in chickens and mice, and an increase in visceral region and total body fat ratio, and a decrease in serum cholesterol and triglyceride levels were detected in those animals. Obesity was observed in 60-70% of the animals in the experiment (Dhurandhar et al., 2000). In another similar study, four weeks old chickens were inoculated with Adv36 to determine the infectivity of the obesity agent and its potential for transmission through blood. After 36 hours, 200 ml of blood was taken from infected chickens and given to healthy animals. As a result of this experiment, the causative virus was isolated from the adipose tissues of the animals that received blood transfusions. When the obtained

data of this study were evaluated, it also revealed the fact that obesity may be an infectious disease with an infectious etiology along with other causes (Dhurandhar et al., 2001).

In order to examine the effects of Adv36 in humans, blood samples were collected from 502 obese and non-obese volunteers in Wisconsin, Florida and New York, and Adv36 antibody positivity were screened by serum neutralization test. The prevalence of antibody positivity was 30% in obese volunteers, while it was 5% in non-obese volunteers. In the same study; antibody screenings were also performed for Adv2, Adv31 and Adv37 antibodies, and it was reported that only five individuals with antibodies positive for Adv37 were detected. In order to understand the metabolic and molecular mechanism of Adv36 triggering adipogenesis, the effect of virus on 3T3-L1 pre-adipocyte cells and primary human pre-adipocyte cells was investigated under in vitro conditions. As a result, it was determined that Adv36 caused differentiation by increasing the level of glycerol 3-phosphate dehydrogenase (GPDH) enzyme in 3T3-L1 pre-adipocyte cells. These data show that the presence of Adv36 antibodies is associated with obesity in humans (Vangipuram, Sheele, Atkinson, Holland, & Dhurandhar, 2004).

Mechanism of Viral Obesity

Leptin is a 167 amino acid hormone secreted largely from adipocytes in white adipose tissue, which reduces appetite and increases energy expenditure. It is known to decrease food intake and increase metabolic rate by primarily acting on hypothalamic receptors. It has also been shown that fat storage is more in case leptin is not produced at an adequate level (Heymsfield et al., 1999). In order to investigate the relationship between leptin secretion/lipid storage and Adv2, Adv9, Adv36, Adv37 viruses, some in vitro studies were carried out on 3T3-L1 pre-adipocyte and A549 cells. The findings showed that Adv2 has no effect on cells; however, the others (Adv9, Adv36, and Adv37) decreased leptin levels by suppressing leptin mRNA synthesis, and thus increased lipid accumulation. Thus, it has been thought that hypoleptinemia may play a role in obesity because of the size and localization of adipocytes for leptin production. In conclusion, hypoleptinemia formation and differentiation of Adv36-infected cells in adipose tissue may be associated with increased fatty acid synthesis and lipid storage (Vangipuram et al., 2007).

On the other hand, many cytokines that affect pre-adipocytes and adipocytes are secreted from adipose tissue. In obesity, tumor necrosis factor-alpha (TNF-alpha) is secreted from adipose tissue and may be responsible for insulin resistance. Increased levels of interleukin-6 (IL-6) and C-reactive protein (CRP) have also been observed in obese individuals

(Argiles, Lopez-Soriano, Almendro, Busquets, & Lopez-Soriano, 2005). Generally, IL-12 stimulates interferon-gamma (IFN-gamma) and creates a T helper-1 (Th-1) immune response together with IL-18 during the infections. However, Adv36 can reduces the Th-1 response by suppressing the production of these cytokines in the early stages of infection. The reduction of this cytotoxic immune response causes the cell survival in Adv36-infected adipose tissue. According to the literature, it is thought that the Adv36-related obesity is due to the effect of the open reading frame 1 early region 4 gene (E4Orf1) (Krishnapuram, Dhurandhar, Dubuisson, Hegde, & Dhurandhar, 2013). In another study to prove this, it was observed that Adv36 increased fat accumulation when the E4Orf1 gene was transferred to retroviruses and entered into pre-adipocytes (Z. Q. Wang et al., 2008).

Future perspective

According to the current literature, it is not difficult to predict that there will be much more studies on "infectobesity" in near future. These studies will not only investigate the relationship between all potential adenoviruses strains and human obesity, but also molecular mechanisms regarding how all related adenoviruses lead to lipid differentiation and accumulation. Therefore, it is hoped that new therapeutic agents and vaccines will be developed that may slow the increasing rate of obesity worldwide.

