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Research & Reviews in Health Sciences - I

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gece kitaplığı

CONTENTS

Chapter 1

TRICHOTILLOMANIA

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INTRODUCTION

Trichotillomania is a mental disorder that was first defined by dermatologist, Hallopeau in 1889 as a result of the examination of a patient who plucked his/her hair in pinches, and was evaluated as a type of alopecia (Keser et al., 1999). Trichotillomania is a Greek word and was formed by the combination of the words of hair (trich), and of plucking (tillo), and of disease-level impulse (mania).

It is a disorder in which the individual plucks his/her hair in a recurring way and in a manner that prominent balding areas can be formed, and a feeling of tension is felt before plucking them, and a sense of pleasure is observed while plucking them. The majority of trichotillomania sufferers are adults and the average age of onset is 13 (Annagür, 2010). In Trichotillomania sufferers, mostly hair plucking, and less plucking bristles from different parts of the body such as eyebrows, eyelashes, beard-mustache, armpits and groin is observed. In the vast majority of cases, it is observed that such accessories as wigs, hats, headscarves, etc. are used in order to hide hair loss.

The frequency of the plucking of action varies from time to time. In the course of trichotillomania, sufferers sometimes manage to stop the act of plucking their hair almost completely, but this is usually not permanent. Trichotillomania negatively affects the life and functionality of the individual on account of both the plucking and the consequences of the action. Individuals may experience shame and loss of self-confidence on account of their inability to control their plucking behavior and the differences that plucking creates in their appearance.

The strategies to be applied so as to hide the negative consequences of plucking behavior from their relatives and surroundings can limit individuals' communication, and might isolate them. In severe cases, these hidings can turn into social isolation.

It is in the cope of in DSM-5, "Obsessive and Compulsive Disorders and Related Disorders". Trichotillomania diagnostic criteria; recurrent hair plucking resulting in hair loss, recurrent attempts to reduce or stop hair plucking and pulling hair cause clinically significant distress or decline in social, occupational related areas or other important functioning areas, hair plucking or hair loss cannot be attributed to another health condition (e.g. a dermatological condition), hair plucking cannot be better explained by the symptoms of another mental disorder (e.g. attempts to correct a defect or disability perceived in terms appearance in body perception disorientation) (DSM-5 p-133) .

In this article, it is aimed to examine the sociocultural and socioeconomic aspects of trichotillomania, which is not a common disorder in daily life, and to reveal the etiology and treatment of the disease.

ETIOLOGY

The exact cause of trichotillomania is unknown. Many etiological factors have been suggested as the cause. Some of those can be counted as life events such as parents' divorce, moving from one's living area to another place, changing schools, physically abuse, trauma, loss or perception of loss (Konkan et al., 2011). The presence of trichotillomania or similar other psychiatric disorders in family members of trichotillomania sufferers suggested that genetic predisposition may have an effect.

TREATMENT

As it is known, there are such treatment models as habit reversal training (HRT), especially SSRIs used, in the medical sense, by physicians and drug treatments with clomipramine.

However, when considered as non-physician therapy models, it was observed that, in Behavior Therapy-Weighted (CBT) Cognitive-Behavioral Therapy, especially imagination works, with its effectiveness, is close to getting results, and that Hypnotherapy, especially by the Traditional and Complementary Medicine and Psychology, plays a very active role, and that, with the therapeutic approach, the treatment, as both indirect suggestion and direct suggestion, is faster and more result-oriented.

THE EFFECT OF ENVIRONMENTAL MODIFICATION ON CHILDREN IN TRICHOTILLOMIA

Many families can experience a very difficult process when they move on account of financial or moral reasons. Leaving a familiar environment and moving to a different place can have many kinds of spiritual effects on children. They exhibit coping behaviors in response to stress so as to adapt to a different place. Behavioral disorders, hyperactive, touchy behaviors, low self-perception, depressive state, shame, and aggressive behaviors can be seen in children of many families moving from one place to another one. Along with these behaviors, feelings of unhappiness, guilt, anger, anxiety, tension, and sadness can also be experienced. Environmental modification is not only related to moving, but also changing kindergarten and nursery schools can also cause the same behavioral disorders. Their classmates, teachers are new. Each child tries to cope with a foreign environment with a different behavior and emotional status.

FINDINGS

The results, we achieved through the 40-person survey, we conducted and interviewed, are as follows: The fact that 100% of the individuals, between the ages of 0-10, were those who changed their social environment, is seen as the first cause of Trichotillomania. In line with the answers to the survey questions and our interviews, we see that the beginning of school (nursery school or primary school), neighborhood and environmental changes, in terms of social environment and socio-cultural terms, causes the beginning of Trichotillomania disease, as being Obsessive-Compulsive-Disorder (OCD), together with anxiety disorder, or that the foundations of this disorder are laid. In the survey study accordingly conducted, we see that the initial bases of the disease occur in a rate of 18.4% of the individuals, between the ages of 0-10, with trichotillomania. When considering the age range of 10-18, we encounter that this rate is 50%, together with the exposure to the same situation, following the first initial foundations are laid. In the individuals, between the ages of 18-30, we see that the disease has already started in the rate of 18.4%, as a result of exposure to the same situation. The reason for this is that the psychological problem experienced by the person appears as an escape by the unconscious mind, and when the same problem is encountered in the future, it takes place as Obsessive-Compulsive-Disorder (OCD). In line with our research, we can say that trichotillomania disorder emerges before the age of 20. We observe that trichotillomania emerges, with a higher incidence rate of (86.8%), more in women than in men. Loss, harassment, etc. in the emergence of trichotillomania. It has been observed that such traumas as loss, abuse are not effective in the emergence of trichotillomania. It has been observed that traumas are not effective. trauma is not effective. In our study, it has been observed that trichotillomania sufferers had social environment changes on account of different reasons in a high rate (73.7%). It has been observed that personality traits (social, asocial, etc.) do not affect the onset of the disease, and that the majority of trichotillomania sufferers feel themselves as worthless, inadequate, hopeless and helpless.

Its Importance

We always make environmental changes in our lives. Those children, at the ages between 0-6, cannot easily adapt to a new house or school as adults do. In this process, we need to understand their feelings correctly. We must observe the children correctly, and support them in this adaptation process. We should establish effective communication with them and we should listen to their problems. It is important that we increase their motivations and self-confidences. In this difficult period, the feelings of inadequacy, unsociality, helplessness, loneliness, stress and anger, especially during the

change in the social environment experienced by children, are equivalent to the emotions, which are experienced by trichotillomania sufferers.

Assumptions

It is assumed that randomly selected trichotillomania sufferers completed the scale honestly and objectively.

Purpose of the research

This research has two purposes.

1- To search and find out the cause of the onset of trichotillomania, which is under the scope of title of obsessive-compulsive disorders,

2- To suppress trichotillomania or to provide its treatment by developing the method related to the treatment/therapy process of trichotillomania.

METHOD

Research Design

In this research, by using the relational research model, which is a quantitative research model, the relationship between the emotional and behavioral disorders of 0-6 years old childhood with the disease, together with the change in the environment and changes in the school or home environment of the trichotillomania sufferers during their childhood, was investigated.

Population/Sampling

The study included 40 patients, 86% of whom were female, and 14% of whom were male, who were diagnosed with trichotillomania according to DSM-V-TR diagnostic criteria. The patients were informed about the study and their consent was obtained.

Data Collection Tools

“Personal Information Form” is the form including the participants’ personal information such as age, gender, etc.

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Chapter 2

IMMATURE NEURONAL MARKERS: NEUROD1, DOUBLECORTIN, PSA-NCAM AND THEIR USE TO IMMUNOHISTOCHEMISTRY

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1. IMMATURE NEURONAL MARKERS: NeuroD1, DOUBLECORTIN, PSA-NCAM AND THEIR USE TO IMMUNOHISTOCHEMISTRY

1.1.NeuroD1

The development of the nervous system is achieved by the gradual and gradual expression of certain gene clusters. Neurogenic Differentiation 1 (NeuroD1, also called Beta2) is a member of the basic helix-loop-helix (bHLH) transcription factor family that has been shown to play an important role in the development of the nervous system and the formation of the retinal, olfactory, and endocrine system. The human gene is encoded by NEUROD1 (1-5).

NeuroD1 plays a role in the development of neuronal tissues, including the hippocampus and auditory/vestibular system, in the developing central nervous system (6-8). Sensorineural deafness due to the loss of sensory neurons in the inner ear has been observed in rats that NeuroD1 knock-out (9). NeuroD1 plays a role in the development and differentiation of neuroendocrine cells in the pancreas as well as in the central nervous system. It has been reported that mice with the knocked out NeuroD1 gene born diabetic (10).

The expression of NeuroD1 in the development of the nervous system continues throughout postnatal development. It also remains at constant levels in the adult peripheral and central nervous system. This is thought to occur because NeuroD1 is active in both the mitotic phase and the post-mitotic phase of the cells (11). In line with these findings, it can be said that NeuroD1 plays a role not only in the development of the central and peripheral nervous system but also in maintaining its stability and functioning. In one study, it was observed that mice whose NeuroD1 gene was deleted died of diabetic ketoacidosis shortly after birth. However, it has been shown that the heterozygous loss of NeuroD1 in mice does not cause mortal diabetes, unlike knockout mice. These mice have been reported to exhibit pre-diabetic activities (12).

NeuroD1 is important for the development of the cerebellum, hippocampus, and Dentate Gyrus. Studies in NeuroD1-deficient mice have revealed that NeuroD is critical for proliferation and postnatal differentiation of neurons in the Dentate Gyrus (13).

NeuroD1 is usually observed in regions of the developing nervous system where differentiating post-mitotic neurons are distributed (14). However, it has been found that ectopically expressed NeuroD1 can transform epidermal cells and non-neuronal cells of the neural crest into neurons (15). Considering this information, it should be considered

that NeuroD1 may affect different phases in central nervous system development such as migration, proliferation, and differentiation. In the research, it has been observed that the overexpression of NeuroD1 in the F11 neuroblastoma cell line contributes to neurite growth. The data obtained show that NeuroD1 has an important role in providing terminal differentiation of neuroblastoma cells (16). In addition, P19 embryonal carcinoma cells have been reported to have high NeuroD levels (17). In mutant mice with a homozygous deletion in the NeuroD gene locus, defects in the development of granule cell layer in Dentate Gyrus, one of the main structures of the hippocampal formation, have been detected. These disorders lead to malformation of the dentate granule cell layer and excessive cell death. NeuroD mutant mice have also been reported to exhibit seizure activity with increased excitability in the hippocampus and cortex (18). Since, in another study, reduced cell volume and hypoplasia were observed in NeuroD knockout mice in regions adjacent to the Dentate Gyrus CA3 area. Although the number of cells decreased, it was found that existing cells showed signs of maturation (19). These findings show that NeuroD1 plays an important role in the development of the hippocampus and dentate gyrus. However, it may be said that dentate gyrus granule cells can also mature in the absence of NeuroD.

In a study on NeuroD1 knock-out mice, it was shown that vestibular and cochlear afferents enter the cochlear nucleus as a single mixed nerve in the absence of Neurod1 in the ear. In addition, it was observed that the peripheral innervation of the remaining sensory neurons was irregular and neuronal terminations projected more than one end-organ (20). These findings show that NeuroD1 is not only essential for the survival of neurons in the central nervous system, especially in the ear, but is also critical in regulating the central projection of afferents.

In a study conducted in 2017, the transformation of non-reactive astrocytes into striatal neurons was observed by intravascular injection of NeuroD1 with Adeno associated virus 9 (AAV9) in mice (21). In another study, it was aimed to transform astrocytes into functional neurons with a "chemical cocktail" that is a mixture of NeuroD1 and Neurogenin. It was observed in the study that transformed neurons could survive more than 5 months in vitro. In addition, it has been reported that neurons that survive for more than 1 month in vivo can function by being included in local neuronal circuits (22). In a study conducted in 2020, NeuroD1 injection was applied to ischemic damage mouse brains with the AAV9 gene therapy method. It has been reported that one-third of the neurons lost as a result of ischemic damage was rescued after the application and another third of the damaged neurons were also improved (23). These findings make NeuroD1 an important target for the development of regenerative therapies for the

treatment of pathologies that cause neuronal loss, especially ischemic neuron damage.

In summary, NeuroD1 is an important transcription factor that plays a role in the development, differentiation, and migration of neurons in the central and peripheral nervous system. Its relationship with the pancreas, auditory system, and olfactory system has been shown, and its deficiency causes the development of pathologies in these systems. Recent studies show that NeuroD1 can also be targeted therapeutically. Especially, its application after ischemic brain injury is promising in terms of minimizing neuronal loss. Accordingly, similar therapeutic approaches can be evaluated in terms of NeuroD1-associated sensorineural hearing loss and diabetes mellitus.

1.2. Doublecortin

Doublecortin is a microtubule-related phosphoprotein that stimulates tubulin polymerization, regulates the organization and stability of microtubules. It is encoded by the DCX gene. It is seen as a marker of development in migrating neuroblasts, young neurons, and adult neurogenesis in the hippocampus (24). The function of doublecortin and related molecules is generally considered as regulation of microtubule dynamics, modulation of cytoskeletal structure, mitotic spindle formation, and microtubule-based transport. In addition to microtubules, Doublecortin-like kinase 1, 2, and 3 (DCLK1, DCLK2, and DCLK3) encode serine/threonine kinase domains that show homology to Ca^{2+}/calmodulin-dependent protein kinases (25-27).

Doublecortin expression is known to be specific for newly generated neurons. The reason for this is that many of the doublecortin-positive cells in the Dentate Gyrus also express other early neuronal antigens (28). However, not all neurons newly formed in the brain express doublecortin. While expression was observed in newly formed hippocampal, striatal, and olfactory neurons, it was reported that doublecortin was not found in interneurons in the neocortex and striatum (29). In addition, doublecortin expression has been shown to play a role in the functioning of newly formed neurons by joining neuronal networks in adult neurogenesis (30). Initial studies have shown that mutations in the DCX gene cause X-linked lissencephaly and subcortical band heterotopia (SBH) syndromes associated with abnormal migration of cerebral cortical neurons (31).

DCX mutations generally cause anterior dominant lissencephaly in men and SBH in women. In a case report published in 2016, a new DCX mutant causing focal epilepsy with late childhood-onset and anterior dominant pachygyria without SBH was reported in both genders (32). In the light of this information, it is seen that the cortical neuronal organization

is altered as a result of Doublecortin mutation. In addition to this, in another study, in addition to neuronal migration, disorders in neuronal proliferation were observed in embryonic brains with DCX mutations (33). These findings suggest that Doublecortin's functions are not limited to neuronal migration but are also critical for cellular proliferation that occurs during neurogenesis.

Studies have reported that DCX mutations lead not only to cortical malformations but also to the development of blindness-related retinitis pigmentosa and dyslexia (34, 35). Retinitis pigmentosa causes night blindness, progressive loss of the peripheral visual field, and pigment deposits. This results in severely reduced vision or blindness in patients. In addition, there are studies on the role of the RP1 gene in this retinopathy (36). In addition, in addition to RP1, DCLK1 and DCLK2 have been reported to be expressed in the mouse photoreceptor complex (37). Considering the localization and similar functions of these proteins, the roles of Doublecortin and Doublecortin related molecules in the pathogenesis of Retinitis Pigmentosa can be investigated in more detail with tubular functions.

In a study conducted in 2015, DCLK1 overexpression was shown in renal cell carcinoma (RCC) cells. In the study, it was shown that invasion, migration, focal adhesion, drug resistance, and clonogenic capacity in tumor cells decreased significantly after the deletion of DCLK1 using small-interfering RNA (siRNA). In addition, it has been reported that epithelial-mesenchymal transition and tumor stem cells decrease pluripotency factors, which are associated with increased recurrence and decreased survival (38). These findings show that DCLK1 is an important target for new studies aiming to suppress epithelial-mesenchymal transmission, metastasis, and drug resistance in RCC. In another study, overexpression of DCLK1 and decreased expression of microRNA-424 (miR-424) were detected in ovarian clear cell carcinoma cells compared to non-tumor counter cells. In addition, it has been reported that in these cells, suppression of DCLK1 by miR-424 stops growth by suppressing tumor cell viability and invasion (39).

In a study conducted in 2021, the relationship of DCLK1 with small extracellular vesicles in tumor development and spread in gastric cancer cells was examined. Suppressing the DCLK1 and small extracellular vesicle functions with a DCLK1 inhibitor, showed that suppress tumoral activity (40). These findings show that the effects of Doublecortin and its related kinase molecules on the cytoskeleton are important for the development of new approaches in cancer treatment.

In summary, Doublecortin plays a role in neuronal development,

especially in providing cortical organization, as well as in immature neuronal migration and proliferation. Mutations in DCX, the gene encoding doublecortin, might lead to cortical malformations, epileptic syndromes, and visual disorders such as Retinitis pigmentosa. In addition, Doublecortin-like kinases have been shown to be involved in the development, invasion, metastasis, and drug resistance of solid tumors. Accordingly, it may be an important goal to develop therapeutic approaches by investigating this cytoskeleton-related molecule in other cancer types.

1.3. PSA-NCAM

Polysialic acid (PSA) is a linear glycopolymer consisting of multiple α2,8-linked N-acetylneuraminic acid units that bind to the neural cell adhesion molecule (NCAM) as a post-translational modification (41).

Neural cell adhesion molecule (NCAM) belongs to the immunoglobulin (Ig) superfamily of glycoproteins (42). NCAM mediates Ca2 + -independently homophilic / heterophilic cell-cell and cell-extracellular matrix interactions. It also plays a role in neurite outgrowth, cell migration, synapse formation, and axonal branching. The polysialylated form, PSA - NCAM, shows adhesion-reducing properties (43). In addition, NCAM and PSA-NCAM play an important role in synaptic plasticity, learning, and memory in the adult nervous system (44).

PSA-NCAM is highly expressed during brain development. It has been shown that PSA-NCAM is highly associated with progenitors associated with neural stem cells (45). In addition, it has been reported that newly formed and developing granular cells in Dentate Gyrus express PSA-NCAM at a high rate (46). In addition, most of the cells expressing PSA-NCAM were also found to be positive for NeuroD and Doublecortin (47).

During embryonic development, PSA-NCAM is widely distributed throughout the nervous system (48). However, in adults, neurogenesis has been found to be expressed in regions that show structural and synaptic remodeling (49). During adulthood, PSA-NCAM is expressed in major regions such as the olfactory nucleus where the neuronal formation is provided (50). In addition, it has been shown that the enzymatic suppression of PSA prevents the neuronal progenitor cells produced from progressing into the lesion (51). In addition to the role in providing memory, learning, and plasticity, PSA-NCAM is thought to be associated with neurological and neurodegenerative diseases.

Decreased expression was observed in PSA-NCAM in the Dentate Gyrus and Hilus regions of the hippocampus of schizophrenic patients compared to the control in a study. It has been reported that this decrease may be related to the decrease in synaptogenesis and plasticity related to

the pathophysiology of schizophrenia (52). In another study, a high level of PSA-NCAM expression was observed in the hippocampus and entorhinal cortex of patients with epilepsy. It has been reported that this may be related to new axon formation and neurogenesis that occur after epileptic seizures (53). It has been reported that the levels of markers such as PSA-NCAM, Doublecortin, and NeuroD increase in the new neurogenesis that occurs after neurons damaged in the hippocampus in patients with Alzheimer's disease. In this context, it has been suggested that these molecules can be therapeutically induced (54). These findings show that the role of PSA-NCAM in neurogenesis and plasticity may be related to the pathophysiology or prognosis of the diseases.

In a study conducted in 2021, the PSA-NCAM levels in the hippocampus in mice with a neonatal hypoxia-ischemia model were examined. In the findings obtained, an increase of PSA-NCAM in the dorsal hippocampus and serum levels of mice observed. It has been reported that this increase may have developed as an acute response to neurodegeneration in the hippocampal tissue (55). Accordingly, PSA-NCAM levels that change in response to plasticity and neuronal damage should be examined in detail. In another study conducted in 2021, the neuroprotective effect of 5-noniloxytryptamine (5-NOT), a PSA-NCAM mimetic, against glutamate toxicity was investigated. It has been reported that 5-NOT treatment in cerebellar neurons upregulates synaptic plasticity showing decreased expression and expression of cell survival pathway proteins (56). In a study conducted in 2020, it was shown that cells expressing cholecystokinin and co-localized with PSA-NCAM increase the efficacy of serotonergic antidepressants (57). With this information, it may be said that PSA-NCAM should be evaluated not only in terms of neurogenesis but also in terms of therapeutic approaches to diseases. In another study, PSA-NCAM expressing cells in the hippocampus obtained from epilepsy patients were analyzed. While the number of PSA-NCAM decreased in patients with granule cell dispersion, the presence of abnormal PSA-NCAM + cells was observed (58). These findings show that PSA-NCAM can be an important target for the evaluation of neurogenesis and neuronal damage in epilepsy.

In summary, PSA-NCAM is a molecule that regulates the interaction between cells and the cell-extracellular matrix in the brain. It also plays a role in neuronal development and the functioning of progenitor cells. In addition, PSA-NCAM's role in plasticity-related mechanisms makes it an important target in the pathophysiology and treatment of diseases that cause neuronal damage. New therapeutic approaches can be targeted by providing neuroprotective effects, especially with PSA-NCAM mimetics.

Conclusion

NeuroD1, Doublecortin, and PSA-NCAM are important molecules involved in the development of the central and peripheral nervous system. In addition to its effectiveness in neuronal development and differentiation processes, recent research has begun to target its therapeutic effects. In this respect, it is important to clarify the roles of NeuroD1, Doublecortin, PSA-NCAM, and many different "progenitor" molecules in basic mechanisms, in terms of future treatment approaches. NeuroD1 targeted therapies after neuronal formation and brain damage should be evaluated with further studies and more effective vectors. Similarly, it is important to target the potential anti-tumoral effects of Doublecortin clinically, supported by further in vivo research. In conclusion, the functions of the basic molecules involved in neurogenesis should be investigated and the mechanisms should be elucidated. Understanding the pathophysiology of neurodegenerative diseases in the clinic and developing new treatment approaches can be made possible with the mechanisms that have been elucidated.

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Chapter 3

NURSING MANAGEMENT OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Rheumatoid Arthritis Definition and Epidemiology

Rheumatoid Arthritis (RA) is a chronic, systemic inflammatory disease of unknown cause. Although the disease mainly affects the joints, extra-articular findings such as skin, lungs, and heart can also be seen (Boldt, 2013). RA is the most common inflammatory joint disease in the world and our country, and the prevalence of the disease varies between 0.5-1%. The prevalence of RA in our country is 0.36% (Akar et al., 2004). The disease is seen at all ages, regardless of race, ethnicity, and geographical region, and increases markedly between the ages of thirty-five and sixty. It starts at an earlier age in women and is 2-3 times more common than men. While the incidence increases with age, the male/female ratio converges (Helmick et al., 2008; A. M. Wasserman, 2011; White & Kocijan, 2016).

Etiology of Rheumatoid Arthritis

RA is an autoimmune inflammatory rheumatic disease of unknown etiology that mainly affects the synovial joints, can cause loss of physical function, is characterized by symmetric joint involvement, has a chronic course, and affects more than one joint and system (Brasington Jr, 2015). Some genetic and environmental factors play a role in the emergence of the disease. Although the disease is not exactly hereditary, there is a lot of evidence that genetics plays a role. For example, first-degree relatives of patients with RA have a 1.5-fold increased risk of developing the disease. The most emphasized environmental factors are infections, hormones, and smoking (Zyrianova, Kelly, Sheehan, McCarthy, & Dinan, 2011). As RA affects the joints, it can also cause damage to different organs. RA is a long-term disease that can affect psychological and social functions as well as physical health (Ryan, 2014). Therefore, it includes a treatment that includes both medical treatment and social support.

Rheumatoid Arthritis Physiopathology

Tissue Antigens (MHC) play an important role in susceptibility to RA. HLA DR4 significantly affects disease severity. Socioeconomic status, living conditions, psychological factors affect the course of the disease. Smoking is considered a risk factor for the development of RA (Padyukov, Silva, Stolt, Alfredsson, & Klareskog, 2004). Infectious agents have been investigated as the cause of RA, but no definitive evidence has been found (Schellekens et al., 2000).

Synovial inflammation plays a fundamental role in the pathogenesis and course of RA. Synovial tissue increases, inflammatory cell infiltration is evident, synovial cell hyperplasia becomes evident, blood vessels increase (angiogenesis), and the characteristic picture is settled with invasion into cartilage and bone. The characteristic histopathological appearance of RA

is called pannus (Schellekens et al., 2000; Vallbracht et al., 2004).

Cytokines, especially TNF-a, play a role in the pathogenesis of RA. Interleukin-1 (IL-1), IL-6, colony-stimulating factor-1 (CSF-1) synthesized by macrophages and fibroblasts and IL-2, IL-3, IL-4 produced from T cells are other effective cytokines in the pathogenesis of RA. . Rheumatoid factor (RF) developing against the Fc part of IgG is not specific for RA, but if it is positive at high titer and this high titer persists despite treatment, it is a poor prognosis sign for the disease. RF; It can also be positive in Sjögren's syndrome, systemic lupus erythematosus, myxoconnective tissue disease, scleroderma, polymyositis, reactive arthritis, osteoarthritis, and even in healthy people. Anti-Cyclic Citrullinated peptide (anti-CCP) is an antibody considered highly specific for RA (Schellekens et al., 2000; Vallbracht et al., 2004).

Rheumatoid Arthritis Symptoms and Signs

The symptoms of RA differ from patient to patient. Approximately 70% of patients have an insidious onset spread over several weeks or months. During this period, patients may develop a mild fever, weakness, fatigue, weight loss, and pain in one or more small joints (Gabriel & Crowson, 2015). In addition, after sleeping or resting for a long time, a feeling of stiffness occurs in the joints or around the joints and defined as morning stiffness (Özsoy, Altınel, Çağrır, Çavuşoğlu, & Dinçel, 2006). During the active phase of the disease, it can last longer than an hour. Patients increasingly state that they have extreme difficulty in doing their daily work and their joint functions are decreasing.

The earliest and most important finding of joint inflammation in RA is joint tenderness and swelling. There may be an increase in temperature in the joint, but there is no erythema. The sensitivity of the joint is revealed by applying direct pressure to the joint during palpation. Symmetrical involvement of the hand joints is characteristic in RA. Arthritis of the distal interphalangeal joint is rarely seen in RA, and this feature is considered important in differentiating it from osteoarthritis (Magyari et al., 2014). Uncontrollable inflammatory synovitis of the proximal interphalangeal and metacarpophalangeal joints causes ulnar deviation as a result of subluxation with button marrow and gooseneck deformities. Involvement of the cricoarytenoid joint can cause hoarseness, and involvement of the ossicles in the ear can cause hearing problems (Nam et al., 2010). Involvement of the cervical vertebrae is common, but the thoracic and lumbar regions are rarely affected. Sacroiliac joints may be involved in advanced RA patients. Knee joint involvement is common in RA and significantly reduces the patient's quality of life. Popliteal Baker's cyst is one of the most common problems associated with the knee joint in RA patients, and

when the cyst ruptures, it causes a clinical picture indistinguishable from true thrombophlebitis (Magyari et al., 2014; Nam et al., 2010; Schaible, Schmelz, & Tegeder, 2006; Schultz. 2005).

The onset of the disease varies depending on factors such as the patient's age and gender. Although RA clinic is seen in some patients within weeks, it can settle insidiously within months in some patients. Insidious onset is more common (Shim, Stavre, & Gravallese, 2018). There may also be RA patients with a single joint involvement. This difference at the beginning is also seen in the course of the disease. Apart from a disease that lasts at least 1 year with remission and attacks, it can also be mentioned that there is a disease clinic that gradually restricts functions with short remissions and exacerbations, or 3 different disease courses that show rapid and severe joint involvement (Zhernakova, Withoff, & Wijmenga, 2013). Severe joint involvement at the beginning, uncontrollable polyarthritis, high titer positivity of RF, presence of HLA-DR4 are the predictors for the poor prognosis of the disease. RA, which progresses with complaints and findings completely related to progressive synovitis in the joint in the early period, causes the development of extra-articular systemic organ involvement in nearly half of the patients (Salamanna, Veronesi, Frizziero, & Fini, 2019).

In the hematologic system, changes occur in all three cell types in RA patients. Anemia develops due to many factors. Iron use may be insufficient in RA. Megaloblastic anemia may also develop with anemia of chronic inflammation and iron deficiency due to the drugs used. Thrombocytosis accompanies active disease and thrombocytopenia is rare. Leukopenia is part of Felty syndrome. These patients also develop splenomegaly (Moutsopoulos, Zampeli, & Vlachoyiannopoulos, 2018). In the active RA period, the liver enzymes of the patients are high. In addition, drugs that modify the disease, especially nonsteroidal anti-inflammatory drugs, also cause liver side effects (Mills et al., 2018).

Respiratory system involvement is common in RA. Although the disease is more common in women, lung involvement can be more common in men. The pleural fluid, which is characterized by cell infiltration dominated by lymphocytes, may have transudate characteristics. The lactic dehydrogenase and protein content are high, the glucose content is very low, and the fluid can be mostly unilateral. Pulmonary nodules are larger than 1 cm (maybe as large as 8 cm) and may occur in large numbers. The patient may also have nodules in the periphery (Gono, Tokuda, Sakai, & Takemura, 2018). Laboratory findings of RA vary according to the duration and clinical course of the disease. In the initial period and the exacerbation periods of the chronic disease, the acute phase response is high in positive correlation with the severity of the inflammation.

High erythrocyte sedimentation rate and especially C-Reactive Protein (CRP) levels are accepted as the most sensitive tests showing disease activity and response to treatment (Guo et al., 2018).

Rheumatoid Arthritis Treatment

In the past 10 years, a good understanding of the pathogenesis of the disease has prepared new treatment approaches. First, early diagnosis and therefore early treatment is considered the most important step in the treatment of RA. After the diagnosis of the disease, disease-modifying drugs (DMARDs) can be started. Because joint damage develops early in the course of RA (Akıcı & Aydın, 2018). Radiologic findings of joint erosions occur at the time of diagnosis in one-quarter of the patients. Erosion develops in more than half of the patients in two years. Bone erosions and deformities are irreversible. From this point of view, early diagnosis becomes very important. The diagnosis is not based on a single laboratory test, the classification criteria determined by the American Rheumatism Association are carefully used (Akıcı & Aydın, 2018; Bullock et al., 2018). Joint involvement may resemble RA in many other diseases. Therefore, clinical criteria for RA are sought for at least six weeks. Anti-CCP (cyclic citrullinated peptides) is very specific for the disease (90-98%), although it is initially found at a rate of 50-65%, it can be used for early diagnosis. These antibodies can be found in the serum of patients long before the onset of the disease (Harrold et al., 2019). RA treatment aims to improve the symptoms and signs of the joint and to make them functional. The treatment is decided by considering the number of swollen and tender joints at the beginning, the markers of inflammation in the serum, the functional status of the patient, and the accompanying diseases. Drugs used in the treatment of RA are collected in three groups: NSAIDs, steroids, and DMARDs (Arayssi et al., 2018).

- NSAIDs do not affect the course of the disease. They are only effective as long as they are taken. It does not suppress sedimentation rate and CRP. These drugs do not change the course of the disease. There is no difference between them in terms of their effects.

- Steroids have strong anti-inflammatory effects, but they also have serious dose-dependent side effects.

- The use of steroid therapy without DMARD in the treatment of RA is not considered correct (Akıcı & Aydın, 2018; Arayssi et al., 2018; Harrold et al., 2019).

The main drug in the treatment of RA is Methotrexate (MTX). In treatment guidelines, MTX is the first drug that can be started in the treatment of RA, and if it is not started as the first drug, it is the drug that should be

added immediately when there is no response to the drug used. It is used alone or in combination with other DMARD drugs, orally or parenterally (Allen, Carville, & McKenna, 2018). TNF-α inhibitors used as biological agents in the treatment of RA are infliximab, etanercept, and adalimumab, golimumab, certolizumab pegol drugs. Each is effective in the treatment of RA as monotherapy (single drug therapy). TNF-α inhibitors reduce the symptoms of RA in the majority of patients and stop the progression of bone erosions. However, none of them provide a complete remission of the disease, and joint symptoms can relapse a few weeks after the drug is stopped in most patients (A. Wasserman, 2018).

Effects of Rheumatoid Arthritis on Joints

Typical joint involvement in RA is usually symmetrical and pain, swelling, tenderness, and loss of function occur in many joints at the same time. Joint pain is the most important problem of patients (A. M. Wasserman, 2011). One of the criteria used in determining the course of the disease and evaluating the response to treatment is joint pain. The inflammatory nature of the pain is understood by the presence of morning stiffness, and prolonged morning stiffness is a typical finding of RA. Joint tenderness is easily detected by palpation. Joint swellings can be caused by the presence of fluid or by synovial proliferation. Synovial proliferation is palpable between the skin and underlying bone, and its consistency is pasty (Ye, Kalichman, Spittle, Dobson, & Bennell, 2011). In the early stages of RA, insidious progressive atrophy begins in the muscles adjacent to the inflamed joints. As a result, the patient's pain and weakness occur. Deformities develop over time due to damage caused by inflammation (Kobayashi et al., 2008).

Metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints are involved in most patients. Although the disease can involve all synovial joints, it usually starts in the MCP, PIP, and metatarsophalangeal (MTF) joints, then wrists and ankles, elbows, shoulders, knees, and hips are involved (Kobayashi et al., 2008). Joint stiffness is one of the most prominent symptoms of RA. It occurs early in the day and affects life activities. Morning stiffness is thought to be caused by inflammation and edema in the synovium. Morning stiffness regresses and disappears during the remission period of the disease. In RA, signs of inflammation such as pain, swelling, temperature increase, and loss of movement are seen in the clinical picture in the first years. In people whose disease cannot be adequately controlled, deformities and loss of function due to joint instability occur in later years (Ryan, 2014).

Hand involvement

MCP joints, PIP joints, and wrist joints are the most frequent and earliest involved joints in RA. Symmetrical fusiform swelling in the PIP joints and accompanying swelling in the MCP joints are the typical manifestations of RA. Involvement of the distal interphalangeal joints is rarely seen alone and is not the first site of involvement. DIF joint involvement may be due to concomitant osteoarthritis, especially in geriatric RA patients (Cutolo et al., 2006). Studies in which the involvement of the wrist joints in RA are followed radiologically in the long term have shown that joint damage develops in the first three years, especially in the first year, and then disease progression slows down (Qwaider & Abu Naser, 2017).

Initially, swelling, pain, and limitation of movement are predominant due to synovium hypertrophy, while deformities typical for RA develop over time. Swan neck deformity results from flexion of the DIF and MCP joints and hyperextension of the PIP joint. The hyperextension of the DIP joint with flexion of the PIP joint is called the buttonhole (boutonniere) deformity. Two deformities that develop due to MCF joint involvement are volar subluxation and ulnar deviation of the fingers relative to the metacarpals. With ulnar deviation, it is mostly seen together with radial deviation of the wrists. The ulnar collateral ligament is stretched by the proliferative synovium of the radioulnar joint, resulting in ruptures or destruction. The head of the ulna slides upward into the dorsal prominence. It can be easily pressed with the examiner's fingers and fluctuates (Brenton-Rule, Dalbeth, Menz, Bassett, & Rome, 2017). Since tendons are covered with synovium, trigger finger due to flexor tenosynovitis, tendon ruptures and swellings on the dorsal aspect of the wrist due to extensor synovitis. may occur. Median nerve compression may occur in the carpal tunnel due to tenosynovitis, and bilateral carpal tunnel syndrome often develops (Gutierrez et al., 2016).

Three types of deformities have been described for the thumb:

Type I: MCF inflammation causes a stretch-induced buttonhole-like deformity in the joint capsule.

Type II: Carpometacarpal joint (CMC) inflammation leads to volar subluxation if adductor hallucis contracture is present.

Type III: After long-term involvement of the MCP joints, excessive adduction of the first metacarpal, flexion of the MCP joints, and hyperextension of the DIF joints develop due to the need for a thin grip (Cutolo et al., 2006).

Elbow involvement

The most common findings in the elbows in RA are synovitis or inability to fully extend the elbow due to effusion, the presence of periarticular cysts associated with effusion, and rheumatoid nodules. Flexor contracture in the elbows may develop early in the disease and patients may not be aware of it (Prodinger, Hammond, Tennant, Prior, & Tyson, 2019).

Shoulder involvement

More than two-thirds of RA patients have shoulder complaints. It has been observed that shoulder involvement is more common especially in geriatric patients and those with RF positive. RA can cause the involvement of all components of the shoulder joint. Involvement occurs in the acromioclavicular and glenohumeral joints, in the subacromial region, and to a lesser extent in the sternoclavicular joint. Involvement of the shoulder causes pain, loss of range of motion, and dysfunction (Prodinger et al., 2019).

Cervical spine involvement

In a study in which the hand, foot, and cervical spine radiographs of RA patients were followed annually, it was shown that the involvement of the cervical vertebrae progressed in parallel with the involvement of the hand and foot joints. The most frequently involved part of the cervical vertebrae is the occipital atlantoaxial area (Joaquim, Ghizoni, Tedeschi, Appenzeller, & Riew, 2015). The atlantoaxial joint is a synovial joint and, like other synovial joints, can undergo proliferation and instability. Subluxation may develop due to erosion formation and ligament damage. In the presence of atlantoaxial subluxation, there is a danger that the odontoid process may compress the spinal cord during neck flexion. Head and neck pain, paresthesia, weakness, transient ischemic attack, relaxation of the bladder and anus sphincter may occur (Carotti, Salaffi, Di Carlo, Sessa, & Giovagnoni, 2018). Thoracic, lumbar, and sacral portions of the spine are usually spared. However, the apophyseal joints are the exception; Synovial cysts that rarely occur in the apophyseal joints may protrude into the spinal cord, causing pain and neurological deficits (Yoshihara, Yoneoka, Margalit, & Zuckerman, 2016).

Hip involvement

The early signs of hip joint involvement are pain with rotation or when a load is placed on it, and difficulty in walking. Pain is usually felt in the groin and inner thigh. Significant acetabular protrusion occurs in 5% of all patients with RA. (Yoshihara et al., 2016).

Knee involvement

Knee involvement is common in RA, it can be encountered in the early period, sometimes as the first joint involved, and is a good indicator of disease activity. Effusion in the knees, atrophy, and functional deformity may develop due to the deterioration of the function of the quadriceps muscle. Loss of knee extension movement can be seen in the early stage of RA. Another condition related to the knee joint is secondary osteoarthritis due to progressive synovitis (White & Kocijan, 2016).

Ankle and foot involvement

Involvement of these joints is as common as the hand joints, and the first erosions are observed in the MTF joints in 10% of patients. With MTF joint involvement, gait may change because pain develops in the push-up phase of gait. After MTF joint involvement, dorsal subluxation of the metatarsal heads occurs, flexion of the fingers may develop to compensate for this situation (hammer finger). As the metatarsal heads become a weight-bearing surface, callus development under the metatarsal heads and even ulcers may occur in later periods. The subtalar and talonavicular joints are frequently affected in RA. Synovitis developing in these joints causes pain and stiffness. It can sometimes lead to subtalar dislocation (White & Kocijan, 2016).

Nursing Care in Rheumatoid Arthritis

With the emergence of new treatment strategies in rheumatology and the professionalization of nurses, the nurse's care role in rheumatology is more needed. Nurses, who take on advanced and expanded roles today, achieve great success in the care of rheumatology patients (Kelly et al., 2015). Nurses' caring roles include patient self-management support, patient education and counseling, information on intra-articular injections, medication management, coordination with other healthcare professionals, the convenience of telephone consultation, and education about biological treatments (Garner, Lopatina, Rankin, & Marshall, et al. 2017; Hill & Hale, 2004; Schiff et al., 2017).

Nursing care in rheumatology patients; consists of subjects such as disease management, patient education, pain management, supporting life activities, continuation and follow-up of medical treatment, supporting and increasing physical strength, preserving tissue integrity (Bands, 2007). Nursing management includes diagnosis, practice, evaluation, and patient education. In the diagnosis, a comprehensive history is taken including the sociocultural characteristics of the individual, the presence of secondary disease, the course, and duration of the disease. After taking the patient's history, the physical diagnosis of the individual should be started. Especially

deformities of the affected joint, joint patency should be checked. Each joint should be monitored for symmetry, appearance, heat and temperature, color, form. The patient's pain should be carefully examined. It should be tried to understand the other experiences of the individual about the disease (Meadows & Sheehan, 2005).