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Chapter 17

ANXIETY DISORDERS FROM PAST TO PRESENT

Gülay EKİNCİ[1]

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INTRODUCTION

Anxiety is an emotional condition that characterized by fear and worry. Anxiety is a generalized and unfocused feeling of restlessness and worry that is often an overreaction to a situation seen as threatening [1]. This mood could lead to shortness of breath, chest tightness, sweating et al. on the individual. Experiencing anxiety without a concrete danger, increasing the frequency and severity of this situation and starting to affect the normal life of the person suggests that the individual has an anxiety disorder [2]. Anxiety disorder is considered a problem only when it begins to negatively affect a person's activities in daily life. For the diagnosis of anxiety disorders, evaluation is made within the framework of the following criteria:

- Anxiety creates difficulties in professional and family life in the invidual.
- Causes problems in relationships with close environment and family members
- Occupying one's mind for most of their daily time
- Difficulty controlling fear and anxiety
- This condition has been ongoing for at least 6 months.

Anxiety disorders include panic attacks, agoraphobia and its types, specific phobia, social phobia, obsessive compulsive disorder, stress disorder, generalized anxiety disorder, general medical condition anxiety disorder and anxiety disorders caused by substance use. Such disorders are believed to affect 18% of the population [3]. About 3.7% of the world's population suffer from anxiety disorders[1]. there was no notification about the direct anxiety-related death data in the statistics. However, anxiety is effective on diseases such as the cardiovascular system, respiratory system, etc, and is also accompanied to psychiatric disorders.

With this study, it was aimed to analysis the current situation of the anxiety disorders according to globally. This study was a descriptive-retrospective study. The study was designed in two sections. First section anxiety disorders were analysed according to income group countries and the level of globally. From this perspective anxiety disorders for the years 1990-2019 were analyzed retrospectively. In the first stage, we evaluated the prevelance rate[2], incidence rate, the Disability-Adjusted Life Year

1 The mean prevalence of anxiety disorders at the global level was calculated by proportioning the population for the year of 2019.

2 Acoording to CDC; a basic measure of disease frequency is a rate, which takes into account the number of cases or deaths and the population size. For example, if a anxiety disorders incidence rate is 500 per 100000, it means that 500 new cases of anxiety disorders were diagnosed for every 100000 people; rate per 100k

(DALY) from anxiety disorders. The data (anxiety disorders) were taken from Global Burden Of Diseases Database [4]. The second stage the current situation of the anxiety disorders in Turkey over the years in total.

1. 1.Basic Concepts Of The Analysis

Prevelance is obtained by dividing the number of all (old and new) cases detected in a population in a given time period by the number of people at risk at the same time. It refers to the prevalence of a disease in the community at that time. Prevalence helps to measure the number of diseases in a population and determine healthcare needs.

Incidence is the number of new infections (cases) or the subsequent cases for each health outcome in the population, stratified by age and sex. Incidence is the rate obtained by dividing the number of new cases of a disease by the population at risk within a certain period of time (day, week, month, year). Incidence indicates the determination of etiological factors and in the probability of developing a specific diseases. Prevalence and incidence are important criteria determining the disease level.

The disability-adjusted life year (DALY) is a health gap metric, measuring the healthy life years lost due to diseases, injuries or risk factors [5]. According to WHO (2013); DALYs are calculated by adding the number of years of life lost due to premature mortality (YLL) and the number of years lost due to disability (YLD) [6]:

$$DALY = YLL + YLD$$

YLL is the product of the number of deaths (M) and the average remaining life expectancy (RLE) at the time of death:

$$YLL = M \text{ x } RLE$$

YLD, calculated from an incidence perspective, is defined as the product of the number of incident cases (N), the average duration until recovery or death (D), and the disability weight (DW), which reflects the reduction in health- on a scale from 0 (no impact on full health) to 1 (death):

$$YLDinc = N \text{ x } D \text{ x } DW$$

YLD calculation in other words [7];

• Years lost due to injury or complete loss of health and well-being.

• Number of new cases X disability coefficient X Duration of illness or disability – until death or recovery.

• If a person who died at the age of 75 was diagnosed with diabetes at the age of 50, the YLD lost by this person is 25.

1.2. Evaluation Of The Burden Of Anxiety Disorders

In this section, the prevalence rate, incidence rate, and disease burden of anxiety disorders were examined under subheadings. The analysis was made in four stages. In the first stage; information was given about the basic concepts subject to analysis than the other each stage; the prevalence, incidence, YLDs' values belonged the anxiety was examined.

In the analyzes, the data was analyzed by classifying them into five categories according to income status. Abbreviations for the countries used in the analysis;

- High-Income Countries (HIC)
- Low-Income Countries (LIC)
- Upper Middle-Income Countries (UMIC)
- Lower Middle-Income Countries (LMIC)
- Global level
- Turkey level

1.2.1. Prevelance Rate of Anxiety Disorders

The world average of anxiety disorders prevalence rate was 3774.15/ per 100k while the prevelance rate of anxiety disorders in LMIC, LIC countries was below the world average; HIC, UMIC and Turkey have a value above the average rate at thirty years. Prevalence rate of anxiety disorders has been increased about 6.8 % globally in 30 years. While this increase was around 5.2 % in high-income countries; in the upper-middle-income, lower middle income and low-income countries this increase was calculated as above 10 % in 30 years (Table 1). In Turkey, the prevalence of anxiety disorders rate has increased by approximately 19% in 30 years.