Nurses can take interventions by evaluating the general health status of patients with RA, and thus they can increase the treatment compliance and quality of life of patients by reducing the complications that may develop due to RA (Kaya 2006; Dirik and Karancı 2010; Pehlivan et al. 2010; Pehlivan et al. 2015). The use of models and theories while providing care and education to their patients by nurses has been suggested and seen to be beneficial for individuals with RA (Naranjo et al., 2014). Individuals need to adapt to the disease, treatment, and drugs. It has been argued that the needs of individuals with RA should be met by producing diagnoses and interventions by nurses in terms of the care needs of individuals with RA (Hill & Hale, 2004).

After the diagnosis, the nursing diagnoses for the patient with RA are stated as follows:

- Chronic Pain

- Lack of Self-Care (Bathing)

- Lack of Self-Care (Toilet, Hygiene)

- Lack of Self-Care (Nutrition)

- Lack of Self Care (Dressing)

- Disruption in Sleep Pattern

- Tiredness

- Impaired Physical Mobility (ineffective management of chronic pain secondary to RA)

- Impairment in walking (related to chronic pain in RA)

- Deterioration in Maintaining the Home

Relief of pain: The drugs prescribed to the patient are given according to the doctor's request. Hot or cold application is made to the patient. The warm application can give comfort to the patient within twenty minutes and it can be ensured that the patient can do his exercises more easily. If there is acute inflammation, the cold application should be applied. As walking aids reduce the weight on the joints, they also reduce pain.

Self-care inadequacy-(bath): The patient's ability to use assistive devices is evaluated. Body cleanliness and oral mucous membrane are

evaluated daily. The condition of the skin is evaluated during the bath. The patient and his family are directed to alternative methods of bath and oral hygiene. It is recommended to take painkillers before taking a bath. In the planning of the patient's care interventions, support is obtained from physiotherapy and occupational therapy specialists for materials that can be used. The patient is encouraged to be independent in maintaining bathing and oral hygiene, the patient is assisted only when necessary. The patient is encouraged to set his own pace during self-care. The family is involved in providing and maintaining care. As much as possible, the patient's preferences and needs are regulated (eg, taking a bath, etc. shower, and what time of the day he wants to take a bath). The patient is assisted until he fully takes care of himself. Towels, soap, deodorant, shaving materials, and other necessary materials are placed at the head of the bed or where they can easily reach the bathroom. When necessary, the patient is supported to brush their teeth. It is recommended to install a non-slip floor and grab handles in the bathroom. Referral to home health care services when appropriate. Bathing skills are taught to caregivers when necessary (Wilkinson & Ahern, 2016; Wilkinson, Barcus, Meneghetti, & Rigon, 2017).

In the elderly: The ability to perform life activities independently using appropriate scales is evaluated. Cognitive and physical changes that increase self-care inadequacy are evaluated. Strength-enhancing exercise and walking are encouraged. It is ensured that there is a non-slip floor and grabs handles in the bathroom. Use no-rinse cleansers and warm water instead of soap. The bathroom environment is kept warm and only the washed area is left open. To prevent skin dryness, take a full bath once or twice a week, and a partial bath the other days. Bathing and drying are done gently to protect sensitive skin. The independence of the elderly is supported to the extent possible (Wilkinson & Ahern, 2016; Wilkinson et al., 2017).

Lack of self-care (toilet): The patient's ability to walk independently and safely is evaluated. The patient's ability to straighten his clothes is evaluated. The patient's ability to use assistive devices (eg walker, cane) is evaluated, and activity intolerance and energy level are monitored. The progress of the patient's ability to meet his/her own toilet needs is monitored. Sensory, cognitive, and physical deficiencies that may limit self-wasting are evaluated. Transfer and movement techniques are taught to the patient and his family. Adaptation activities and the use of assistive tools are shown. Painkillers are recommended/applied before going to the toilet. Physiotherapy and occupational therapy resources are used in planning patient care interventions and providing necessary auxiliary tools. When necessary, it helps to meet the basic care and toilet needs.

The patient is supported to wear clothes that are easy to manage, and he is helped to dress when necessary. At certain intervals, the patient is assisted in going to the toilet, sitting on the potty chair, using the slider and the duck. Toilet cleaning is facilitated after emptying is complete. Unloading tools (potty, slider) are cleaned. After evacuation, the patient's clothes are dressed. Privacy is protected during discharge. Items that prevent going to the toilet are removed (eg loose rugs and small movable furniture). Family and relatives are informed about the arrangement of the home environment for patient safety (Wilkinson & Ahern, 2016; Wilkinson et al., 2017).

In the elderly: Cognitive deficits are managed (eg, verbal directions are kept short and simple). To prevent reluctance and fatigue, sufficient time is allocated to meet the toilet needs. Strength-building exercise is recommended and assisted. The patient is helped to move for a few minutes when he gets up for the toilet. A potty chair or a toilet stool is used when the knees need to be elevated above the hips (Wilkinson & Ahern, 2016; Wilkinson et al., 2017).

Lack of self-care (nutrition): The ability to use assistive devices is evaluated. The energy level and activity tolerance are monitored. Improvement or deterioration in self-feeding ability is observed. Chewing and swallowing skills are evaluated. Nutritional intake for adequate nutrition is evaluated. Adaptation activities and the use of assistive tools are shown. Alternative methods of eating and consuming liquids are taught to the patient, and the method and education plan are determined. Adequate pain relievers are administered before meals when necessary. One nutrient at a time is given in small amounts. The patient's independent eating and fluid intake are supported, assisted only when necessary. To increase the patient's independence, meals that are eaten by hand (eg fruit, bread) are preferred. A pleasant environment is created while eating. Processes such as cutting the meat and peeling the eggs are done on the tray and the food is arranged. Pipettes are available for liquids if necessary or if the patient wishes. Assistive devices such as long handles, large round handles, or small strapping utensils are provided to support patient self-feeding when needed (Wilkinson & Ahern, 2016; Wilkinson et al., 2017).

Inadequate Self-Care (Dressing): The patient's ability to use assistive devices is evaluated. The energy level and activity tolerance are monitored. Sensory, cognitive, or physical deficiencies that may cause the patient to have difficulty in dressing are evaluated. Pain medication is recommended/applied before dressing and preparation. The patient is given one piece of clothing at a time during dressing. The dressing is started in the same order every day, as the patient can easily do. Velcro straps and locks are used on the patient's clothes. Opportunity is created and highlighted for small successes. The patient is encouraged to dress and prepare at his own

pace. The patient is assisted in choosing easy-to-wear and loose-fitting clothes. The patient's clothes are kept in a place where he can reach them in the order required for dressing. The privacy of the patient is protected while dressing. Laces, buttons, and zippers are assisted when necessary. If appropriate, extension materials such as long-handled shoehorns, button hooks, and zipper pullers are used during dressing. The patient's effort to dress is supported. The patient is encouraged to do strength-enhancing exercises and walking (A. M. Wasserman, 2011; Wilkinson & Ahern, 2016; Wilkinson et al., 2017; Williams et al., 2011).

Disruption in Sleep Pattern: Environmental factors such as noise and light that affect sleep are defined. The effect of the drugs used by the patient on the sleep pattern is evaluated. The patient's sleep/activity pattern is evaluated. The patient's sleep pattern and sleep hours are monitored or recorded. The factors that cause sleep pattern disorder (eg, psychological, physiological, lifestyle, frequent shifts at work, rapid time-region change, long working hours, and other environmental factors) are taught to the patient and his partner. The patient is taught about factors that facilitate the transition to autogenous muscle relaxation or other non-pharmacological sleep. When problems with sleep arise, the doctor is consulted for a change or review of the treatment plan. The doctor is consulted about the use of drugs that do not suppress rapid eye movement (REM) sleep.

When necessary, symptoms related to sleep patterns such as drowsiness, restlessness, and inability to concentrate are treated. Loud sounds are prevented, the overhead light is used during sleep, a quiet and calm environment is provided, and sleep interruptions are reduced. The patient is helped to identify the factors that cause insomnia, such as fear, unresolved problems, and conflicts. The patient is informed that conditions such as irritability and mood swings may be due to interrupted sleep. When necessary, the patient is asked to limit sleeping during the day and to do activities that provide wakefulness. Relaxing practice such as massage, positioning, and touching is initiated. Short-term sleep is provided to meet the need for sleep during the day (A. M. Wasserman, 2011; Wilkinson & Ahern, 2016; Wilkinson et al., 2017; Williams et al., 2011).

Fatigue: The patient is observed for signs of physical and emotional fatigue. The cardiorespiratory responses of the patient to the activity are observed. The patient's sleep hours and sleep times are examined and recorded. The nature and location of the discomfort or pain experienced during movement and activity are observed. Food intake is monitored to ensure adequate energy supply.

The patient and his relatives are informed about the signs and symptoms of fatigue that may require reducing activity. To prevent fatigue,

time management techniques and planning activities are taught in order of priority.

If fatigue significantly affects the patient's relationships, psychiatric help is sought. The patient and family are encouraged to express their feelings for life changes caused by fatigue. The patient is assisted in identifying measures to increase concentration; It is supported in ordering the tasks that need to be done according to their priorities and starting important activities after the rest period.

The patient is encouraged to specify the characteristics of the pain that initiates fatigue (intensity, location, causative factors). Activities to reduce fatigue are planned with the patient and his family. The prepared plan may include:

- Cognitive function and physical disturbances that may interfere with self-monitoring or regulated activity are reduced.

- The patient and his relatives are assisted in setting realistic activity goals.

- Calming activities such as reading, talking to others are recommended for relaxation.

- Activity restriction (eg increasing the number of rest periods) and bed rest are provided in the specified rest interval.

- Environmental stimuli are restricted to facilitate relaxation. Visitors are restricted if possible.

Opinions are exchanged with the patient and his family on arrangements that can be made in the home environment to continue their daily activities and reduce fatigue. The home environment is evaluated in terms of factors that increase fatigue. If chronic pain is the etiologic factor causing fatigue, it is directed for effective management of pain. Priorities are determined based on the activities that the patient and family can perform with the patient (Wilkinson & Ahern, 2016; Wilkinson et al., 2017).

Impaired Physical Mobility (ineffective management of chronic pain secondary to RA): The patient's need for durable medical devices and home health support is evaluated. The use of mobility aids (eg, cane, walker, crutches, or wheelchair) is observed and the patient is educated about them. Physiotherapist support is provided for an exercise program. Positive support is provided throughout the activities. The patient is informed about the use of non-slip and supportive shoes for walking. The patient is informed about how to maintain good posture and body mechanics during any activity. The patient is encouraged and informed about active and passive exercises to develop and maintain muscle strength

and endurance. The patient is informed about the correct body posture.

The motivation level of the patient is determined to restore or maintain the mobility of the joints and muscles. Occupational therapists and physiotherapists are assisted in the planning of patient care activities. The patient and family are encouraged to see their limitations realistically. Positive support is provided throughout the activities. Before starting the exercises, analgesics are given.

Environmental factors such as stairs and uneven floors that impede mobility at home are evaluated. He is taught to get out of bed slowly.

In the elderly: Complications related to immobility such as pneumonia and pressure sores, which develop more rapidly in geriatric individuals, are observed. The patient is evaluated in terms of cognitive impairment and depression. The patient is observed for orthostatic hypotension. When lifting from the bed, it is ensured that the feet are dangling before standing up (Wilkinson & Ahern, 2016; Wilkinson et al., 2017).

Impairment in walking (related to chronic pain in RA): The patient is supported positively during the interventions. Collaborate with a physiotherapist to increase strength, balance, and flexibility. The patient's need for medical equipment and home care services is evaluated. The patient's need for walking aids such as crutches is evaluated. In case the patient decides to move, the supply and use of assistive equipment (eg cane, walker, wheelchair) are assisted. The patient is helped to choose suitable shoes to facilitate walking and prevent falls. The patient is encouraged to act independently in a safe manner. The patient is taught active or passive exercises and encouraged to do so. Information about weight-bearing is given. Informed about correct body posture.

The patient is encouraged about weight lifting or trapeze work to increase and maintain the strength of the upper extremities. Family support is provided so that the patient can be walked at a specified distance. Occupational therapy and physical therapy are used when planning the care of the patient. Considering the limitations of the patient, the patient, and his family are encouraged. During the interventions, positive support is provided to the patient. Passive or assisted exercises are applied. Suggested analgesics are applied before starting exercise and walking. To prevent falls, the home environment is evaluated and potential hazards (eg, slippery floors, foldable rugs, etc.) are removed from the environment. If necessary, the intake of calcium and vitamin D in the diet is increased. Home care services are applied to patients who cannot fulfill their life activities and whose walking is impaired. It is checked whether the auxiliary equipment used is working safely. The patient is taught the necessary precautions to prevent falling (Wilkinson & Ahern, 2016; Wilkinson et al., 2017).

Impairment in Providing Home Care: The need for follow-up after discharge is evaluated and recorded together with the community health nurse. A realistic plan for home care is determined by contacting the health worker or social worker planning discharge. Information on the patient's temporary care is provided when necessary. Home cleaning services are provided when necessary. Facts about the state of the house are accepted and supported without judgment. The patient and family are assisted to identify hazards and difficulties at home that may hinder home care. The household is assisted to identify strengths within the family and support systems to assist with home care. Since the illness of one of the family members may affect the home care management, information is obtained about the health status of the patient and his family and all family members. The patient and his family are included in the decision-making process for home care needs. Suggestions are made regarding the structural changes necessary for easy access to the home. Suggestions for pest control services are made when necessary. Suggestions for home improvement services are made when necessary. Information is provided on the cost of maintenance needed and the resources available.

In the elderly: It is evaluated whether the patient can move independently at home (eg, his vision, hearing, movement, and other functional skills are evaluated). and household cleaning services. It is ensured that family members are aware that the patient needs help to continue living at home.

The patient is assisted in identifying community resources to assist with instrumental living activities (eg, catering establishments/businesses, home care nurses, home catering).

Evaluate the home environment for security threats (eg, the presence of handrails on the stairs and in the bathroom, and adequate light). Elder abuse status is evaluated (Wilkinson & Ahern, 2016; Wilkinson et al., 2017).

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Chapter 4

ATRIAL FIBRILLATION AND RISK FACTORS FOR POST-CORONARY ARTERY BYPASS GRAFTING

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Atrial fibrillation (AF); is a type of arrhythmia characterized by completely irregular atrial depolarization of 350-600 per minute without effective atrial contraction. The electrocardiogram (ECG), has small, irregular, variable atrial amplitude and morphology. Although it attracted the attention of doctors for a long time due to the irregularity in the pulses of patients, AF was first described by Lewis in 1909 with ECG. AF is the most common acquired cardiac arrhythmia, occurring in 1-2% of the general population. More than 6 million Europeans are followed up with this arrhythmia, and the prevalence is estimated to double at least in the next 50 years (Stewart, 2001).

Early diagnosis of AF cannot be made because of the silent course of rhythm disorders. Approximately one-third of patients with this arrhythmia are unaware of the disease and AF in this period is called "silent AF". Early recognition of arrhythmia will protect the patient from the consequences of arrhythmia and will not allow the arrhythmia to progress to a more malignant level. Numerous non-pharmacological interventions have been tried over the past decade to prevent the development of AF. Ablation techniques with a percutaneous catheter have provided significant success in the treatment of AF, especially by reducing the symptoms accompanying arrhythmia and providing a complete cure in some patients. It is obvious that more successful results will be obtained in the treatment of AF when applied together with newly developed medical treatments such as anti-thrombotic and anti-arrhythmic agents. Characteristic features of AF; irregular "RR" intervals and the absence of a prominent "P" wave on the ECG. Regular atrial activity can be observed, mostly in V1. The atrial cycle length is variable and is often less than 200 ms (Silverman, 1994).

The incidence of AF in the general population is 0.4%, and its incidence increases with age. Postoperative AF (POAF) is the most common type of arrhythmia after coronary artery bypass grafting (CABG), and the incidence has been found to be approximately 35%. The incidence of POAF is usually highest on the second and third postoperative days (Furberg, 1994; Greenberg, 2017).

Compared to advances in cardiac surgery and myocardial preservation techniques, no significant reduction in the incidence of newly developing AF has been achieved. POAF that develops in the early period after CABG is mostly short-lived, but it can sometimes resolve spontaneously. It is not a fatal complication, but it is an important medical condition as it causes severe hemodynamic deterioration, thromboembolic events, and more serious arrhythmias. It causes an increase in hospital costs and a prolongation of hospital stay (Zaman, 2000).

Although there is no complete agreement between studies, many causes have been defined for the development of AF after CABG. Some of those; rheumatic heart disease, chronic kidney disease (CKD), hemodialysis therapy, diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), hypertension (HT), peripheral vascular disease (PVD), left ventricular hypertrophy (LVH), preoperative digoxin use, prolongation of the aortic cross-clamp period, discontinuation of beta-blocker therapy in the preoperative period, increased sympathetic activation in the postoperative period, insufficient atrial protection, advanced age, and fluid-electrolyte disorders (Guo, 2002).

Many causes have been identified for the development of AF after cardiac surgery. These factors are divided into three classes as preoperative, intraoperative, and postoperative. Among the preoperative factors, it was stated that advanced age may have a significant effect on the development of POAF. In many studies, the incidence of AF according to age groups was less than 1% in the population under 60 years of age, while it was found to be more than 6% in the population over 80 years of age (Greenberg, 2017; Allessie, 2001).

Among the cardiovascular risk factors associated with POAF is HT especially with LVH. This may be related to fibrosis and dispersion of atrial refractoriness. HT is the most common and independent risk factor for the development of POAF with the highest potential for treatment. In the Framingham study, 60% of the patients who developed POAF were found to have HT, and in the ARMYDA-3 study of Patti et al., systemic hypertension was shown to be a factor that increases the risk for POAF (Kannel, 1982; Patti, 2006).

Male gender increases the risk of developing AF after CABG. The difference in the effects of ion channel expression and hormonal effects on autonomic tone in males and females may be the main reason for AF (Michelena, 2000). Among the intraoperative factors, some studies have shown a relationship between aortic cross-clamp time and increased AF development. The likely mechanism is prolonged atrial ischemia due to prolonged cross-clamping. Localization of venous cannulation is also associated with the development of AF. It has been shown that bicaval cannulation, which avoids atrium incision, can reduce the frequency of AF (Mathew, 1996). Ventilation of the heart via the right upper pulmonary vein is the cause of increased AF (Rousou, 1985). Among the postoperative factors, there are studies showing that pneumonia and COPD are related to the development of postoperative AF (Guo, 2002).

The need for a postoperative atrial pacemaker is also associated with an increased incidence of AF, indicating underlying sinus node

dysfunction and the use of rate-controlling drugs (Mathew, 1996). Some authors have reported that cardiopulmonary bypass is an important factor in the development of POAF (Zaman, 2000). In studies conducted in the following years, oxidative stress and inflammatory infiltrate were detected histopathologically in patients who developed POAF, and the relationship between AF and inflammation was revealed (Khairallah, 2004).

Many independent risk factors have been identified that are thought to have an impact on the development of AF after CABG. The most emphasized risk factor is age and it has been defined as a factor that is thought to have a significant effect on the development of POAF. In many studies, it has been well defined that the incidence of AF increases in older age groups and that this increase is associated with aging leading to degenerative changes in the atrial myocardium, decreased response to oxidative stress, and changes in the electrical properties of the sinoatrial node and atrioventricular node and the atrium (Spach, 1986 ; Gasparova; 2017).

Incidence: Although different values are reported for the incidence of AF development after CABG operation, depending on the variety in the definition of this arrhythmia, patient characteristics, type of operation, and monitoring method, this rate is accepted as 20-40% (Villareal, 2004).

Aranki et al., in a study published in 1996, evaluated 570 patients who underwent CABG for a 12-month period in 1994 for the development of postoperative AF. They found the incidence of AF development as 33% after isolated CABG (Guo, 2002).

Creswell et al., in a study published in 1993, evaluated 4507 patients who underwent cardiovascular surgery between January 1, 1986, and December 31, 1991. They found the incidence of postoperative arrhythmia to be 31.9% in patients who underwent isolated CABG (Creswell, 1993).