Table 1: The Descriptive Analysis of Prevalance Rate of Anxiety Disorders

	Global	**HIC**	**UMIC**	**LMIC**	**LIC**	**TR**
1990*	3650.04	4982.96	3789.8	2900.52	3031.01	4060.29
2019*	3898.26	5240.39	4174.36	3299.63	3267.72	4832.29
Average**	3774.15	5111.68	3982.08	3100.07	3149.37	4446.29

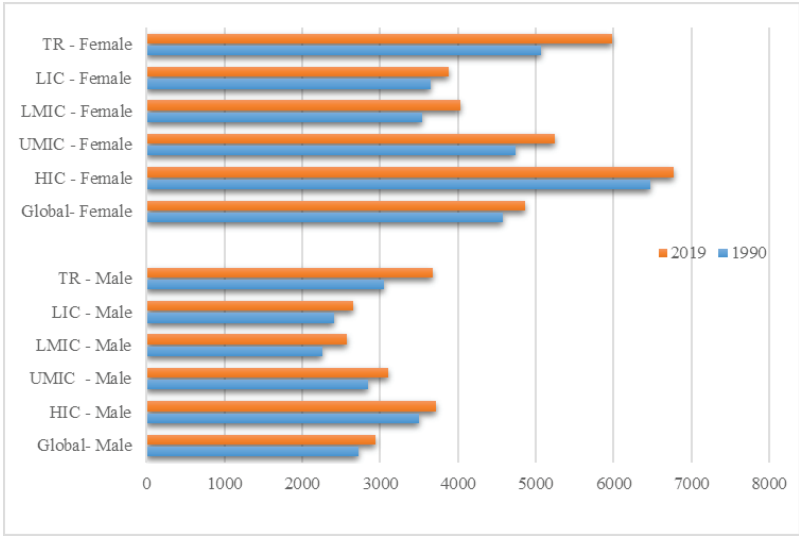
% Change	% 6.8	% 5.2	% 10	% 13.7	% 7.8	% 19

*The prevalence rate of the relevant year was calculated by taking the average of the female and male groups' prevalence rates / *rate per 100k
** While calculating the average value. the average prevalence rate for the years 1990 and 2019 was taken.

Source: This table was prepared by the author using the data from the Global Burden Of Diseases Database

According to distribution by Income Group and Gender, the highest prevalence rate of anxiety disorders was determined in the upper-income group countries and in the female group. In addition, regardless of income groups, the prevalence rate of anxiety disorders was detected higher in women than in men.

Graph 1: Distribution Of The Prevalence Rate Of Anxiety Disorders Rate By Income Groups -Gender, 1990-2019



Source: *This graph was prepared by the author using the data from the Global Burden Of Diseases Database*

Prevelance rate of anxiety disorders in Male group has been increased about 7.6 % globally in 30 years. While this increase was around 7.5 % in upper middle and high income countries; in the low income group countries this increase was calculated as 11.7 % in 30 years. In Turkey, the prevalence of anxiety disorders has been increased by approximately 20.7 % in 30 years. In addition, the increase in Turkeys' prevalence rate of anxiety disorders in the Male group was detected higher than in the other countries.

Table 2: Prevelance Rate Of Anxiety Disorders According To Income And Gender Groups

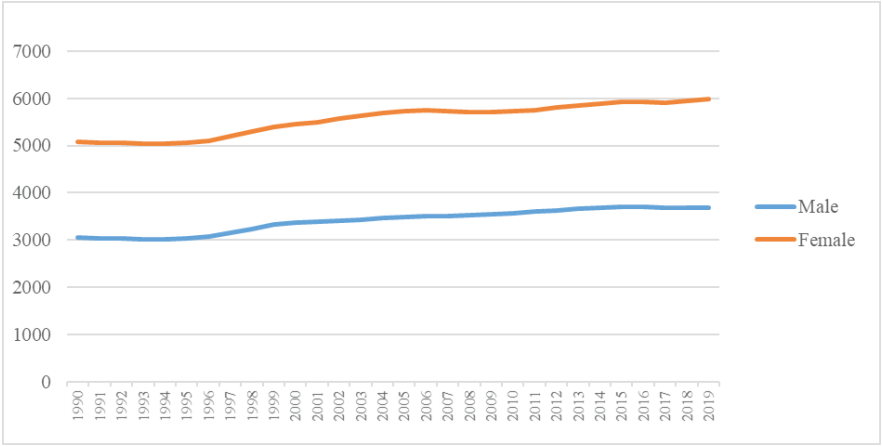
	1990*	**2019***	**% Change**
Global- Male	2726.06	2933.64	0.0762
HIC - Male	3495.36	3713.46	0.0624
UMIC - Male	2845.64	3099.9	0.0894
LMIC - Male	2262.75	2566.24	0.1341
LIC - Male	2413.67	2657.33	0.1009
TR - Male	3047.87	3681.23	0.2078
Global- Female	4574.01	4862.88	0.0632
HIC - Female	6470.56	6767.33	0.0459
UMIC - Female	4733.96	5248.81	0.1088
LMIC - Female	3538.28	4033.01	0.1398
LIC - Female	3648.35	3878.11	0.0629
TR - Female	5072.71	5983.36	0.1795