Villareal et al. in 2004, published a study in which they evaluated 6475 patients who underwent isolated CABG between January 1993 and December 1999 for the development of postoperative AF. AF was observed in 994 patients in the postoperative period and they determined the incidence of AF as 16% (Villareal, 2004).

Age: In AF developing after CABG, advanced age has been accepted as the most influential independent risk factor in almost all of the literature. The fact that the patient's age affects the development of postoperative AF to this extent has been explained by dilatation, muscle atrophy, reduction in conduction tissue, and fibrosis in the atrium due to advancing age (Furberg, 1994; Greenberg, 2017).

In a 1990 study by Leitch et al., in which they investigated the factors affecting the development of postoperative AF in 5807 patients who were in preoperative sinus rhythm and underwent isolated CABG, AF was observed in 17.2% of the patients. While 3.7% of the patients who developed arrhythmia were 40 years or younger, 27.7% were 70 years and older ($p<0.001$). They found that the most statistically significant parameter among the factors affecting the development of AF was the age of the patient (Leitch, 1990).

In the 2000 study by Zaman et al., examined the development of AF after CABG in 358 patients. AF was detected in 92 patients in the postoperative period. While the mean age in the AF group was 65.9, it was 61.7 in the sinus rhythm group (OR=1.07 (1.04–1.11), p=0.0005) (Zaman, 2000).

Gender: Most studies in the literature have reported that the male gender is an independent risk factor in the development of AF after CABG. They explained that the development of postoperative AF is less common in female patients compared to males, with the difference in the effects of ion channel expression and hormonal effects on autonomic tone in males and females. Fuller et al., in the aforementioned study published in 1989 and performed on 1666 patients, it was shown that the male gender constitutes an independent risk factor for the development of AF in the postoperative period (Fuller, 1989).

Aranki et al. In their aforementioned study published in 1996, reported that male gender constitutes an independent risk factor for the development of postoperative AF (P= 0.01) (Guo, 2002).

Borzak et al. in the study published in 1998, examined the factors affecting the development of AF in the postoperative period in 436 patients who underwent isolated CABG between December 1994 and May 1996. They observed that 101 (23%) patients developed AF in the postoperative period. They found that 72% of both AF and sinus rhythm groups were male and presented that gender was not a determining risk factor for the development of postoperative AF (p=0.95) (Borzak, 1998).

Zaman et al. in the study conducted in 2000, it was determined that AF developed in 92 patients in the postoperative period. The number of male patients in the AF group was 81 (88%), and this value was found to be statistically significant (P<0.01) (Zaman, 2000).

Previous percutaneous coronary interventions: The majority of patients who underwent percutaneous coronary intervention are people who apply to cardiology centers with the acute coronary syndrome. This indirectly indicates myocardial tissue under threat of severe

ischemia. Oliveira et al. in a study conducted on 1490 patients, the rate of AF development after CABG operation in patients with a history of percutaneous coronary intervention was found to be statistically significant (Oliveira, 2007).

Left atrium diameter: Zaman et al., found the left atrium diameter to be 38 mm on average in the AF group, 39 mm in the sinus rhythm group, and they did not obtain statistically significant results between the two groups (p=0.831) (Zaman, 2000).

In their study, Nardi et al. investigated the effect of left atrial volume on the development of postoperative AF in 220 patients and found the mean left atrial volume to be 70.6 ± 28.1 mL in the AF group and 59.0 ± 18.3 mL in the sinus rhythm group (p. =0.0004) (Nardi, 2012).

Gabrielli et al. in a prospective study conducted with 70 patients in 2011, evaluated the effects of left atrium volume and left atrial tension on postoperative AF in patients who underwent coronary artery surgery. AF developed in 26% of the patients in the postoperative period. While the left atrium volume was 30 ± 4 mL/m^2 in the AF group, it was 23 ± 1 mL/m^2 in the non-developing group ($p<0.001$). In the same study, left atrial strain s wave (LASs), left atrium strain rate s (LASRs), and a wave (LASRa) values were measured with Doppler echocardiography, and left atrial wall tension was determined preoperatively and postoperatively. They found that LASs, LASRs, and LASRa values decreased significantly in the AF group (LASs ($p<0.001$), LASRs ($p<0.001$), LASRa ($p<0.001$)), which shows us that left atrial wall tension is an important factor in triggering the development of AF. (Gabrielli, 2011).

Presence of right coronary artery distal anastomosis: Koletsis et al. in 2011, a group of 157 patients who developed AF after CABG and the second group of 191 patients with normal sinus rhythm after CABG were evaluated in order to examine the factors affecting the development of postoperative AF after CABG. While the presence of proximal left anterior descending (LAD) artery and proximal circumflex (Cx) artery lesions did not differ significantly between the two groups, proximal right coronary artery (RCA) stenosis was observed in 42 patients in the AF group, while it was observed in 32 patients in the sinus rhythm group (p=0.023) (Koletsis, 2011).

Sedimentation and C-reactive protein (CRP): CRP is the acute phase protein that increases most rapidly after inflammation or tissue damage and decreases the fastest during recovery. Measurement of preoperative CRP level can be used to identify patients at risk of developing a range of complications, such as infection after cardiac surgery. Cappabianca et al. in a study published in 2006, evaluated the effect of preoperative CRP

values on the postoperative period in the medium term in 597 patients who underwent cardiac surgery between August 2000 and May 2004. AF developed in 154 patients in the postoperative period and the incidence was found to be 25.8%. Preoperative CRP value was found to be above 0.5 mg/dL in 65 of these patients (26.7% of the group with high inflammatory response). In 89 patients (25.1% of the group with a low inflammatory response), it was found below 0.5 mg/dL and was not statistically significant (p=0.65) (Alasady, 2011).

Gabrielli et al. in a prospective study conducted with 70 patients in 2011, they evaluated the effects of left atrium volume and left atrial tension on postoperative AF in patients undergoing coronary artery surgery. While the preoperative mean hsCRP value was 6.8 ± 1.4 mg/dL in the AF group, it was 5.7 ± 1.2 mg/dL in the sinus rhythm group and was not found to be statistically significant (p>0.05) (Gabrielli, 2011).

Aortic cross-clamp time and total cardiopulmonary bypass time: There are studies in the literature showing that prolonged aortic cross-clamp time is an independent risk factor for the development of postoperative AF, as it also means prolonged atrial ischemia. Almassi et al. in their study published in 1997, determined the total bypass time as 2.0 ± 0.8 hours in the sinus rhythm group and 2.1 ± 0.9 hours in the AF group and revealed a statistically significant relationship (p=0.0001) (Almassi, 1997).

Triglyceride level: Nisanoglu et al. in their study, investigated hyperlipidemia in the history and found that 20% of the AF group and 24% of the sinus rhythm group had previously diagnosed hyperlipidemia. They found no significant difference between the two groups (p=0.759) (Nisanoglu, 2007).

Intraoperative positive inotropic therapy (PIT) and Intraaortic balloon pump (IABP): Koletsis et al. in their study, 33 patients in the AF group received PIT higher than 15 mcg/kg/min, while only 13 patients in the sinus rhythm group received PIT higher than 15 mcg/kg/min (p=0.016). No statistically significant difference was found between the two groups at doses lower than 15 mcg/kg/min (Koletsis, 2011).

Almassi et al. in their study published in 1997, evaluated the application of mechanical or medical PIT as a risk factor for 30 minutes after the termination of intraoperative cardiopulmonary bypass. PIT was 44% in the AF group, this rate was 33.83% in the sinus rhythm group and it was observed to be statistically significant (p<0.001) (Almassi, 1997).

Nardi et al. evaluated the use of IABP in their study published in 2009. While using the IABP support was 0.5% in the population, it was 0.6% in the AF group (p=1.00), and they concluded that IABP support was not a

risk factor for the development of postoperative AF (Nardi, 2012).

Previous myocardial infarction (MI): There are different opinions in the literature on the effect of previous MI history on the development of AF after CABG. The history of MI is a clear indication of the underlying myocardial ischemia. Myocardial ischemia, especially intraoperative atrial tissue ischemia, has been associated with the development of postoperative AF.

Creswell et al. in their study published in 1993, stated that a history of myocardial infarction was an important risk factor for AF seen after cardiac surgery ($p<0.01$) (Creswell, 1993).

Villareal et al., in the study published in 2004, the history of myocardial infarction was 17.4% in the AF group, while this rate was 15.4 in the sinus rhythm group ($p=0.12$). The history of myocardial infarction was not evaluated as a risk factor for the development of postoperative AF (Villareal, 2004).

In conclusion, AF is the most common rhythm disorder after CABG. It is an important risk factor for stroke and death. Identifying patients with a high probability of developing AF after CABG will facilitate the selection of the revascularization method and the planning of the treatment to be applied afterward. In this context, although preoperative treatments, including β-blockers and amiodarone, are recommended for patients at high risk of AF, randomized trials are still needed to examine whether there is a benefit in patients who develop AF after CABG surgery. Given the bleeding risk of chronic oral anticoagulation, the benefits of such treatments carry a relatively low risk profile.

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Chapter 5

SHOULDER IMPINGEMENT SYNDROME

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Introduction

The subacromial space is located between the coracoacromial arch and the humeral head. Rotator cuff, long biceps tendon head, and subacromial bursa are the main anatomic structures in this region. Shoulder pain is one of the most common disorders for orthopedic clinics, and shoulder impingement is the commonest underlying diagnosis, accounts 48% of all complaints about the shoulder[1]. Shoulder compression is the result of painful compression of soft tissues within the subacromial space. Increasing pain in the shoulder when raising the arm is the characteristic symptom of the syndrome.

Etiology

The etiology of subacromial impingement syndrome consists of two types: Primary and secondary. The structural changes which narrow the subacromial space are called primary impingement. Bony or soft tissue can cause this type of impingement. Acromion type (Type III) [2] (Figure 1) or the prominence of the tuberculum majus due to a past fracture are some reasons for bony impingement. Calcific tendonitis of the supraspinatus or inflammation of the bursa can lead to soft tissue impingement. Secondary impingement occurs due to functional imbalance, such as there is a loss of muscle strength centering the humeral head to the glenoid, and the humeral head is elevated. So that during shoulder elevation, the humeral head migrates upward, and soft tissue compression occurs between os acromion and humeral head. The weakness of the rotator cuff can lead to this type of impingement.

Neer classification of impingement is one of the milestones in this syndrome [3]. This classification has three stages due to the patients' age and the changes in the subacromial space. In this classification, impingement syndrome is thought to occur due to a bone spur on the lower surface of the acromion, which leads to narrowing of subacromial space and results in damage to the rotator cuff (Table 1) [2,4]. Therefore excising this bony prominence called 'acromioplasty' would solve the problem and reduces the contact between the humeral head and the acromion, as Neer described.

Another theory about the SAIS is related to the damage mechanism and development of impingement. The advocates of the 'extrinsic mechanism' stated that extrinsic compression to the rotator cuff leads to the Supraspinatus tendon's damage. On the other side, according to the 'intrinsic mechanism', the event that initiated the impingement is the damage to the rotator cuff tendons, and this degeneration leads to defects on the rotator cuff tendons [5,6]. The subacromial space gets narrower secondarily because of the rotator cuff tendon damage. This theory became more popular in the last years [7]. The vascular blood supply weakness can cause insertional

damage to the tendon and cause degenerative rotator cuff tendon tears. This type of tears is more common in patients with rheumatoid arthritis and diabetes, and also, it is increasingly seen in elderly patients [8].

Many other extrinsic factors such as smoking, heavy physical activities, some medicines like fluoroquinolones, vibration injury are blamed as suspected factors leading to rotator cuff tears and impingement [9].

Epidemiology

Neer first described the shoulder subacromial impingement syndrome (SAIS) in 1972 [3]. Bhattacharyya et al. reported that 44%-65% of all shoulder complaints were accounted for SAIS [10]. The sixth decade is the time when the incidence of shoulder impingement is the highest [11]. People who engage in strenuous overhead activities due to sports and work are most commonly seen with SAIS, like a volleyball player or a wall painter.

History and Clinical Findings

The complaints of patients with SAIS are generally similar. Patients usually complain of shoulder pain that starts mildly and increases over time. Pain begins in the acromion's anterior and lateral and typically spreads to the arm's lateral side. Pain is more common at night and may increase due to sleeping position as sleeping with the arms overhead and head placed on the painful shoulder. Overhead activities cause pain in the shoulder. In time, muscle weakness and stiffness may develop in the shoulder due to disuse due to pain.

Patients are generally 40 years and older. Typically, they cannot remember a trauma history or an event that initiates the pain. There is a complaint of pain that increases with the forward and lateral lifting of the arm. It was described as a 'painful arch', which refers to pain between 60 and 120 degrees when raising the arm.

Physical Examination

After inspection, palpation, assessing range-of-motion passively and actively, using special clinical tests is useful for diagnosing SAIS. It is also essential to check scapular dyskinesia or hyperlaxity by assessing subluxation or luxation of the shoulder. Clinical tests have low specificity, but it helps the diagnose when used together [12].

i.Neer Test: The examiner fixes the scapula with one hand and elevates and internally rotates the arm to the maximal forward flexion point with the other hand. The pain arises because of narrowing the subacromial space between the greater tubercle and the coracoacromial ligament. The sensitivity and specificity of the test is low as average 76,21% and 35,02% [13-15]

ii. Hawkins Test: While the examiner is stabilizing the humerus, as the position, the shoulder and elbow are flexed to 90 degrees, and passively internally rotates the arm. Positive when pain over acromion arises. The specificity of the test is about 41±19%

iii. Jobe Test: The examiner elevates the patient's shoulder to 90 degrees of abduction and internal rotation. Then asks the patient to elevate the arm against the examiners' resistance. If the pain occurs, then the Jobe sign is determined to be positive.

iv. Modified Neer Test: Guosheng et al. reported this new modified test's diagnostic accuracy rate as 90.59%, and the specificity was 95.56% in 2017 [15]. This test consists of two steps. The first step is similar to Neer's test, but the elbow is flexed to 90, and the palm down. The second step is to abduct the affected arm and then to rotate it externally to 90 and elevate the affected arm again. If the paint feels pain, then the test is determined to be positive. The Neer test was incorrectly positive in patients with frozen shoulder. This Modified Neer test correctly tested negative for these patients. So that, this test may be beneficial at differentiating subacromial impingement from frozen shoulder.

Imaging

The diagnosis is confirmed by imaging the patient, who is diagnosed or suspected to a great extent by physical examination.

Standart shoulder radiographs should include anteroposterior (AP) view, transaxillary, and outlet (Y) views. The physician can assess the bony spur, type of acromion (Figure 1), the centralization of the head of the humerus on the glenoid, integrity of the Arch formed by the medial proximal humerus and the scapulas' lateral border (Shenton line), the Acromioclavicular joint, arthritic changes of the glenohumeral joint and width of the subacromial space.

Also, the AP view is useful to determine the critical shoulder angle (CSA) and the acromiohumeral index (AI) (Figure 2).

Another important imaging technique in SAIS is MRI (Figure 3). The physician can assess the acromion, acromioclavicular joint, soft tissues as bursae, rotator cuff, joint capsule and labrum, acromioclavicular space, degenerative changes of the joints of the shoulder, and muscle fatty degeneration.

The diagnostic use of ultrasound and computed tomography in SAIS is limited, and they are not often preferred.

Treatment

The decision of surgical or conservative treatment is directly related to the severity and duration of the pain, as well as the functional loss and functional status of the patient. The primary goal of the treatment is reducing the pain while restoring the function of the shoulder.

A recent review stated that no statistically or clinically significant difference was observed when comparing the surgical and conservative treatment of SAIS [16]. In a 2020 study, no difference was detected between the surgical and conservative treatment of extrinsic subacromial impingement on pain and function [17].

The conservative treatment of SAIS consists of rest, NSAIDs, physical therapy, and subacromial joint injections (corticosteroids, prolotherapy, etc.). Garofalo et al. stated that 70% to 90% good results could be achieved with conservative treatment [18]. In a recent systematic review, exercise therapy was recommended to improve pain, mobility, and function in patients with subacromial impingement. Adding manual therapy to the treatment program is reported beneficial [19]. A recent randomized controlled trial stated that both ultrasound-guided and blind corticosteroid and local anesthetic injections improve shoulder function, pain, and range of motion (ROM). None of them was found superior to others [20]. The effect of Kinesio-taping and subacromial corticosteroid-local anesthetic injection was compared in SAIS, and injection was found with better outcomes in pain at rest, ROM, and disability [21]. When conservative treatment fails, then surgical intervention is resorted by many surgeons.

Arthroscopic subacromial decompression (ASD) (Figure 4) consists of acromioplasty of the anterolateral acromion, bursectomy, and coracoacromial ligament resection, which is the gold standard surgical treatment of SAIS [22]. Early results of arthroscopy seemed superior; however, long-term results are equal and effective when open vs. arthroscopic acromioplasty was compared [23,24]. Lower morbidity, infection rates, and complications are the advantages of arthroscopic intervention than open surgery. Complication rates are low with arthroscopic acromioplasty; between 0.76% to 6.5%, rates of complication were reported [25,26]. Insufficient acromioplasty, infection, chondrolysis, and fractures are some of these complications.

Tables

Table 1: Subacromial impingement classification (Neer's classification)

Stage	*Description*
Stage I	Edema and hemorrhage of the bursa, subacromial space, and rotator cuff, reversible inflammation, age <25 years, Conservative treatment
Stage II	Partial-thickness tear, recurrent pain with activity; irreversible rotator cuff fibrosis and tendinitis, age 25-40 years, conservative / surgery
Stage III	Partial / full-thickness rotator cuff tear, bone spurs, age>40 years, Surgery
Stage IV	Rotator cuff tear arthropathy, progressive disability, age>40 years, Surgery

Figures and Legends

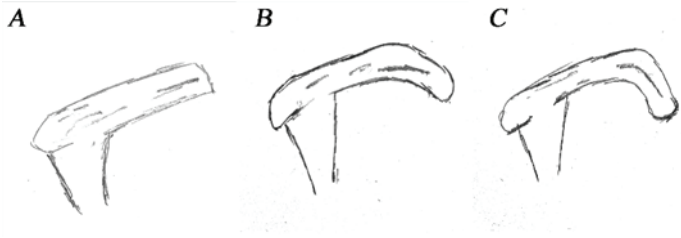

Figure 1*: *Types of the acromion,* A*.Type I acromion, flat* B*. Type II acromion, curved* C*. Type III acromion, hooked.

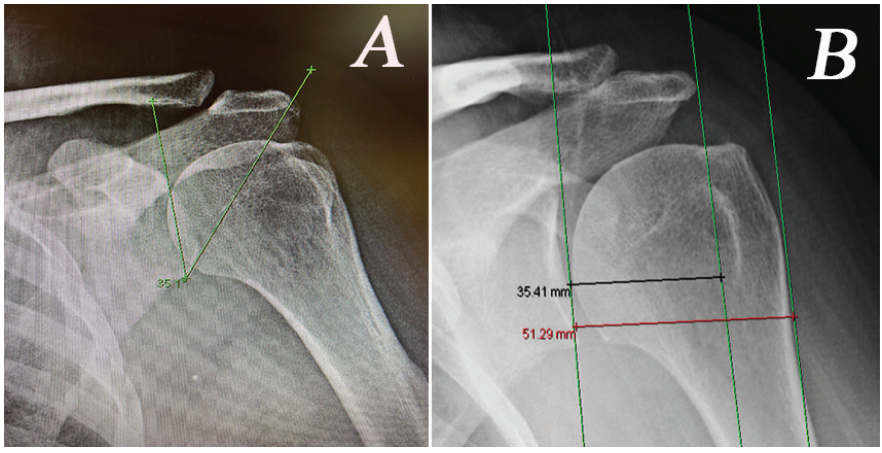

Figure 2*: *A.Critical Shoulder Angle (CSA) and B.Acromiohumeral Index (AI) angles. The angle between the glenoid inclination and lateral border of the acromion is determined as CSA. If CSA>35 degrees, then the risk of the rotator cuff lesion is higher. The quotient of the glenoid- lateral acromion distance and glenoid- humeral head lateral border distance is determined as AI, and higher AI indicates the lateral extension of the acromion and shows a higher risk of rotator cuff lesion.

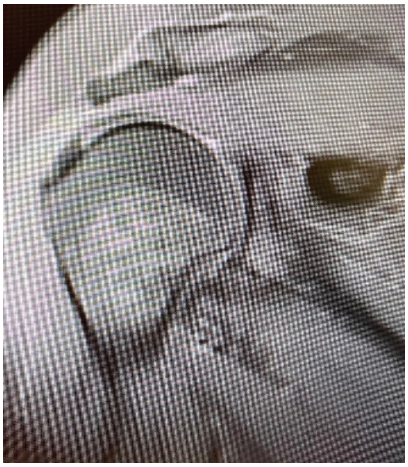

***Figure 3**: MRI is the most valuable imaging method in SAIS, as it allows to determine the narrowing of the subacromial space and degeneration of the coracoacromial joint; at the same time assessing the rotator cuff tendons, glenohumeral joint, labrum, bursa, and atrophy of the muscles should be useful for the treatment of the other pathologies of the shoulder.*

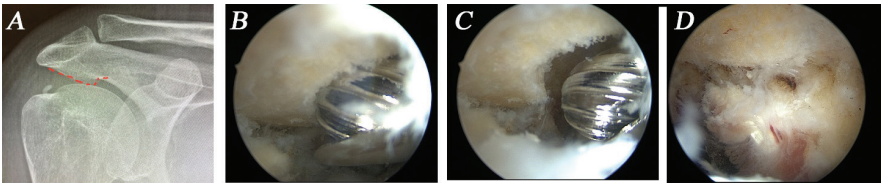

***Figure 4*: *A**. AP view of the X-Ray showing the spur of the acromion. ***B**. Spur of the acromion in the arthroscopic view. Acromioplasty starts with arthroscopic burr ***C**. Arthroscopic image shows how the burr resects the lateral portion of the acromion ***D**. The arthroscopic image after the subacromial decompression (SAD). The subacromial space has become wider after SAD*

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Chapter 6

DENTAL CARIES VACCINES

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1. INTRODUCTION

Dental caries remains a chronic, multifactorial and infectious disease, despite of the availability of various preventive measures (Chatterjee, 2019). In a Brazilian day-care center, at least 25% of children aged three years and older have dental caries. Epidemiologists have reported that more than half of five-year-old children have dental caries in China. Root surface caries occur in the elderly. 70 billion dollars are spent annually in the United States for treatments resulting from caries (Smith, 2003). The World Health Organization (WHO) has reported an incidence of dental caries in more than 5 billion people (Smith, 2010).

Clearly, dental caries remains a chronic infectious disease that is prevalent not only in industrialized countries but also in the world's poorest developing countries. The World Health Organization (WHO) describes dental caries as an important public health problem (Taubman & Nash, 2006). Many studies have been made to understand the etiology of caries. Miller, who made the connection between the main components (carbohydrate, bacteria, time, tooth) that cause dental caries, made an important discovery for this disease (Smith, 2003). In the 20th century, Streptococcus mutans isolated by Clarke, which causes dental caries, one of the greatest breakthroughs in modern dental research. In the second half of the twentieth century, experiments in the USA and Scandinavia have demonstrated the karyogenicity, transmissibility and worldwide distribution of S. mutans (Smith, 2003). (Smith, 2003). Later, other researchers discovered the virulence characteristics and biochemistry of S. mutans. The complete genome sequence of S. mutans was reported in 2002 (Ajdić et al., 2002).

According to the results of these studies; how should this disease be treated and what are the ways of protection? There are many treatment approaches for this disease and it is used routinely today. The use of fluoride in its various forms, pit and fissure sealants, and dental care are among treatment options. These approaches are effective methods to stop and control dental caries. However, economic, behavioral or cultural differences perpetuate dental caries disease (Smith, 2003).

Vaccination strategies have recently become a frequently used method to reduce or prevent the impact of infectious diseases in young people. Vaccines are safe and suitable for public health practice. (Smith, 2003).

Injectable vaccines containing lactobacilli were introduced in the 1940s with limited success. However, in the 1940s, there was insufficient information about the molecular pathogenesis and immune mechanisms of S. mutans. Most of the virulence characteristics were unclear, except for the ability of the acid-producing ability of S. mutans. Technological

developments allow us to explore the treatment of dental caries with vaccines more effectively (Smith, 2003). The purpose of this book chapter is to examine the latest developments in vaccines to prevent dental caries.

2. HOW DOES STREPTOCOCCUS MUTANS INFECT THE ORAL ENVIRONMENT?

Dental caries vaccine therapy began in the late 1960s when William Bowen used S. mutans to immunize irus monkeys (Bowen, 1969; Smith, 2003). In the 1960s, it was known that most people's dental plaques are caused by S. mutans. However, once S.mutans colonizes dental plaque, it is extremely difficult to remove it from dental plaque. Therefore, most experimental dental caries vaccine approaches have attempted to replace initial infection with S. mutans. Explaining this approach to people required us to know when children were first infected with S. mutans and from whom these infections came (Smith, 2003).

When the research on streptococci family is examined; permanent colonization of S. mutans has been observed in children under normal diet and exchange conditions between the middle of the second year and the end of the third year of life. This period is called the infectiousness window and the primary source of this infection is mothers (Caufield, Cutter, & Dasanayake, 1993).

When more sensitive techniques such as DNA probe technology are used for microbial detections, low levels of streptococcal mutans can be detected in the oral cavity (Milgrom et al., 2000). However, S. mutans detected at an early age does not colonize ecological habitats. Based on these data, it is thought that a "window of opportunity for vaccination" of twelve to eighteen months may exist for most populations and also if S. mutans colonization can be prevented by measures such as vaccination in early childhood, there will be benefits in the longer term (Smith, 2003).

3. MOLECULAR PATHOGENESIS OF STREPTOCOCCUS MUTANS

The molecular pathogenesis of S. mutans, which offers targets for immunological intervention, includes several stages (Smith, 2003). This acid-producing S. mutans needs the hard surfaces of the teeth for colonization and proliferation. The first attachment of S. mutans to the tooth occurs by the interaction of the components in the pellicle and the bacterial proteins. These bacterial adhesins were identified by Russell and Lehner (M. Russell & Lehner, 1978) and are designated antigen I/II on the surface of S. mutans. These bacterial adhesins found in parotid and submandibular saliva are acidic, mucin-like glycoproteins (Smith, 2003). These adhesins are found in salivary pellicles that cover both the tooth

surface and early colonizing bacteria (Smith, 2003).

One of the main components of early dental plaque is Dextran. S. mutans produces an enzyme called Dextranase. Thanks to this enzyme, bacteria can invade early plaques rich in dextran. For to prevent colonization of bacteria in early dental plaque; dextranase enzyme can be used as an antigen (Gupta & Mankel, 2020; Shah et al., 2018; Yesh et al., 2018).

The major pathogenicity of S.mutans is caused by lactic acid, a metabolic end product of bacterial development, demineralizing hydroxyapatite in tooth enamel. Excessive accumulation of acidogenic streptococci in tooth plaque, on the other hand, produces high damaging quantities of this acid. The activity of extracellular glucosyltransferases (GTF) released by S. mutans starts the accumulation process (Smith, 2003).

GTFs, high molecular weight, branched extracellular glucans, are found in three forms in the presence of dietary sucrose. These are (Gupta & Mankel, 2020; Shah et al., 2018; Yesh et al., 2018);

• Enzyme synthesizing water-insoluble glucan: GTF-I

• Enzymes synthesizing water-insoluble and water-soluble glucan: GTF-S-I

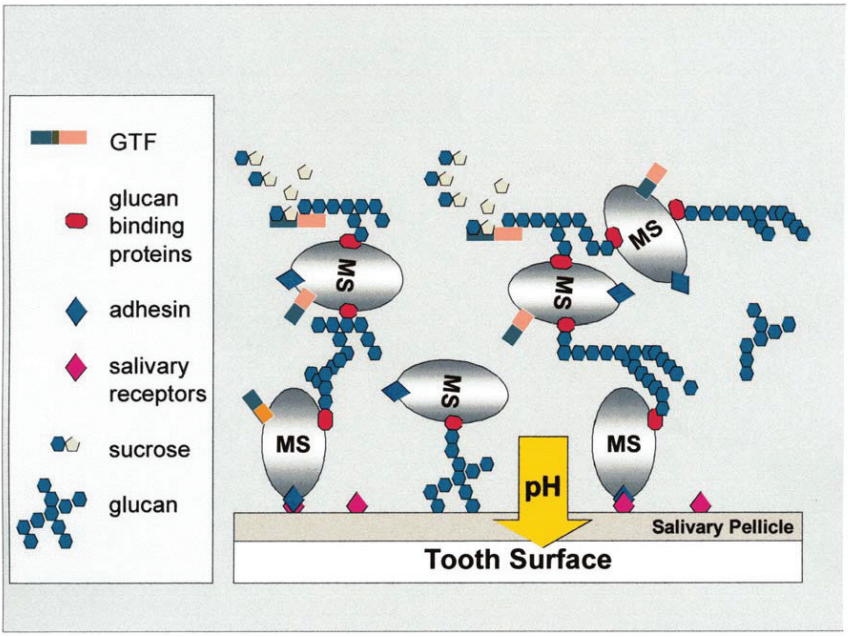
• Enzymes synthesizing water-soluble glucan: GTF-S

The GTF-B, GTF-C, and GTF-D genes, which encode the GTF-I, GTF-S-I, and GTF-S components, are referred to as GTF-B, GTF-C, and GTF-D, respectively. In pathogen-free mouse model systems, all of these genes are implicated in the production of smooth surface caries (Gupta & Mankel, 2020; Shah et al., 2018; Yesh et al., 2018).

GTFs that synthesize water-insoluble glucan forms are closely associated with pathogenicity. Glucose polymers interact with glucan-binding proteins (GBP) to form the scaffold for the aggregation of S.mutans and oral streptococci. Furthermore, glucans increase the availability of nutrients for bacterial metabolism by altering the porosity of the dental biofilm (Smith, 2003). The metabolic activities of colonized S. mutans are thought to cause the next stage of disease. Therefore, several stages in the molecular pathogenesis of dental caries are susceptible to immune interference. Microorganisms in the salivary phase in the oral environment can cause antibody-mediated aggregation. In addition, antibody; It blocks receptors in the dental biofilm required for colonization (adhesin) and accumulation (GTP, GBP). Immune inactivation of GTP enzyme will prevent the formation of the glucan matrix. Furthermore, synergy with endogenous immune components such as mucins or lactoferrin can improve the antibacterial action of salivary antibodies (Figure 1), (Smith, 2003).

Immunological intervention to many of the above-mentioned components has had experimental success and experiments are continuing.

Figure 1: Molecular pathogenesis of S. mutans (MS), (Smith, 2003).



4. WHAT IS VACCINE?

The vaccine is an immuno-biological substance that helps produce a protective antibody and other immune mechanisms. Vaccines are a mixture of live, inactivated, or dead microorganisms, modified microorganism-extracted cellular fractions, toxoids, or a combination of these ingredients (Yesh et al., 2018).

4.1 DENTAL CARIES VACCINE

Dental caries vaccine; can improve by determining the specific bacteria of dental caries and the function of the effector region of the mucosal immune system of the salivary glands (Yesh et al., 2018).

5. IMMUNE RESPONSE

5.1 PRIMARY RESPONSE

When an antigen first enters the body, it has a latent period of 7-10 days. Then, antibodies of the IgM type are developed primarily. In the following 2-3 days, this antibody level reaches its highest level and then decreases. If the antigen stimulation is sufficient; After 7-10 days, IgG antibodies

increase and these antibody levels decrease slowly and gradually over months. After this immunity, immunological memory cells are formed by B and T lymphocytes (Park, 2005; Shivakumar, Vidya, & Chandu, 2009).

5.2 SECONDARY RESPONSE

The secondary response differs from the initial response in several ways. Both IgG and IgM antibodies increase in the subsequent reaction. Immunological memory necessitates collaboration between B and T cells. While IgM antibodies are formed temporarily; IgG antibodies stay in the body longer. This cooperation of primary and secondary response is called immunological memory and forms the basis of vaccination (Park, 2005; Shivakumar et al., 2009).

6. MECHANISM OF ACTION OF DENTAL CARIES VACCINE

Saliva includes 1-3 percent immunoglobulin concentrations, predominantly secretory IgA. The gingival sulcus fluid contains immunoglobulin IgG and IgM antibodies, which are found in saliva. Additionally, immune system cellular components including as lymphocytes, macrophages, and neutrophils can be detected in the gingival sulcus. Antibodies can limit bacterial growth in a variety of methods, as listed below (Shivakumar et al., 2009):

• Salivary immunoglobulin may function as a particular agglutinin that binds to the bacterial surface receptor and prevents caries from forming following colonization. Also, they can inactivate surface glucosyltransferase and this will reduce plaque formation by reducing the synthesis of extracellular glucans (Shivakumar et al., 2009).

• Direct vaccination of gut-associated lymphoid tissue (GALT), where susceptible B-cells can host the salivary glands, causes the salivary glands to manufacture secretory IgA antibodies. Salivary IgA antibodies interact with the tooth surface directly. They can prevent S. mutans from adhering to the enamel surface or inhibit dextran formation by inhibiting GTF activity (Shivakumar et al., 2009).

• There are components of the systemic and humoral immune system in the gingival sulcus fluid. There's enough evidence to speculate about what might happen following a S. mutans subcutaneous vaccination. The microbe is phagocytosed and antigenically processed by macrophages in the gingival sulcus. In lymphoid tissue, T and B lymphocytes inhibit antigen HLA class-II complex and IL-1 is released. The CD-4 helper and CD-8 cytotoxic suppressor cell responses are induced when IL-2 receptors are activated. Intercellular interactions are crucial in the production of IgG, IgA, and IgM antibody classes, as well as B lymphocytes (Shivakumar et al., 2009).

7. ADMINISTRATION METHODS OF DENTAL CARIES VACCINE

The general mucosal immune pathway has two important routes of administration. These are (Gupta & Mankel, 2020);

1. Systemic administration

2. Active gingival-saliva administration.

General mucosal immune system:

This method is preferred for induction of secretory IgA antibodies. The following methods are used for this immune system;

Oral Administration

It is based on the induction of immunity in lymphoid tissue linked with the gut (GALT). Nutrition, gastric intubation or vaccine-containing capsules or liposomes are the best forms of administration for antigen (Gupta & Mankel, 2020). Lehner T. (Thomas Lehner, 1992). Oral immunization with S. mutans does not significantly generate secretory IgA responses, according to T. Lehner (Thomas Lehner, 1992). This may limit the efficacy of oral immunization.

Intranasal Administration

The intranasal administration procedure is preferred to induce immunity against bacterial antigens to prevent colonization and aggregation of microorganisms (Gupta & Mankel, 2020Katz J et al. (Katz et al., 1993) showed that intranasal immunization of rats with S. mutans antigen I/II combined with the B subunit of cholera toxin resulted in a protective salivary immune response with a reduction in S. mutans accumulation and dental caries.

Minor Salivary Gland Administration

The Minor Salivary Gland administration is considered one of the best-practice routes, takes less time, and has wide channels that allow retrograde transition to bacteria and their products (Gupta & Mankel, 2020).

Tonsil

Repeated administration can induce IgA antibodies salivary glands, both major and minor (Gupta & Mankel, 2020).

Rectal

The lower intestinal tract has the highest concentration of lymphoid follicles and is therefore known as the inductive location for immune responses (Gupta & Mankel, 2020).

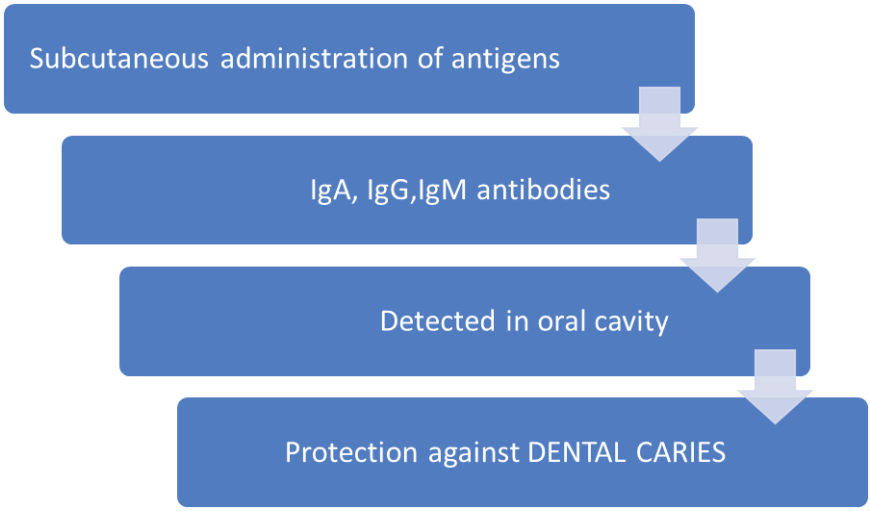
Active Gingival-Saliva Administration

Administration via GCF is considered the best route for immune induction to reduce side effects. Both IgA and IgG antibodies are induced by this method (Yesh et al., 2018).

Systemic Administration

IgG, IgM and IgA antibodies enter the oral cavity via the GCF, protecting against dental caries (Figure 2). Increased serum IgG antibodies, on the other hand, were mostly linked to caries prevention (Gupta & Mankel, 2020).

Figure 2: Systemic Administration (Abraham et al., 2018).



8. PASSIVE ADMINISTRATION/ VACCINATION

A different way to develop antibodies against dental caries is by passive oral administration of the antigen. A different way to develop antibodies against dental caries is by passive oral administration of the antigen. Active immunization risks are less than passive immunization. Since no immunological memory is produced, its effect in plaque only lasts for a few hours to 3 days (Abraham et al., 2018).

1. Monoclonal Antibodies:

These antibodies against S. mutans surface Ag I/II were examined. In locations where S. mutans was present, topical application was more affected and showed a significant decrease (Yesh et al., 2018).

2. Bovine Milk:

This milk reduces dental caries as it contains polyclonal IgG Abs. When used as a mouthwash, it helped reduce the percentage of S. mutans in plaque (Yesh et al., 2018).

3. Egg Yolk Antibodies:

Caries reduction was noticed due to the presence of formalin-killed cells and cell-associated GTFs (Yesh et al., 2018).

4. Transgenic Plants:

The vaccine is tasteless, colorless and can be applied to teeth. It is the first plant-derived vaccine derived from genetically modified plants (Yesh et al., 2018).

9. NEW MOLECULAR VACCINES

Recently, scientists have begun to apply molecular biology techniques and other options for the development of an anti-caries vaccine. Some of the important and recent developments in this field are as follows:

9.1 New Fusion Anti-Caries DNA Vaccine:

A novel DNA vaccination has been developed by researchers at the Wuhan Institute of Virology in China. The cell surface protein Pac and GTFs are two key virulence factors in S. mutans. In gnotobiotic animals, a fusion anti-caries vaccine, pGJA-P/VAX, which targets two major antigenic domains of S. mutans (PAc and GLU), has been beneficial in lowering caries levels. However, its protection against S. sobrinus infection has proven weak (Giasuddin, Huda, Jhuma, & Haq, 2017).

9.2 Subunit Vaccines:

For a variety of reasons, subunit vaccines comprising structural members of the Ag I/II adhesion family, GTFs or GbpB, have been developed. By creating subunit vaccines containing single or multiple epitope copies, researchers have been able to improve immune responses to functional epitopes, glucan-binding activities related with salivary binding catalytic processesMultivalent subunit vaccines can also be made up of numerous epitopes that target various activities on the same or different components. The removal of areas that may produce unwanted antibody specifications is also possible when vaccines are designed this way (Jalewa & Pandey; Smith, 2010).

9.3 Synthetic Peptides:

The alanine-rich repeat region of Ag I/II is immunogenic and produces protective immunity, according to synthetic peptide techniques. Antibodies

are produced by synthetic peptides in both the gingival sulcus fluid and saliva. The GTF enzyme was utilized to create the synthetic peptide (Jalewa & Pandey).

9.4 Recombinant Vaccines:

Synthetic peptides cannot host bigger functional domain segments, hence recombinant techniques allow for the expression of larger functional domain segments. Because avirulent Salmonella strains are an efficient vaccine vector, fusions based on recombinant methods have been employed (Jalewa & Pandey).