* rate per 100k

Source: This table was prepared by the author using the data from the Global Burden Of Diseases Database

The prevalence rate of anxiety disorders in the Female group has been increased about 6.3 % globally in 30 years. While this increase was average around 5.4 % in high and low-income countries; in the low and upper-middle-income countries, this increase was calculated as an average of 12.4 % in 30 years. In Turkey, the prevalence rate of anxiety disorders in the Female group has been increased by approximately 17.9 % in 30 years. In addition, the increase in the Turkeys' prevalence rate of anxiety disorders in the Female group was detected quite high from the world average rate with 17.9 %.

Graph 2: Prevalence Rate Of Anxiety Disorders According to Gender in Turkey, 1990-2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

1.2.2. Incidence Rate of Anxiety Disorders

The world average anxiety disorders incidence rate was 587.51/ per 100k while the incidence rate of anxiety disorders in LMIC and LIC countries was below the world average; HIC, UMIC and, Turkey have a value above the average rate at thirty years. The incidence rate of anxiety disorders has been increased about 1.7 % globally in 30 years. While this increase was around an average of 7.1 % in LMIC-LIC; the incidence rate in the high-income countries was decreased average of 1.9 % in 30 years (Table 3). In Turkey, the incidence rate of anxiety disorders has been increased by approximately 6.18 % in thirty years.

Table 3: The Descriptive Analysis of Incidence Rate of Anxiety Disorders

	Global	HIC	UMIC	LMIC	**LIC**	**TR**
1990*	582.54	683.96	607.92	509.46	537.67	682.18
2019*	592.49	670.93	612.63	550.82	571.17	724.34
Average**	587.51	677.44	610.28	530.14	554.42	703.26
% Change	% 1.7	% - 1.9	% 0.77	% 8.1.	% 6.2	% 6.18

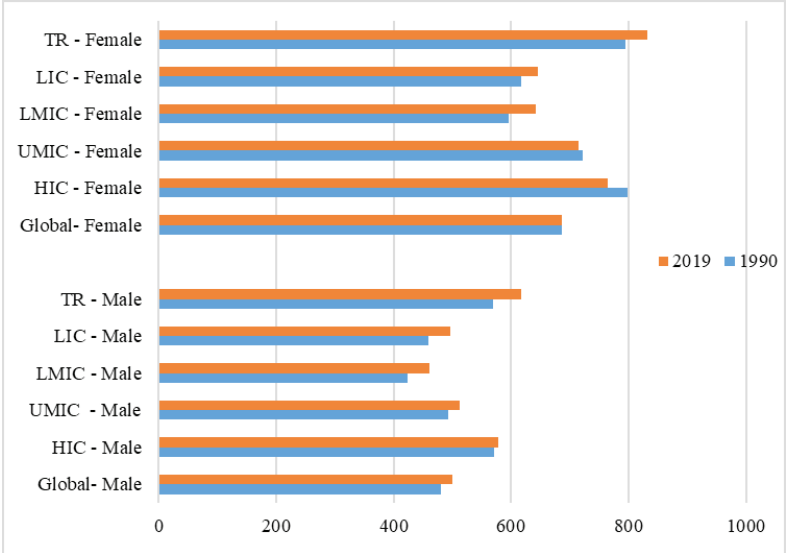
*The incidence rate of the relevant year was calculated by taking the average of the female and male groups' incidence rates / * rate per 100k

** While calculating the average value. the average incidence rate for the years 1990 and 2019 was taken.

Source: This table was prepared by the author using the data from the Global Burden Of Diseases Database

According to distribution by Income Group and Gender, the highest incidence rate of anxiety disorders was determined in the upper-income countries and in the female group. In addition, regardless of income groups, the incidence rate of anxiety disorders was detected higher in women than in men generally between the genders.

Graph 3: Distribution Of The Incidence Rate Of Anxiety By Income Groups-Genders, 1990-2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

The incidence rate of anxiety disorders in the Male group has been increased about 4.0 % globally in 30 years. While this increase was average around 2.4 % in UMIC and HIC countries; in the LMIC and LIC countries, this increase was calculated as 8.5 % in 30 years. In Turkey, the incidence rate of anxiety disorders has been increased by approximately 8.3 % in 30 years. In addition the increasing in Turkeys' incidence rate in Male group was detected more high than the female group.