9.5 Liposomes:

M cell absorption is aided by liposomes, which are hypothesized to improve mucosal immune responses by promoting antigen transfer to lymphoid parts of inductive tissue. Humans have higher levels of IgA antibodies (Jalewa & Pandey).

9.6 Microcapsules and Microparticles:

Because of their capacity to control the rate of release, escape pre-existing antibody clearance mechanisms, and decay slowly without evoking an inflammatory response to the polymer, microspheres and microcapsules constructed of polylactide-co-glycolide (PLGA) have been employed as local delivery systems. As determined by their ability to generate a diffused mucosal IgA anti-toxin antibody response, oral vaccination with these microspheres is efficiently administered and released in gut-associated lymphoid tissue (Jalewa & Pandey).

9.7 Cholera Toxin and E. Coli Heat Resistant Enterotoxin:

Cholera toxin (CT) is a mucosal immunity agent that protects against a wide range of bacterial and viral infections. IgA responses are rarely elevated or sustained after mucosal administration of a soluble protein or peptide antigen. Small quantities of CT added to enterotoxins, on the other hand, can significantly boost mucosal immune responses to S. mutans antigens or peptides derived from these antigens when delivered intragastrical or intranasal. S. mutans colonization was effectively suppressed by the protein's interaction with the non-toxic unit of cholera toxin (Gupta & Mankel, 2020).

9.8 Conjugate Vaccines:

Chemical conjugation of functionally relevant protein/peptide components with bacterial polysaccharides is another vaccination technique that can prevent various elements of S. mutans molecular pathogenesis. In addition, the presence of T-cell-independent polysaccharide improves the immunogenicity of the vaccine by conjugating the protein with the

polysaccharide (Giasuddin et al., 2017).

10. RECENT DEVELOPMENTS REGARDING VACCINE:

A protein known as p1025 has just been discovered as a vaccine. S. mutans is fooled by this protein (Pathak, 2016).

11. RISKS OF USING DENTAL CARIES VACCINE (Jalewa & Pandey):

a) Serological cross-reactivity between heart tissue antigen and specific antigens generated from hemolytic streptococci has been observed in the serum of certain rheumatic fever patients.

b) Streptococcal cells' ability to produce cardiac reactive Ab has piqued researchers' curiosity in the development of a subunit vaccination to prevent caries.

12. ADVANTAGES of THE DENTAL CARIES VACCINE (Jalewa & Pandey):

• Prevents disease in children.

• It can be included in the universal immunization program.

• It will be cost effective in the long run.

• It will provide lifelong immunity.

13. DISADVANTAGES of THE DENTAL CARIES VACCINE (Jalewa & Pandey):

• There may be a risk of hypersensitivity.

• There may be a risk of microbial resistance.

• Some Antigenic components of S. mutans may have cross-reactivity with heart tissue.

14. EXPERIMENTAL STUDIES

In animal models, previous experimental research have shown that establishing protective immunity against S. mutans and subsequent development of dental caries is possible. Several small-scale experiments on adult volunteers were also used to gather data, confirming its relevance to people (Giasuddin et al., 2017).

14.1 Animal Studies

Ever since Edward Jenner discovered in 1779 that smallpox could be prevented by grafting pus from the pustules of cowpox patients, a large number of effective and safe vaccines have been created that are routinely used all over the world. Similarly, the fact that dental caries is caused

by certain pathogens has led scientists to explore the possibility that this disease can be prevented by active immunization (Giasuddin et al., 2017).

A substantial amount of work has been done on active immunization against caries in either rodents (mouse, rat, and hamster) or monkeys (rhesus, Macaca hybrid and irus, Macaca fascicularis). Until the role of S. mutans in dental caries is clarified, researchers have tried to reduce cavities by immunizing against Lactobacilli and other organisms, but very not success. Most immunization studies in recent years have been carried out with vaccines prepared from various strains of S. mutans (Gambhir et al., 2012; T Lehner, Challacombe, & Caldwell, 1975). While some of these studies used bacterial cell vaccines, others used bacterial components such as Glycosyl-Transferase (GTF) preparations. Researchers in these studies sought to stimulate either systemic or local antibody production both, by injecting vaccines into different sites near the salivary glands, either intravenously, intramuscularly or submucosal. Attempts have also been made to induce an immune response by injecting adjuvant into antigens. While some researchers have used the hamster model with a mixed immunization, the rat model immunization studies have been more widely used. Although many of these studies have shown a caries protective effect with various immunization regimens, some negative and valuable results have been obtained in different experiments (Giasuddin et al., 2017).

Monkeys are probably the best experimental model for dental caries, because their tooth structure and disease pattern are closer to those of humans. Various immunization studies have been performed in monkeys, including various cell vaccines and vaccines consisting of purified bacterial antigens. Several researchers have shown that immunity to against caries can be passively transmitted. Although caries reduction in monkeys is not achieved by the transfer of immune serum alone, administration of whole immune serum with transfer factor immunized the monkeys (Giasuddin et al., 2017). When these findings are examined, it is seen that both humoral and cellular immunity may play a role in providing a protective effect against dental caries. In fact, recent studies have shown that not only sensitized lymphocytes, but also polymorph nuclear leukocytes can be involved and help to provide protection by actively phagocytizing microorganisms, including S. mutans (Giasuddin et al., 2017). One approach that has been tried for passive immunity was monoclonal antibodies. The use of transgenic plants to deliver antibodies is a recent advancement in passive vaccination. A caries vaccine made from a genetically modified (GM) tobacco plant has been produced by researchers. Active and passive vaccination techniques that target critical aspects in S. mutans' molecular pathogenesis could help control the disease. Many of the world's children can be protected from dental caries disease by incorporating immunization

measures into broad-based public health programs, with those at high risk benefiting the most (Gambhir et al., 2012).

Researches that effective, reliable and acceptable vaccines against dental caries will not be far away offer great hope for the future. However, parameters such as optimum dose, route of immunization, immunization schedule, need for adjuvant and other practical considerations have yet to be determined. Only when such information is available would it be appropriate to consider clinical trials on human subjects.

14.2 Human Studies

Several small-scale human trials in adults have demonstrated that it is possible to increase salivary S-IgA antibody levels against S. mutans and interfere with S. mutans colonization.

Dental caries vaccine can also be given to children before their artificial teeth erupt, along with other vaccines such as diphtheria and tetanus, to ensure optimal caries suppression. An increase in salivary IgA antibody response was found after 14 participants were given GTF from S. sobrinus coupled with Aluminum Phosphate (AP) orally in capsules (Giasuddin et al., 2017; MW Russell, Childers, Michalek, Smith, & Taubman, 2004). Furthermore, oral immunization with this antigen was successful in preventing S. mutans from repopulating the oral cavity. The antigen dose, frequency of administration, composition, route of administration, or presentation of the antigen to appropriate antigen-presenting cells can all considerably improve the intensity and length of the response, despite the fact that these effects are relatively short-lived. GTFs from S. sobrinus were administered topically to the lower lips of young people in another investigation, and they were found to stimulate local antibody production in minor salivary glands and delay S. mutans oral decontamination. Seven adult volunteers were given an enteric-coated capsule containing 500 micrograms of S. mutans GTFs, which resulted in increased salivary IgA antibodies (Giasuddin et al., 2017). Salivary IgA antibodies were equally enhanced when comparable antigen preparations were administered intranasally or topically to the tonsils, in soluble form or integrated into liposomes (Childers et al., 2002; Li et al., 2003). As a result, more clinical investigations in younger age groups are needed to offer solid proof that the responses produced can prevent S. mutans from colonizing the mouth.

Today, in addition to caries prevention methods, dental caries vaccines have the potential to make an invaluable contribution to disease control. Also, basic research into the mode of action of the dental caries vaccine continues in the form of new, more effective and possibly multivalent vaccines (vaccines that can protect a person against more than one type of the causative agent).

15. CONCLUSIONS

Because dental caries is a multifactorial illness, there are a variety of ways to prevent it, including fluoridation, mechanical and chemical plaque reduction, and pit and fissure sealants. The creation of safe and efficient dental caries vaccines appears to be the main emphasis of dental research. Animal research, subunit vaccinations, and recombinant DNA vaccines have all showed promise in the development of a caries vaccine, although few human trials have been done to far.

For caries protection, there are considerable variations of opinion as to whether the antibody should be in the IgG or IgA class. Immunization techniques that target key aspects of S. mutans' molecular pathogenesis, whether active or passive, have shown promise. Making immunization against dental caries feasible, it will depend on clinical trials aimed at determining whether findings from animal experiments can be successfully transferred to humans. Also, it is important to evaluate the pros and cons of the various route of administration for the vaccination of dental caries vaccine in humans. However, removing human heart cross-reactivity and other risks, such as rheumatic fever, from any dental caries vaccine would increase the safety of the dental caries vaccine globally. The development of adequate oral health behaviors will continue to play an important role for good oral health and reduction of dental caries.

In fact, the world waited more than a century for a vaccine against typhoid after the active ingredient was identified. It took nearly half a century to develop vaccines against polio and measles. Searching and researching an HIV/AIDS vaccine is a much bigger challenge than sending a human to the moon. Despite many difficulties; scientists are cautiously optimistic that a dental caries vaccine will be available sooner for global human consumption.

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Chapter 7

ORIGINAL RESEARCH ASSOCIATION OF *INTERLEUKIN-1B* -511C/T POLYMORPHISM (RS16944) WITH CORONARY ARTERY DISEASE IN POPULATION FROM SOUTHEAST TURKEY

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INTRODUCTION

Coronary artery disease (CAD), which appears as a result of atherosclerosis within coronary arteries, can lead to cardiovascular diseases such as myocardial infarction, ischemic stroke, and renal failure. Due to these serious complications, CAD is the leading cause of deaths in developed countries (Öngen & Yılmaz, 2006). Through last two decades in developed countries, although the rate of cardiovascular diseases in older people (>50-year-old) declined, it increased in young adults (between 18-50 years old) due to prevalence of high risk factors such as obesity, physical inactivity, and inadequate diet (Andersson & Vasan, 2018). It is estimated that cardiovascular diseases will be one of the primary cause of deaths because of continual increase in the rates of obesity and diabetes in the next 10 years (Kablak-Ziembicka et al., 2011).

Previous studies revealed that inflammatory molecules play critical roles in atherosclerotic plaque formation and progression of CAD (Arman et al., 2008; Hansson, 2005; Shimizu et al., 2013). Besides, it was reported that mRNA levels of IL-1β and TNF-α in CAD patients were quite high (Jing et al., 2014).

IL-1β is a polymorphic gene and a member of IL-1 family genes comprising cytokine gene cluster on chromosome 2. One of the most common IL-1β gene polymorphisms (rs16944) is the C/T exchange at -511 nucleotide position (Tsai, 2017). Whereas several studies showed that -511 C/T single nucleotide polymorphism (SNP) on IL-1β gene is closely related with the IL-1β protein levels and cardiovascular diseases (Arman et al., 2008; Enquobahrie et al., 2009; Rios et al., 2010), some other studies contradicted these results reporting that there is no such correlation between this particular SNP and the IL-1β protein levels and cardiovascular diseases (Ren & She, 2015; Vohnout et al., 2003).

The aim of our study is to investigate the relationship of -511 C/T polymorphism with CAD in people living in Adiyaman, Southeast of Turkey, who were diagnosed as CAD patients angiographically.

METHODS

Study Population

In the study, for patient group, 87 people diagnosed as stable CAD patients with ≥ 60 % stenosis post cardiac catheterization were included in the study, selected out of 298 people who came to the cardiology polyclinic for CAD suspicion at Adiyaman 82nd Year State Hospital. The patients who had the following diseases in the past 1 month were excluded from the study; acute coronary syndrome, chronic heart failure, valvular heart disease, congenital heart disease, cardiomyopathy, chronic renal

failure, hepatic dysfunction, pulmonary disease, stroke, acute and active infectious disease. For control group, 79 volunteers outpatients who and whose families had no cardiovascular or chronic diseases such as diabetes, asthma, hypertension, etc. were included. The people, who are smokers, drink alcohol, and whose CRP levels were >15, were excluded from the study.

This study was approved by the "Adiyaman University Biomedical Researches Ethical Committee" (2012/04-1.4). Blood samples were collected and other clinical tests were performed after the written informed consents from all patients were obtained according to World Medical Association Declaration of Helsinki.

Blood Assays

Patient bloods were centrifuged at 1,600 g for 15 min by using a cooling centrifuge (Hermle ZK510, Gosheim, Germany) to provide 4°C. Thereafter, the plasma layers were collected and analyzed to measure high-density lipoprotein (HDL), low-density lipoprotein (LDL), plasma total cholesterol, and triglyceride by using commercial kits (Roche Diagnostics, Mannheim, Germany) with an auto analyzer (Roche/Hitachi Cobas c Systems, Basel, Switzerland). The measurements were expressed as mg/dl.

DNA Extraction and Genotyping

DNA isolation from patient blood samples were carried out with salting out procedure as described previously (Miller, Dykes, & Polesky, 1988). In order to detect IL-1β gene -511 C/T polymorphism (rs16944), the target DNA region was amplified with F: 5'- TGGCATTGATCTGGTTCATC -3' and R: 5'- GTTTAGGAATCTTCCCACTT-3' primer set by using conventional PCR method as described previously (Ferri et al., 2000). To summarize shortly, 50 ng genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 100 mol dNTP mixture, 50 pmol of each primer and 0.5 U DNA Taq polymerase in a total volume of 25 µL. The cycling conditions of PCR were as following: 7 min at 94°C predenaturation, 35 cycles of; 40 s at 94°C denaturation, 40 s at 51°C annealing, and 40 s at 72°C extension, followed by a final extension step for 7 min at 72°C using a thermal cycler (Applied Biosystems Veriti).

After PCR reactions, 304 bp-long PCR amplicons were treated and cut with 10 U/µl (per sample) *Ava I* endonuclease restriction enzyme (Fermentas, St. Leon-Rot, Germany) in Buffer R (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C overnight. After the reaction, enzyme-treated PCR products were run on 2 % agarose gel electrophoresis under 120V electrical current and thereafter the gel was visualized and analyzed. After the treatment with restriction enzyme to detect IL-1β gene -511 C/T

polymorphism, 190 bp and 114 bp DNA fragments for C allele, and 304 bp DNA fragment for T allele were detected on the gel (gel image was not shown).

Statistical Analysis

The non-categorical values (age, total cholesterol, etc.) were expressed as mean±standard error of mean (SEM) while categorical ones (sex, allele, geneotype) as frequency or percentage. Shapiro-Wilk test was used to check normal distribution of the values. The categorical values between groups were compared with Pearson Chi Square Test. Non-categorical values between groups showing normal distribution were compared with two independent sample t test but the values showing no normal distribution were tested with Mann-Whitney U. Categorical values showing normal distribution between groups were tested with one-way ANOVA whereas Kruskal Wallis test was used for comparison of values having no normal distribution. 95 % was used as confidence interval and the *p* values less than 0.05 were considered as significant. All statistical analyses were performed with SPSS version 18.0 softaware (IBM, Chicago, IL, USA).

RESULTS

The patient and control groups were compared with respect to individual features (Table 1). In patient group, systolic pressure (p=0.011) and HDL (p=0.001) values were significantly lower compared to the control group.

Table 1. *Comparison of CAD patients and controls regarding clinical and individual features*

Clinical and individual features	Controls (n = 79) (mean ± SEM)	CAD patients (n = 87) (mean ± SEM)	*p* value
Sex (female/male)	33 (41.7 %) / 46 (58.2 %)	33 (37.9 %) / 54 (62.0 %)	0.496
Age (years)	55.35 ± 1.27	58.54±1.20	0.952
BMI (kg/m^2)	27.26 ± 0.43	27.00±0.49	0.429
Systolic BP (mmHg)	125.12 ± 1.35	119.46±2.38	**0.011***
Diastolic BP (mmHg)	78.58 ± 1.07	73.74±1.44	0.300
Total cholesterol (mg/dL)	198.63 ± 3.98	185.93±4.97	0.055
HDL cholesterol (mg/dL)	47.26 ± 1.76	38.83±1.05	**0.001***
LDL cholesterol (mg/dL)	115.78 ± 3.98	111.05±4.39	0.114
Triglyceride (mg/dL)	183.38 ± 14.54	165.70±10.48	0.151

Mean: arithmetic average, SEM: standard error of mean. BMI: body mass index, BP: blood pressure, HDL: high-density lipoprotein, LDL: low density lipoprotein, CAD: coronary artery disease. * p<0.05.

The genotype and allele frequencies of IL-1β gene -511 C/T polymorphism for patient and control groups were demonstrated in Table 2. In patient and control groups, the genotype frequencies of IL-1β gene -511 CC, CT, and TT were at Hardy-Weinberg equilibrium (Table 2, for control group p=0.253, for patient group p=0.487). When patient and control groups were compared, there was no significant correlation of CAD with allele or genotype frequencies (Table 2, for genotype frequency p=0.653, for alel frequency p=0.405). Furthermore, when we analyzed dominant and recessive models, there was also no significant difference between patient and control groups (Table 3).

Table 2. *Genotype distributions and allele frequencies of IL-1β -511 C/T polymorphism in CAD patients and controls*

IL-1β -511 C/T	Controls (n = 79)	CAD patients (n = 87)	*p* value
Genotypes			
CC	1 (1.3 %)	1 (1.2 %)	
CT	26 (32.9 %)	23 (26.4 %)	0.653
TT	52 (65.8 %)	63 (72.4 %)	
Alleles			
C	28 (17.7 %)	25 (14.4 %)	0.405
T	130 (82.3 %)	149 (85.6 %)	

CAD: coronary artery disease, IL-1β: Interleukin 1β

Table 3. *Dominant and recessive models of IL-1β -511 C/T polymorphism in CAD patients and controls*

IL-1β -511 C/T	Controls (n = 79)	CAD patients (n = 87)	Ref	*p* value
Dominant				
TT	52 (65.8 %)	63 (72.4 %)		
CT+CC	27 (34.2 %)	24 (27.6 %)	0.746 (0.385-1.445)	0.404
Recessive				
TT+CT	78 (98.7 %)	86 (98.8 %)		
CC	1 (1.3 %)	1 (1.2 %)	0.918 (0.056-14.922)	1.000

CAD: coronary artery disease, IL-1β: Interleukin 1β.

DISCUSSION

Coronary artery disease is indicated with the atherosclerosis which inhibits blood flow by blocking luminal cavity due to accumulation of lipid and fibrous elements within coronary arteries following the inflammatory complications. Since atherosclerotic plaque, which is formed along with CAD in coronary arteries perfusing and nourishing heart, can result in cardiac arrest, CAD is one of the deadliest diseases worldwide (Drouet,

2002; Lusis, 2000; Shaw et al., 2008).

In our study, the patient group with 87 CAD patients and the control group with 79 healthy people were primarily compared with respect to their clinical and individual features. Systolic pressure and HDL parameters were significantly lower in patient group compared to the control group.

Atherosclerosis is a multifactorial process in which environmental and genetic factors play role. It is established that during the formation of atherosclerosis several risk factors play role in such as hypertension, high cholesterol levels, smoking, obesity, diabetes, genetic factors, age, and gender (Buğan & Çelik, 2014; Graham et al., 2007; Hamm, Möllmann, JP, & Van de Werf, 2009). Hypertension contributes to the pathogenesis of atherosclerosis by affecting endothelial function and is responsible for 35 % of atherosclerotic cardiovascular incidences (Viera, 2017). Previous studies showed that reduced blood pressure leads to reduction in cardiovascular pathologies and besides, balancing blood pressure is an effective way in primary and secondary protection against cardiovascular diseases (Graham et al., 2007; Hamm et al., 2009; Viera, 2017). Histopathological studies demonstrated that inflammatory cells involved in the atherosclerotic lesions, therefore, atherosclerosis is considered as an inflammatory process (Arman et al., 2008; Rios et al., 2010; Vohnout et al., 2003).

IL-1β, an effective pivotal cytokine on inflammatory processes, is implicated in the pathogenesis of atherosclerosis (Baykal, Karaayvaz, & Kutlu, 1998; Peiro, Lorenzo, Carraro, & Sanchez-Ferrer, 2017; Rader, 2012). Despite contradictory results of previous studies, a few polymorphisms associated with CAD were detected in IL-1β gene. Of which, -511 C/T polymorphism in the promoter region of IL-1β gene is the most common one (Arman et al., 2008; Enquobahrie et al., 2009; Rios et al., 2010). Since IL-1β is a polymorphic gene, the association of many SNPs in this particular gene with several diseases other than CAD has been investigated. Among which, -511 C/T polymorphism occupies a significant position in this respect (Borekci, Karakas, Kandemir, Aras, & Yalin, 2014; Iacoviello et al., 2005; Lakhanpal et al., 2014).