Table 4: Incidence Rate Of Anxiety Disorders According To Income Groups, % Change

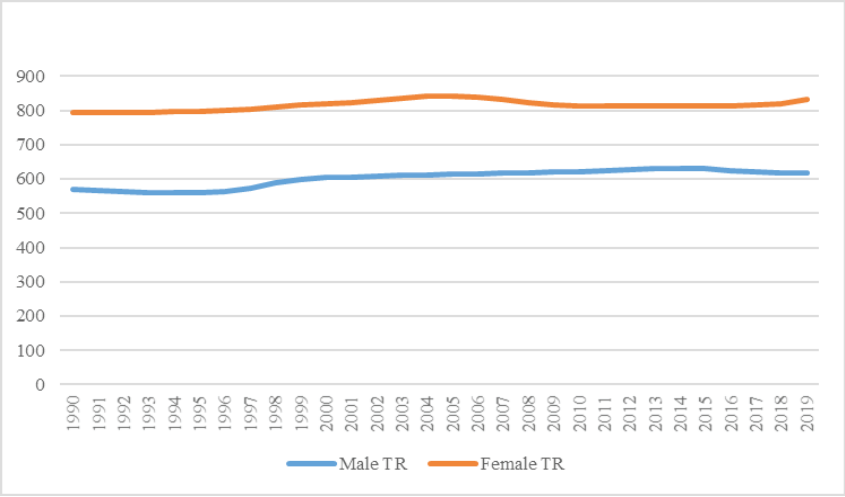
	1990*	**2019***	**% Değişim**
Global- Male	479.8	499.27	0.0406
HIC - Male	570.66	577.73	0.0124
UMIC - Male	493.37	511.4	0.0365
LMIC - Male	422.92	460.56	0.0890
LIC - Male	459.25	496.79	0.0817
TR - Male	569.66	617.39	0.0838
Global- Female	685.27	685.71	0.0006
HIC - Female	797.26	764.12	-0.0416
UMIC - Female	722.47	713.86	-0.0119
LMIC - Female	596	641.08	0.0756
LIC - Female	616.09	645.55	0.0478
TR - Female	794.69	831.29	0.0460

* rate per 100k

Source: This table was prepared by the author using the data from the Global Burden Of Diseases Database

The incidence rate of anxiety disorders in Female group has been increased about 0.064 % globally in 30 years. While this increase was average around 6.1 % in LMIC and LIC countries; in the HIC UMIC countries this rate was decreased about by 2.7 % in 30 years. In Turkey, the incidence rate of anxiety disorders has been increased by approximately 4.6 % in 30 years.

Graph 4: Incidence Rate Of Anxiety Disorders According to Gender in Turkey, 1990-2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

1.2.3. Years Lost Due To Anxiety Disorders

The world average YLD of anxiety disorders was 23668534,46 and increased about 53 % globally in 30 years. While this increase was around 31 % in HIC and UMIC countries; in LMIC and LIC countries this increase was calculated as above 110 % in 30 years (Table 5). In Turkey, the YLD of anxiety disorders has increased by approximately 60 % in 30 years.

Table 5: The Descriptive Analysis of Years Lost Due To Anxiety Disorders

	Global	**HIC**	**UMIC**	**LMIC**	**LIC**	**TR**
1990*	18661018.46	4733590.8	7655472.38	5297903.19	962888.14	234059.55
2019*	28676050.45	5896433.7	10536099.9	9993938.34	2230781.7	375857.06
Average**	23668534.46	5315012.2	9095786.14	7645920.76	1596834.9	304958.31
% Change	0.5367	0.2457	0.3763	0.8864	1.3168	0.6058

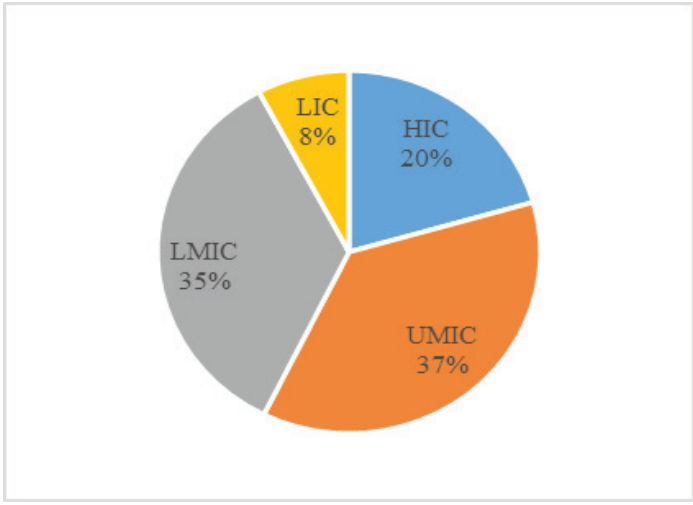
*The YLD of the relevant year was calculated by taking the average of the female and male groups' YLD

** While calculating the average value. the average YLD for the years 1990 and 2019 was taken.

Source: This table was prepared by the author using the data from the Global Burden Of Diseases Database

Globally YLD of anxiety disorders was 28676050.45 had been realized in 2019. The distribution of anxiety disorders YLD according to income groups was evaluated respectively; HIC (20 %), LMIC (35 %), LIC (8 %), UMIC (37 %) in 2019.

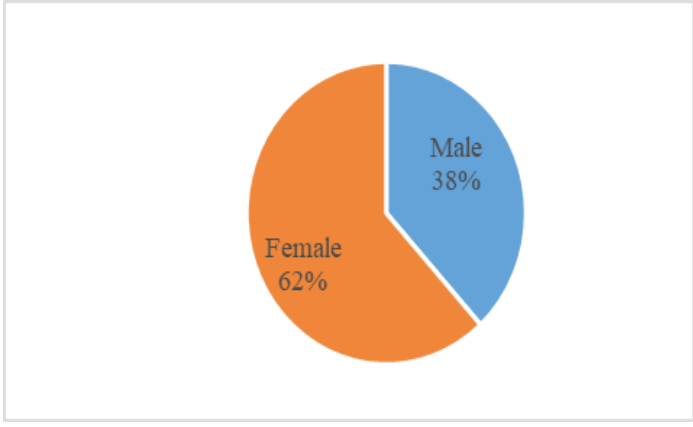
Graph 5: YLD From Anxiety Disorders According To Income Groups, 2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

It has been determined that 62% of the anxiety disorders-related burden of diseases at the global level belongs to women and 38% to men.

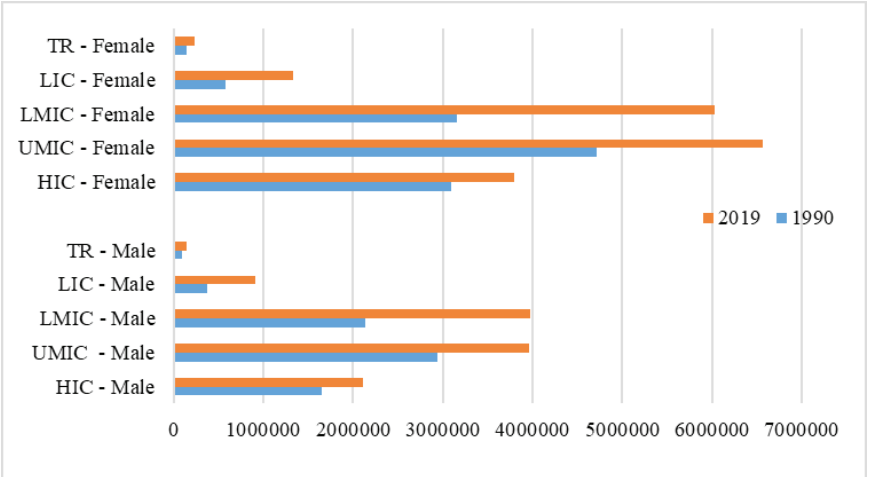
Graph 6: Years Lost Due To Anxiety Disorders According to Gender, 2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

According to distribution by Income Group and Gender, the highest YLD of anxiety disorders was determined in the UMIC and LMIC countries and in the female group. In addition, regardless of income groups, the YLD of anxiety disorders was detected higher in women than in men generally between the genders.

Graph 7: Distribution Of The YLD Of Anxiety Disorders By Income Groups-Genders, 1990-2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

YLD of anxiety disorders in Male group has been increased about 54 % globally in 30 years. While this increase was average around 31.4 % in UMIC and HIC countries; in the low income group countries this increase was calculated as 112 % in 30 years. In Turkey, the YLD of anxiety disorders in the Male group has been increased by approximately 62 % in 30 years.

Table 6: The YLD Of Anxiety According To Income Groups, % Change

	1990	2019	% Change
Global-Male	7107416.89	10955964.05	0.5415
HIC - Male	1646361.19	2104949.15	0.2786
UMIC - Male	2941141.89	3966293.69	0.3486
LMIC - Male	2136459.16	3972436.36	0.8594
LIC - Male	379370.81	905294.35	1.3863
TR - Male	90559.62	147083.22	0.6242
Global-Female	11553601.57	17720086.4	0.5337
HIC - Female	3087229.56	3791484.51	0.2281
UMIC - Female	4714330.49	6569806.21	0.3936
LMIC - Female	3161444.03	6021501.98	0.9047
LIC - Female	583517.33	1325487.31	1.2715
TR - Female	143499.93	228773.84	0.5942