In the present study, there was no significant difference between CAD patient group and control group with respect to genotype and allele frequencies of IL-1β gene -511 C/T polymorphism. In a study with 534 Italian individuals comprising 325 CAD patients and 209 healthy people conducted by Vohnout et al., while they found no correlation between CAD and -511 C/T polymorphism in line with our findings, they highlighted that, on the contrary of our results, C allele frequencies in both patient and control groups (66 % and 68 % respectively) were greater than T allele frequencies (34 % and 32 % respectively) (Vohnout

et al., 2003). There are similar discrepancies among other studies carried out in different populations. Whereas Arman et al. found no correlation between -511 C/T polymorphism and CAD in Turkish population, Zhang et al. reported a significant correlation between the severity of coronary heart disease and -511 C/T polymorphism (Arman et al., 2008; Zhang et al., 2006). According to another notable study with 667 individuals (253 Afro-Brazilians and 414 Caucasian-Brazilians) diagnosed as CAD patient, it was stated that no correlation was detected between CAD and -511 C/T polymorphism in Caucasian-Brazilians while CAD risk increased by 2 fold in -511 CC genotype carrier Afro-Brazilians (Rios et al., 2010). Similar to these findings, in another study conducted with 92 CAD patients in Poland, it was affirmed that -511 CC genotype carriers had significantly higher risk in development of CAD at a level of urgency for surgical intervention (Rechcinski et al., 2009).

As it is known, the frequencies of polymorphisms vary population to population based on ethnic and geographical differences. The possible reasons of why we could not detect any relationship of -511 C/T polymorphism-related allele frequencies with CAD unlike other previous studies (Rios et al., 2010; Zhang et al., 2006). can be due to race-based distribution differences of gene polymorphisms, environmental factors, and the low number of participants in patient and control groups (87 CAD patients in our study).

Considering that genetic homogeneity, we organized our experimental groups from the same clinical center. But regarding the cosmopolitan structure of Turkey and therefore ethnic and geographic differences affecting gene polymorphism frequencies, it will be more convenient to investigate the distribution of IL-1β gene -511 C/T polymorphism at different geographic regions and its effect on CAD development.

In our study, we found T allele more dominant in both control and patient groups. In patient group, we detected C allele as 14.4 % and T allele as 85.6 % and in control group, C allele as 17.7 % and T allele as 82.3 %. In contrary, in most of the previous studies, the wild type C allele frequencies were higher compared to T allele (Arman et al., 2008; Vohnout et al., 2003; Zhang et al., 2006). Arman et al. showed in a study concerning the relationship between CAD and -511 C/T polymorphism in Turkish population that, CC/CT/TT genotype frequencies in control group were observed as ~30/43/26 % and in CAD patient group as ~29/50/20 % respectively (Arman et al., 2008). In the same study, they found C/T allele frequencies in control group as ~52/48 % and in patient group as ~55/45 % respectively. In another study with hepatitis B patients in Turkey, -511C/T genotype and allele distributions were as follows; CC/CT/TT genotype frequencies in control group were as ~44/7/48 % and in patient group as

~50/8/41 respectively while C/T allele frequencies were as ~48/52 % in control group and ~54/46 % in patient group respectively (Borekci et al., 2014). Gehmert et al. carried out a study investigating the association of gastric cancer with IL-1β gene -511 C/T polymorphism in Peruvians and they found that T allele frequency in control group was 80 % which was higher than the previously reported ones in Caucasians and Asians (~30 % and ~46 % respectively) (Gehmert et al., 2009). So it was asserted that all these discrepant results can be explained by considering the involvement of several factors that determine genotypic and allelic frequencies of polymorphisms (Gehmert et al., 2009; Matsukura et al., 2003; Zeng et al., 2003).

CONCLUSION

In the present study with CAD patients in Adiyaman province of Turkey, we found no significant correlation between CAD and IL-1β gene -511 C/T polymorphism. Furthermore, unlike most of the previous studies, the overall -511T allele frequency was more prevalent in the study population compared to the wild type C allele.

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Conflicts of Interest

This study was produced based on the master thesis of Fatima Kaya, as the first author here and all authors declare that there is no conflict of interest.

Authors' Contribution

FK and MUK conceived and designed the study. FK and MYT wrote manuscript. FK, AB, MYT, and MUK drafted and edited the manuscript. OA diagnosed CAD and collected patient samples. FK, MUK, AB, HIG, and MYT performed experiments, conducted analyses, and prepared tables. All authors interpreted the results and revised and approved the content of this manuscript.

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Chapter 8

PROTECTIVE MECHANISM OF HEAT-SHOCK PROTEINS IN MALE REPRODUCTIVE SYSTEM DISORDERS

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1. Introduction

Spermatogenesis is a complex process that occurs in the spermatogenic cell line in the seminiferous tubules and ultimately results in mature spermatozoa. During spermatogenesis, the proliferation of spermatogonia, the differentiation of spermatogonial cells into spermatocytes, the meiotic division of spermatocytes to form spermatids, the maturation of initially round shaped spermatids to form spermatozoa, and the release of highly specialized mature spermatozoa into the testicular tubule lumen occur (Turek, 2012). The entire spermatogenic process is thought to be completed in about 74 days (Heller, 1964; Heller & Clermont, 1963), however, recent studies on healthy men have shown that the total ejaculate sperm production time can range from 42 to 76 days (Misell et al., 2006). The estimated number of spermatozoa produced daily in healthy men is between 150 and 275 million (Amann, 2008; Heller, 1964). Various testicular structures and cells play important roles during spermatogenesis, but in various diseases and damage conditions, damage to the testicular tissue can negatively affect spermatogenesis and thus cause a significant decrease in sperm quality and quantity.

This review focuses on the expression patterns and mechanisms of HSPs in the male reproductive system in various disease, damage and stress conditions. It will review studies reporting how expression levels of members of HSP families are regulated during testicular injury induced by several diseases and stresses. The most important feature of the male reproductive system in mammals is keeping the testicles at lower temperatures than other organs in order to ensure a healthy spermatogenesis. Even a slight increase in temperature within the scrotum causes decreased spermatogenesis, significantly reducing male fertility. In addition, the damage caused by various diseases and damage to the male reproductive system seriously affects male fertility. The protective mechanism of HSPs has been shown in many studies in mitigating and eliminating the damage that occurs in these stress conditions and in various disease and damage situations. Recent studies have suggested that increased testicular temperature and other conditions that cause damage to the testis have inhibitory effects on spermatogenesis, and that HSP expressions increase significantly as a cellular response to this stress.

2. Heat shock proteins (HSPs)

Constitutive and sustained expression of HSPs during spermatogenesis is an important event. However, as in many cell types, HSPs are synthesized in testicular tissue as a rapid response to various stress conditions such as heat or chemical stress and various diseases. Heat stress-induced HSPs play a key role in protecting cellular proteins from irreversible damage

by binding to unfolded or misfolded proteins to delay heat-induced denaturation and aggregation of proteins in many cells. In addition to their stress-induced functions, HSPs also play an active role in many processes within the cell. These processes involve the folding and rectification of newly formed intracellular proteins and their transport in the cytoplasm. Therefore, some HSPs are consistently expressed in testicular tissue, while others are expressed under stress conditions or during cell growth and development and participate in normal cell functions (Nixon, Bromfield, Cui, & De Iuliis, 2017; Santiago, Santos, Fardilha, & Silva, 2020). During spermatogenesis, the levels of protein expression in the spermatogenic cell line are constantly changing. This causes significant differences in the expression patterns of proteins in germ cells (Carrageta et al., 2020; Mäkelä & Hobbs, 2019; Scieglinska & Krawczyk, 2015). It has been suggested that the expression of HSPs in testicular tissue is responsible for the formation of a unique set of proteins in cells in the spermatogenic cell line.

HSPs are divided into several families based on their molecular weight (kDa). These are small HSPs (16-40 kDa), HSP70, HSP90 and HSP110. All members of these families are expressed in constitutively healthy testes, but their expression levels vary due to stress conditions induced by heat or chemical agents and various diseases (Dun, Aitken, & Nixon, 2012).

2.1. Small HSPs

Among HSPs, HSP27 and HSP32 have been reported to play an important role in protecting testicular tissue from heat stress. HSP27 exists in the cell as large or smaller oligomers that act as chaperones (actin-associated protein) combined with actin microfilaments that bind the actin fibers in stressed cells, particularly under the effects of reactive oxygen species (ROS) and TNF-α (L. Li, Han, Li, Jiang, & Wang, 2013; L. Li, Li, Wu, Huang, & Wang, 2014; Tian et al., 2016; Wang et al., 2021). Moreover, the strongest protective activity of HSP27 is provided by phosphorylation of HSP27 oligomers by p38 MAP kinase, losing their ability to bind to actin microfilaments and promoting remodeling of the actin network in the cell (Chebotareva, Bobkova, & Shilov, 2017). Moreover, studies show that HSP27 suppresses lipopolysaccharide-induced inflammation (J. Li et al., 2019). HSP32 (heme oxygenase-1, HO-1), is among the highest differentially expressed HSP in a few hours after heat stress. Moreover, HSP32 overexpression can be triggered by several endogenous and exogenous factors such as heat stress and chemical damage (Erdmann, Grosser, & Schroder, 2005; Gabunia et al., 2012; Goldbaum & Richter-Landsberg, 2001; Morse & Choi, 2002)

HSP40 is a member of the family of low molecular HSPs, and it is also known as DNAJB3. Studies show that HSP40 is highly expressed in testicular tissue, and it has many functions. One of these functions is

that HSP40 plays a crucial role in acrosomogenesis in testicular tissue (Meccariello et al., 2002). Furthermore, human HSP40 expression has been shown to be strikingly inhibited by endoplasmic reticulum stress (ER) stress (Mitsugi, Itoh, & Fujiwara, 2015).

2.2. HSP70 Family

70 kDa heat shock proteins (HSP70s) and their regulatory co-chaperones form multifunctional cellular machinery necessary for proteostasis (protein homeostasis) (Brodsky & Chiosis, 2006; Hartl, Bracher, & Hayer-Hartl, 2011; Kampinga & Craig, 2010; Mayer & Bukau, 2005; Vos, Hageman, Carra, & Kampinga, 2008). HSP70s are essential for the folding, conformational regulation, sorting and degradation of proteins due to their ability to bind hydrophobic protein sequences. Proteostasis capacity is increased under stress conditions such as heat or oxidative stress via overexpression of inducible HSP70s such as HSP60, HSP72, HSP73 and HSP70t (Murphy, 2013; Vos et al., 2008).

Within HSP70 family, HSP60 is a highly conserved mitochondrial matrix protein with a molecular weight of 60 kDa in many cells. HSP60 is essential for folding and maturation of mitochondrial matrix proteins. It is considered among the molecular chaperones functioning in inhibiting aggregation and regulating the regeneration of unfolded or misfolded proteins (Hartl & Martin, 1995). It has been shown in many studies that heat stress significantly increases the expression of HSP60 in the male reproductive system.

Among HSP70s, the HSP72 and HSP73 are both constitutively expressed and stress inducible forms of HSPs in male reproductive system. HSP72 is a special and highly inducible isoform of HSP70 family, and it has several important physiological effects. Intracellular and extracellular HSP72 has inflammatory effects. Intracellular HSP72 serves to display an endogenous activation within a cell. Whereas extracellular HSP72 is detected in serum or plasma with stress conditions and plays an important role in induction of immune response. It is also known that HSP72, like other HSPs, regulates protein conformations in the elimination of impaired proteostasis in various damage situations (Yamada, Amorim, Moseley, & Schneider, 2008). Several studies show that HSP72 is highly expressed in seminiferous epithelium and epididymis tissues in heat treated mice (Zaprjanova, Rashev, Zasheva, Martinova, & Mollova, 2013). The HSP73 isoform is expressed in the reproductive system of both unstressed and heat-treated mice, and its expression is increased following elevated temperature (Tavaria, Gabriele, Kola, & Anderson, 1996). While HSP72 triggers proinflammatory immune response, HSP73 is generally cytoprotective (Asea, 2007). HSP72 has been known to inhibit the

expressions of mitochondrial apoptosis-inducing factors, thereby reducing cellular damage (Ruchalski et al., 2003). HSP72 and HSP73 have %95 sequence identity, and they are found in the cytoplasm and nucleus of many cells, and it is localized nucleus during stress conditions such as heat stress. (Xu, Wright, Higashikubo, & Roti, 1998).

In the HSP70 family, only HSP70t, which is known as HSPA1L, are constitutively expressed in testicular tissue (Daugaard, Rohde, & Jäättelä, 2007; Radons, 2016). HSP70t mutations are hypothesized to be associated with inflammatory bowel disease (Takahashi et al., 2017), male infertility (Ciftci et al., 2015), and spontaneous preterm birth (Huusko et al., 2018).

2.3. HSP90 Family

HSP90 family are known to be an abundant and ubiquitous chaperone family in the body functioning to ensure correction of folding and conformational maturation of cellular proteins. Although, it has chaperone activity, HSP90s also regulates cell signaling and function in many cells (Miao et al., 2008). There are two HSP90 isoforms identified in rodents; these are HSP86 and HSP84 (Moore, Kozak, Robinson, Ullrich, & Appella, 1989). Recent studies have reported increased HSP90s expressions in testicular tissue in various pathological conditions are referred to inflammation, oxidative stress and apoptosis (Öztürk et al., 2020).

2.4. HSP110 Family

The HSP110 family include chaperones with a molecular weight of 110 kDa. The HSP110 family is a large family of chaperones having several important functions in the cells. Four HSP110 family chaperones have been identified in mammals: HSPA4l, HSPA4, HSPH1 (HSP105) and HYOU1 (Vos et al., 2008). Among HSP110s, HSP105, a primary chaperone from the HSP110 family, is known to be highly upregulated in the porcine primordial germ cells against mild heat stress. HSPA4l plays a role in spermatozoa apoptosis and is essential for proper spermatogenesis (Held et al., 2006). In addition, ORP150 is an antiapoptotic ER resistant chaperone that protects cells from apoptosis triggered by ER stress. This protein is activated by stress, such as a lack of oxygen or glucose, and it works by limiting ER stress (Sanson et al., 2008). Taken together, overexpression as a response to mild increase in the scrotal temperature may play a vital role in providing heat resistance to germ cells such as spermatogonia.

3. HSP expressions under stress conditions

3.1. Heat stress response

Temperature is one of the most important environmental factors that have effects on the behavior, growth, reproduction and survival of all

animals (Wojda, 2017). Heat stress, on the other hand, is associated with high temperature, which can cause molecular and physiological changes in mammalian reproductive organs and thus affect their reproduction (Hanafi et al., 2010). Heat stress, which reduces reproductive function by causing adverse effects on both female and male reproductive organs, is responsible for embryonic deaths and deterioration in sperm morphology and physiology (Belhadj Slimen, Najar, Ghram, & Abdrrabba, 2016; Hansen, 2009). Main mechanism against abiotic stress conditions is the induction of overexpression of some key proteins that have a protective role. Among them, heat shock proteins (HSPs) are molecular chaperones that have been detected in almost all types of organisms. These chaperones undertake tasks such as preventing the denaturation of other proteins in stress situations, repairing damaged proteins and ensuring the degradation of irreversibly damaged proteins (Lam et al., 2013). It is known that HSP synthesis increases at high temperatures. However, when the temperature rises and reaches a certain point (eg, over 42 °C), HSP synthesis stops and the ongoing process results in cell death (Kregel, 2002).

Changes in the expression levels of HSP27 and HSP32 in testicular tissue under heat stress have been studied in many studies. Studies with HSP32 show that this heat shock protein is highly expressed under high temperature stress. For example, Maines et al., in their study, showed HSP32 mRNA expressions in testicular tissue at body temperature increased to 41 °C in rats by immunohistochemical methods and northern blot analysis. As a result, they reported that increasing body temperatures increased the expression of HSP32 at both mRNA and protein levels in Sertoli cells and Leydig cells of rats. They suggested that the strong expression of HSP32 in Leydig cells following heat stress points to the role of this enzyme in maintaining steroidogenesis by producing antioxidants and catabolizing the heme portion of denatured hemoproteins. In the same context, they also suggested that the presence of HSP32 protein in Sertoli cells, which form the blood-testis barrier under normal conditions, and its overexpression following hyperthermia may be related to the function of this cell type in the support and maintenance of germ cells and protection under normal and adverse conditions (Maines & Ewing, 1996). In studies investigating the effect of heat stress on HSP32 expressions in isolated Sertoli cells, it was reported that heat stress significantly increased HSP32 expressions in Sertoli cells. Some studies have shown that overexpression of HSP32 protect Sertoli cells from apoptosis via the p38 mitogen-activated protein kinase and guanyl cyclase pathways (L. Li et al., 2014). In addition, in other studies reporting that HSP32 suppresses apoptosis in Sertoli cells, it has been shown that HSP32 severely reduces caspase-3 activity, which is the initiator of apoptosis, and thus protects Sertoli cells from apoptosis (L. Li et al., 2013).

In some studies, the effect of heat stress on the organs of the male reproductive system was investigated in male cavies (*Cavia porcellus*), and it was shown that temperatures up to 39 °C significantly increased serum HSP40 levels in this experimental animal. However, in the same study, increasing the temperature further to 46 °C caused a decrease in serum HSP40 levels. This indicates that HSP synthesis is stopped at temperatures above 42 °C, as mentioned before (Ngoula et al., 2020).

Heat shock proteins (HSPs), originally identified as stress-responsive proteins, belong to the most prominent group of proteins with functions involved in folding and unfolding other proteins (Wechalekar et al., 2010). The 70-kDa heat shock proteins (HSP70s), which comprise the major class of proteins induced by increased temperatures, are chaperones that assist in folding, assembling, and disassembling protein complexes (Dix et al., 1997). HSP70 family includes several types of heat shock proteins with 70 kDa molecular weight functioning as a molecular chaperone in many cells.

As it is known that HSP60 is a mitochondrial matrix protein which is essential for inhibition of aggregation and regulating the regeneration of unfolded or misfolded proteins, many studies have reported that HS60 expressions increased in many tissues and organs under heat stress. For example, some studies reported that HSP60 expressions significantly increased in cultured monkey Sertoli cells treated with 43 °C temperature. They investigated the differences in the HSP60 expressions with both mRNA and protein levels and they observed that the mRNA and protein expressions of HSP60 increased under heat stress. As a result, they suggested that HSP60 acts as a chaperone rather than heat-inducible protein in the testicular tissue under high temperature stress.

HSP72 and HSP73 have %95 sequence identity, and they are found in the cytoplasm and nucleus of many cells, and it is localized nucleus during stress conditions such as heat stress. (Xu et al., 1998). It is shown that HSP72 and HSP73 expressions significantly increase as a response to high temperature in testicular tissue. *In vitro* studies have shown that HSP72 expressions are significantly increased in heat-treated Sertoli cells. In that study, it was suggested that HSP72 expressions increased even more in groups given ameliorating agent, and that this situation protected Sertoli cells from the harmful effects of heat stress by acting as a chaperone (X. Guo et al., 2015). Moreover, in addition to *in vitro* studies, many *in vivo* studies have investigated the expression of HSP72 and HS73 in the rat male reproductive system. For example, in a study investigating the curative effect of red grape extract in heat-treated rat testicles, it was shown that heat stress significantly increased HSP72 expressions (Halder et al., 2018). In another study, in which the expression levels of HSP72 and HSP73 expression in the testicles and epididymis of mice were

investigated under heat stress by electrophoretic and immunocytochemical methods, it was shown that the expression levels of HSP72 and HSP73 at the protein level increased significantly in the testis and epididymis tissue under stress conditions (Zaprjanova et al., 2013). In all of these studies, it was suggested that the protective effect of HSP72 and HSP73 on testicular tissue under heat stress was due to its chaperone activity.