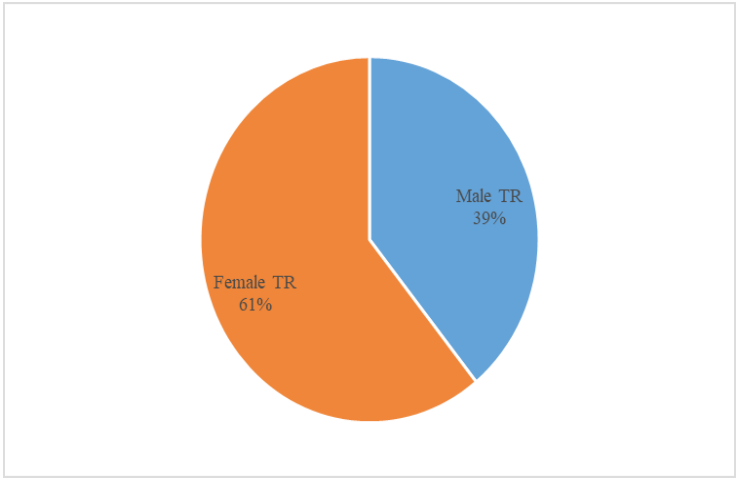
Source: This table was prepared by the author using the data from the Global Burden Of Diseases Database

YLD due to anxiety disorders in the Female group has been increased about 53.4 % globally in 30 years. While this increase was around 31 % in the HIC and UMIC countries; in the low income group countries this increase was calculated as 100 % in 30 years.

In Turkey, the YLD due to anxiety disorders has been increased by approximately 59 % in 30 years. YLD caused by anxiety disorders had been realized 375857,06 in Turkey in 2019. In Turkey, the YLD caused by anxiety disorders share of global anxieties YLD was calculated at 1.3 %.

It has been determined that 61% of the anxiety disorders-related burden of diseases at Turkey belongs to women and 39% to men. It has been understood that this distribution is compatible with the values in the World (Graph 6).

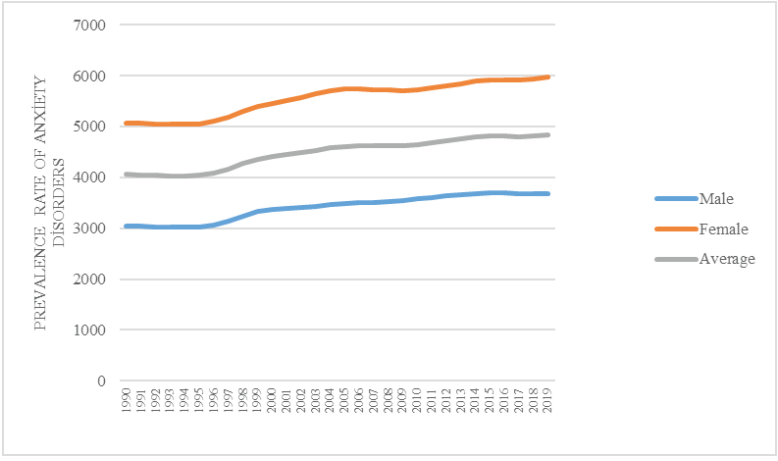
Graph 8: Years Lost Due To Anxiety Disorders According to Gender in Turkey, 2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

The prevalence rate of anxiety disorders in Turkey was lower in men than in women; but the increase of this indicator was found to be higher in men in the thirty years (Table: 2-4-6).

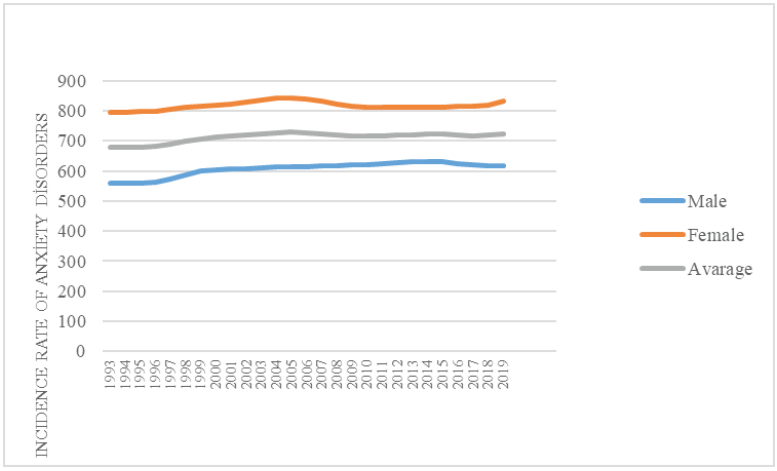
Graph 9: Prevalence Rate Of Anxiety Disorders in Turkey, 1990-2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

The incidence rate of anxiety disorders in Turkey was lower in men than in women; but the increase of this indicator was found to be higher in men in the thirty years as prevalence rate (Table: 2-4-6).

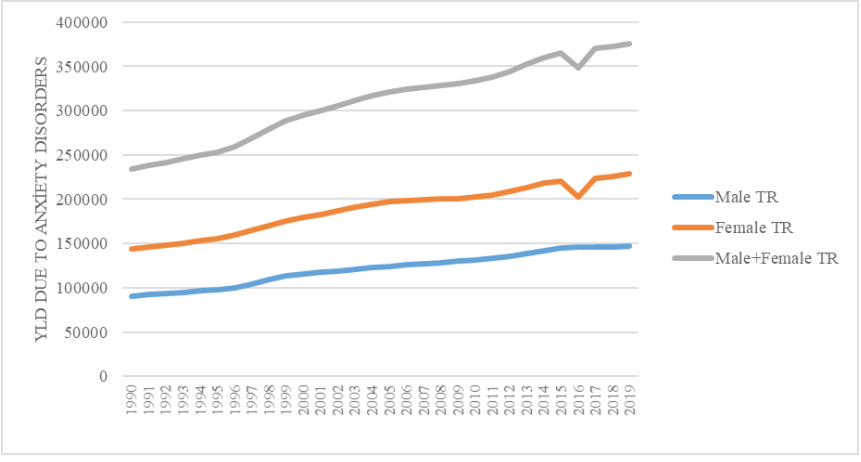
Graph 10: Incidence Rate Of Anxiety Disorders in Turkey, 1990-2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

The YLD values due to anxiety disorders was lower in men than in women; but the increase of this indicators was found to be higher in men in the thirty years (Table: 2-4-6).

Graph 11: Years Lost Due To Anxiety Disorders in Turkey, 1990-2019



Source: This graph was prepared by the author using the data from the Global Burden Of Diseases Database

1.3. Results

The results are discussed under two headings at the global level and Turkey specific.

For Global level;

Anxiety-related prevalence rates are increasing worldwide.

- The prevalence rate of anxiety disorders in LMIC, LIC countries was below the world average; HIC, UMIC, and Turkey have a value above the average rate at thirty years.
- The prevalence rate of anxiety disorders was detected higher in women than in men.
- The incidence rate of anxiety disorders has been increased about 1.7 % globally in 30 years.
- Anxiety-related incidence rates were increasing worldwide without HIC countries. In HIC countries anxiety-related incidence rates decreased in the female group.
- The incidence rate of anxiety disorders in LMIC and LIC countries was below the world average; HIC, UMIC has a value above the average rate at thirty years.
- The world average YLD of anxiety disorders was increased about 53 % globally in 30 years. While this increase was around 31 % in HIC and UMIC countries; in LMIC and LIC countries this increase was calculated as above 110 % in 30 years.
- Globally, the burden of illness due to anxiety disorders is 63% higher in women than in men. This is also true for Turkey.

For Turkey;

- In Turkey, the prevalence of anxiety disorders rate has increased by approximately 19% in 30 years.
- In the Male group the prevalence of anxiety disorders rate has increased more than in the Female group in Turkey,
- Generally the increase in the Turkeys' prevalence rate of anxiety disorders in the Female and Male group was detected quite high from the world average rate (17.9%, 20.7% respectively) (see Table:2).
- The increase in the anxiety prevalence rate in Turkey has realized 1.8 times more than the increase in the world's anxiety prevalence rate
- The increase in the anxiety incidence rate in Turkey has realized 2.6 times more than the increase in the world's anxiety incidence rate

- The increase in the YLD of anxiety disorders in Turkey has realized 13% more than the increase in the world's YLD of anxiety disorders

If the results of this study are evaluated from the perspective of the economy; resource limitations may be the most important cause of anxiety. The population increased from 3,031 billion in 1960 to 7,592 billion in 2018 which amounts to an increase of 151 percent. Life expectancy at birth increased from 52.58 in 1960 to 72.56 in 2018 which amounts to an increase of 38 percent. Besides these results, the growth rate of per capita income in the world has been decreased by 35% following a fluctuating course. The decrease in resources on a world scale and in addition the inadequacies/ inequalities in the current resource distribution make life more anxious. Anxiety is a psycho-social process associated with an individual's self-protection mechanism against any threat. It can be said that the reasons explained above are a determining factor in making life more anxious on a world scale from the economic perspective.

1.4. Recommendations

In high-developed countries, men and women have more equal employment opportunities and women are economically stronger. Since the upper-income group has less share in women's anxiety, women-oriented approaches should be prioritized in the improvements to be made in the anxiety level.

Although the share of anxiety seems to be lower in low-income countries, the fact that the increase rates are much higher than the world average, and the burden of disease is more severe in these countries, in the improvements strategy in the anxiety low-income countries should be handled as the second priority.

The prevalence rate, incidence rate, and the continuing increase in the burden of anxiety-related disease in Turkey indicate that a more anxiety-related structure has developed at the social level. Although this situation is more evident in Turkey, it is valid in the whole world substantially.

It is important to determine the factors that cause the development of anxiety both in the world and in Turkey and recommended to develop plans and programs in order to protect society from the negative effects of this structure.

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