Some *in vitro* studies have shown that HSPA1L increases chaperone activity via MAPKAP kinase 2-dependent phosphorylation, increasing the resistance of male germ cells to apoptosis induced by high temperature (Williams, Kobilnyk, McMillan, & Strochlic, 2019). In some other *in vitro* studies, it has been shown that HSP70t expressions are significantly increased under heat stress in male germ cells. In this study, they suggested that the activation of the p38-MK2 pathway in male germ cells under heat stress and the phosphorylation of HSP70t by stress activated protein kinase MK2 at the serine 241 region protects male germ cells against heat stress-induced apoptosis (Williams et al., 2019).

The harmful effects of heat stress on the male reproductive system and changes in HSP90 expression levels have been investigated in many experimental and wild animals. For example, studies on teleost (*Puntius sophore*) have shown that high temperatures significantly reduce HSP90 expressions in the male reproductive system of these fish in the group exposed to high temperatures for 7 days, while HSP90 expressions increase in the group exposed to high heat for 60 days (Mahanty, Purohit, Mohanty, & Mohanty, 2019). In addition, another study showing the effects of heat stress in rabbit testes showed that chronic heat stress for 9 weeks significantly increased HSP90 expressions in rabbit testes (Pei, Wu, & Qin, 2012). Results from most studies show that the increase in both constitutively expressed and heat-induced HSP90 expressions in testicular tissue occurs in response to heat stress-induced protein degradation and misfolding in testicular tissue.

Studies have shown that a transient increase in temperature induces a serious but reversible damage to spermatogenic cell line in cynomolgus monkeys. It was found that overexpression of HSP105 decreased considerably with loss of spermatids after 3 days following the treatment of 43 °C temperature (Zhang et al., 2005). In other studies, it has been reported that HSP105 binds to p53 and stabilizes cytoplasmic p53, thereby inhibiting possible apoptosis in rats (Kumagai et al., 2000). Researchers investigating the heat stress-induced changes of HSP105 in testicular tissue proposed that HSP105 regulates apoptosis of germ cells and therefore its expression levels are decreased in heat stress-induced apoptosis. In addition, it has been suggested that HSP105 expressions do not increase significantly at moderate temperature increases and therefore this heat shock protein functions only under non-stress conditions with heat.

3.2. Chemical-induced stress response

The effect of environmental toxicants on the health of all organisms and diverse components of the environment is studied in environmental toxicology. Its significance stems from the fact that human survival is dependent on the conservation of other animal and plant species, as well as environmental resources such as clean air, food, and water, all of which are frequently threatened by anthropogenic chemicals that disrupt living organisms and ecological processes (Yu, 2005). Many substances have been linked to negative effects on male reproductive in recent years, prompting increased worry. These substances can cause testicular toxicity and are made up of a variety of molecules that are linked to social habits, living situations, workplace dangers, and the use of pharmaceuticals and medicines (Obregon & Belmar, 2008). Due to the huge number of chemical pollutants, only a handful have been studied for developmental or reproductive toxicity, especially given the financial implications (Obregon & Belmar, 2008).

For example, Wang et al., in their study investigating the effect of selenium against lead toxicity in chicken testicles, reported that lead toxicity significantly increased HSP27 expressions by inducing heat shock response in testicular tissue. They suggested that increased expression of HSP27 against lead toxicity in testicular tissue occurs as a response of HSP27 to inflammation (Wang et al., 2021). In another study performed by the same research team on lead toxicity in spermatogonium and Leydig cells in chicken testicles, they showed that the expressions of HSP27 in both spermatogonium and Leydig cells increased significantly with lead administrations. They concluded that the overexpression of HSP27 was induced by decreased spermatogenesis and testosterone levels as a result of lead toxicity (Huang et al., 2021).

In addition, some studies investigating HSP40 expression levels in lead toxicity in chicken testicles, it was observed that HSP40 expressions were significantly increased as a result of lead toxicity causing activation of the heat shock response (Wang et al., 2021). In another study of same research team, they have reported that HSP40 expressions increased significantly as a result of lead toxicity in spermatogonium and Leydig cells isolated from chicken testes (Huang et al., 2021).

It has been shown in many studies that HSP70 family are overexpressed under heat stress conditions. While investigating the effect of heat stress on male reproductive system in chickens, it was observed that HSP60 increased significantly in testicular tissue (Wang et al., 2021). Likewise, it has been reported that HSP60 expressions in spermatogonia and Leydig cells are significantly increased against heat stress (Huang et al., 2021). Moreover,

some studies investigated the effect of molybdenum and cadmium on the male reproductive system in shaoxing ducks (*Anas platyrhyncha*) and showed that these chemicals significantly increased their HSP60 mRNA expression (Dai et al., 2019). HSP60 overexpression is thought to be induced in the male reproductive system as a defense mechanism against protein misfolding under heat or chemical-induced stress conditions.

In an experimental rat models in which the negative effects of pesticides on the male reproductive system were demonstrated, it was shown that pesticides such as methoxychlor, deltamethrin and lindane significantly increased the expression of HSP70 as a mechanism of self-protection of the cells against damage to the rat testes (Ismail & Mohamed, 2012; Saradha, Vaithinathan, & Mathur, 2008; Vaithinathan, Saradha, & Mathur, 2009). In addition, it has been shown that HSP70 expressions are significantly increased in testicular damage induced by drugs such as testosterone undecanoate in rhesus monkeys and ecstasy (MDMA) in rat testes (Mobaraki, Faraji, Zare, & Manshadi, 2017; Zhou, Zhang, Hu, Zou, & Liu, 2002). It has also been reported that the expression of HSP70s in testicular damage induced by inhalation of chemical gases is significantly increased. For example, studies in rats showed that inhalation of formaldehyde and toluene significantly increased HSP70 expressions in the seminiferous tubules (Ishigami, Tokunaga, Kubo, & Gotohda, 2005; Ozen et al., 2005).

In a study investigating the effects of lead toxicity on the spermatogonia and Leydig cells isolated from the male reproductive system of chickens, it was reported that HSP90 expressions were significantly increased in these cells with lead toxicity (Huang et al., 2021). In another study, it was reported that HSP90 expressions increased significantly under stress conditions caused by molybdenum and cadmium in testes of shaoxin ducks (*Anas platyrhyncha*) (Dai et al., 2019). It is thought that the increased expression of HSP90s under stress conditions induced by heat or chemicals occurs as a response to disruption of proteostasis as a result of these stress conditions, and consequently to the induction of oxidative stress, endoplasmic reticulum stress and apoptosis.

3.3. Chronic exercise response

As competition in competitive sports increased, athletes increased their training load at the same rate. Moderate-intensity exercises that improve cardiopulmonary function also increase the number of mitochondria in muscle cells and the number of capillaries in muscle tissue. However, prolonged and high-intensity exercises may cause health problems in athletes with clinical or subclinical symptoms (Hackney, Moore, & Brownlee, 2005). Many studies have shown that prolonged and high-

intensity exercise affects endocrine function, leading to a health problem called 'exercise-induced hypogonadal male problem'. It has been reported that this health problem also significantly reduces spermatogenesis in male reproductive system (Hackney & Lane, 2015). It is well known that various histopathological changes such as spermatogenic cell line apoptosis and seminiferous tubule atrophy were observed in testicular tissue of experimental animals exposed to chronic exercise stress (Shen et al., 2020).

In some studies, researchers have created chronic stress conditions by making experimental animals swim for certain periods of time daily. In one of these studies, researchers applied rats daily 60 minutes of swimming exercise for 9 weeks and examined the histopathological and biochemical changes caused by this intense exercise in the testicular tissue of the rats. At the same time, the expression of many proteins in proteomic analyzes structures was studied. As a result, it has been shown that intense exercise causes serious damage to the testicular tissue of rats and HSP70 expressions increase in response to decreased spermatogenesis (Y. Guo, Wang, Liu, & Li, 2019). In another study, rats were placed in an acrylic restraint cage for 4 hours a day for 60 days and then forced to swim for 15 minutes daily to create a chronic stress model, and histopathological, immunochemical and biochemical changes caused by chronic stress in the epididymis tissue were investigated. The results of the study show that chronic stress has a serious negative effect on epididymal tissue in terms of immunochemical and biochemical parameters in rats and significantly increases HSP70 expressions. According to their results, they have suggested that increased HSP70 expressions can be used as a potential marker of for male infertility.

3.4. Acute stress response

3.4.1. Testicular torsion

Testicular torsion is a urologic disorder in which the testicles, epididymis, and spermatic cord spin along the longitudinal axis. Indeed, the TT necessitates scrotal examination in an emergency (Yurtçu, Abasiyanik, Avunduk, & Muhtaroğlu, 2008). According to prior clinical findings, there are two forms of testicular torsion: the first occurs during pregnancy and the first year of life, while the second occurs more frequently during adolescence (Filho, Torres, Bordin, Crezcynski-Pasa, & Boveris, 2004; Lorenzini et al., 2012). Several investigations have been conducted to determine the likely mechanisms by which the testicular torsion harms testicular tissue. It has been demonstrated that testicular torsion lowers blood circulation by compressing spermatic veins, resulting in severe hypoxia and oxidative and nitrative stress (Lorenzini et al., 2012; Vigueras, Reyes, Rojas-Castañeda, Rojas, & Hernández, 2004). More research revealed that long-term (i.e.,

30 days) testicular torsion is associated with a considerable drop in testosterone levels in the blood and germinal cell destruction, resulting in subfertility and/or infertility (Turner, Bang, & Lysiak, 2005). Reperfusion and recirculation of the blood flow of the afflicted testis, on the other hand, as occurs in surgical therapeutic intervention, results in spermatogenesis cell loss and, as a result, increases the testicular lipid peroxidation ratio (Turner & Brown, 1993; Yurtçu et al., 2008).

Torsion models generated in experimental animals have been used by researchers for many years to determine the histopathological changes caused by ischemic conditions in testicular tissue. Several of these studies investigated the expression changes of heat shock proteins (especially HSP70) under torsion-induced acute stress conditions. For example, 1.,2.,4. of the torsion model in rats. In a study investigating the negative effects of testicular tissue at 8 and 8 hours, changes in HSP70 expression were shown. When the changes in the mRNA expression of HSP70 were investigated, it was shown that the HSP70 expressions were significantly decreased in the first 8 hours following the testicular torsion compared to the control group (Shamsi-Gamchi, Razi, & Behfar, 2018).

In another study, the role of omerprazole in heat shock proteins was revealed by blocking ATP'ase activity in testicular torsion. In this study, it was shown that the expressions of HSP40, HSP70 and HSP90 were significantly increased following testicular torsion, but the expression of these heat shock proteins decreased with omerprazole treatment. As a result of a detailed literature review, it is seen that the expression levels of heat shock proteins are increased at first but then decreased under acute stress conditions induced by testicular torsion. Studies suggest that decreased expression of these stress proteins leads to apoptosis of cells damaged under acute stress conditions (Güney, Coşkun, & Tutar, 2021).

3.4.2. Vasectomy

Vasectomy is a widely used surgical surgery and long-term family planning strategy (Weiske, 2001). Although vasectomy is considered a safe procedure for male fertility management, it has been linked to a number of negative effects, including duct obstruction, inflammation, and degeneration of the seminiferous tubules, as well as a decrease in spermatogenic cell quantity (Jones, 2004). The male vasa deferentia is cut and knotted or sealed during the surgery to prevent sperm from entering the urethra and so fertilization of a female through sexual intercourse. Vasectomy has been found to cause testicular apoptosis in several studies (Jones, 2004).

In many studies investigating the harmful effects of vasectomy on the male reproductive system, the expression levels of heat shock proteins

(especially HSP100 family) in testicular tissue of experimental animals after vasectomy were investigated. In one of these studies, a vasectomy model was generated in mice and the expression levels of genes belonging to the HSP110 family at the mRNA and protein level were investigated after vasectomy. It has been shown that ORP150, which is a member of the HSP110 family, in the testicular tissue of vasectomized mice increases significantly in the first 8 days following vasectomy and decreases in the following days. However, the expression levels of HSPA41, also known as APG1, were found to be significantly higher than the control group during the first 60 days after vasectomy. The researchers suggested that ORP150 and HSPA41, whose expression levels were significantly changed after vasectomy, play an important role in maintaining the normal histological structure and function of testicular tissue after vasectomy. Studies with the HSP110 family suggest that proteins belonging to this heat shock protein family play an important role in testicular tissue during and after acute stress conditions.

4. HSP expressions in infertility

4.1. Azoospermia or severe oligospermia

Male infertility is a multifaceted clinical illness with a wide range of phenotypic manifestations, ranging from the complete lack of spermatozoa in the testes to significant sperm quality changes (Tournaye, Krausz, & Oates, 2017). Male infertility is caused by genetic factors in at least 15% of cases, and genetic factors play a role in all four key etiological categories: spermatogenic quantitative abnormalities, ductal blockage or malfunction, hypothalamus pituitary axis disturbances, and spermatogenic qualitative defects1 (Table 1). Men with azoospermia have the highest likelihood of becoming carriers of genetic abnormalities (25%) and this risk lowers as sperm productivity increases (Krausz, 2011). In male fertility, azoospermia means no sperm in the ejaculate, whereas oligospermia is considered as cases where sperm concentration <15 × 10/ml and the total sperm count is <39 × 10/ml (Leslie, Siref, Soon-Sutton, & Khan, 2021).

In case of male infertility (especially azoospermia and severe oligospermia), immunohistochemical localizations and expression patterns of heat shock proteins, especially HSP60 and HSP70, have been shown in many studies. Early studies have shown that HSP60 is expressed in the spermatogonia and primary spermatocytes of the seminiferous epithelium of healthy men. Similarly, HSP60 has been shown to be expressed in the mitotic stages of spermatogenesis in rat testicles. In the same study, when the HSP60 immunoreactivity in testicular tissues obtained via testicular biopsy of men with azoospermia was compared with healthy testicular tissue, it was observed that HSP60 expressions were significantly reduced

(Werner, Meinhardt, Seitz, & Bergmann, 1997). Since HSP60 is a member of the family of heat shock proteins, its expression levels are expected to increase in damaged tissues. However, it is thought that the decrease in spermatogenic activity together with the decrease in spermatogonium activity in infertile men causes a serious decrease in HSP60 expressions.

In another study, the expression of HSP70-2 belonging to the HSP70 family was investigated in testicular tissues obtained from men with azoospermia and severe oligospermia suffering from testicular maturation arrest. While it was determined that HSP70-2 expressions were at high levels in healthy testicular tissue, especially in spermatocytes and spermatids, it was reported that HSP70-2 expressions decreased significantly in testicular biopsies with maturation arrest. In line with their results, they suggested that HSP70-2 may be associated with the pathogenesis of male infertility (Feng, Sandlow, & Sparks, 2001).

4.2. Cryptorchidism

Cryptorchidism (failure of testicular descent) is one of the most common congenital deformities in humans, affecting 13% of newborn males. The cryptorchid testis's abdominal position produces a temperature rise, which inhibits spermatogenesis and results in infertility. An increase in scrotal temperature has a significant impact on the cauda epididymidis' sperm storage activities and its ability to maintain sperm survival (Légaré, Thabet, & Sullivan, 2004). In order to demonstrate the negative effects of cryptorchidism on the male reproductive system, many studies have been carried out in humans, experimentally established cryptorchidism models, and transgenic cryptorchid animals. In some of these studies, the expression levels of heat shock proteins (especially HSP70) in cryptorchidism were investigated and the protective role of these chaperone-enhancing proteins in cryptorchidism was revealed.

For example, in a study, changes in the expression levels of HSP70 were investigated in epididymis tissues from cryptorchid donors and healthy human epididymis tissues. Results from the expression of HSP70 at the mRNA and protein level showed that expression of HSP70 in the epididymis in cryptorchidic humans is significantly increased compared to that in healthy humans (Légaré et al., 2004). In another study, in which an experimental cryptorchidic testis model was generated, it was shown that the expression of HSP70 at protein level in rat testis tissue increased significantly (Tekayev et al., 2019). In studies investigating the expression changes of HSP70 in cryptorchidism in the literature, it is seen that HSP70 expressions increase significantly. Studies on this subject suggest that the increased expression of HSP70 in cryptorchid testicular tissue results from exposure of testis to constant body temperature. In addition to being a

heat-induced protein, HSP70 is also thought to be overexpressed in the cryptorchidic testis as a response to the destructive effects of heat stress due to its chaperone activity.

5. HSP expressions in other diseases

5.1. Varicocele

The abnormal growth, elongation, and tortuosity of the spermatic vein, which has been thought to be the principal cause of male infertility, is known as varicocele (Sadek et al., 2011). The pathophysiology of testicular dysfunction, as well as the mechanism of infertility caused by varicocele, are unknown. Many conditions can cause testicular dysfunction, including testicular microcirculation disturbances, vasoactive substance reflux, oxidative stress, nitric oxide (NO), hypoxia, hyperthermia, or apoptosis (Fretz & Sandlow, 2002).

As it is widely known that heat shock proteins are expressed at high levels under chronic physiological conditions and has specific regulatory effects on cell growth, development, differentiation and cell death, several studies aimed to reveal the expression patterns of HSPs (especially HSP70 and HSP90) in patients with varicocele or experimental varicocele model. In a study investigating the expression levels of HSP70 in the seminal plasma of patients with varicocele, which contains the factors/proteins required for the maturation, hyperactivity, capacitation and acrosome reaction of sperm, it was shown that the HSP70-2 protein belonging to the HSP70 family was significantly reduced in patients with varicocele (Panner Selvam & Agarwal, 2021). On the contrary, in another study, it was reported that HSP70-2 expressions increased significantly after 2 months in testicular tissue of rats with varicocele induced. In this study, it is thought that the reason for the significant increase in HSP70-2 expressions is that the expression of HSP70s decreases under acute stress conditions, but then increases significantly in order to ameliorate the damage in the testicular tissue (Afiyani et al., 2014). In another study investigating the effect of varicocelectomy on increased HSP70 expressions with the induction of varicocele, varicocelectomy was performed in male rats 4 weeks after varicocele was induced, and it was observed that HSP70 expressions increased even more when compared to the varicocele group (Ning et al., 2017). Besides HSP70-2, HSP90 is one of the heat shock proteins whose expression levels have been investigated in the experimental varicocele model. Similar to HSP70-2, it was reported that HSP90 expressions were significantly increased at the end of 60 days in varicocele-induced experimental animals compared to the control group (Salmani, Razi, Sarrafzadeh-Rezaei, & Mahmoudian, 2020). These results show that HSP90 expressions in testicular tissue of experimental animals

with varicocele induced increase, like HSP70-2 expressions, in order to reduce the detrimental effects of varicocele in the long term.

5.2. Diabetes

Diabetes is a collection of disorders known as metabolic diseases, which are characterized by elevated glucose levels (hyperglycemia) due to insulin insufficiency, either in terms of production or activity, or both (Atta et al., 2017). Diabetes affects several organs, including the reproductive system, in both gender in many species (Shoorei et al., 2019). On a cellular level, type 1 diabetes has a negative impact on testicular function and testosterone production (Condorelli, La Vignera, Mongioì, Alamo, & Calogero, 2018). The development of oxidative stress, on the other hand, has been demonstrated to decrease testicular function in diabetic patients (Kanter, Aktas, & Erboga, 2012). It has been shown in many studies that heat shock proteins are overexpressed in response to the devastating effects of diabetes on the male reproductive system (Öztürk et al., 2020).

In a study investigating the curative effect of insulin treatment in testicular tissue of hyperglycemic rats, they showed that HSP70-2 expressions decreased in diabetic rat testicular tissues, whereas HSP90 expressions increased (Aeeni, Razi, Alizadeh, & Alizadeh, 2021). The increase in constitutively expressed HSP90 expressions with diabetes proposes that this heat shock protein performs chaperone activity in response to oxidative stress induced by diabetes in testicular tissue. However, it has been shown in many studies that the expression of HSP70-2 first decreases and then increases significantly in case of acute stress. On the other hand, this study suggests that HSP70-2 expressions, which decreased for 8 weeks, do not have a role in maintaining the normal histological structure and function of testicular tissue in diabetic rat testicles.

In another study, it was shown that HSP90 expressions were significantly increased in testicular tissue of diabetic rats. It has been reported that HSP90, which is constitutively expressed at high levels in spermatogonia in the control group, is overexpressed in all spermatogenic cell lines in diabetic testicular tissue. It is known that HSP90 acts as a chaperone in testicular tissue under conditions of diabetic stress and is overexpressed in response to oxidative stress-induced protein degradation and misfolding. In addition, in this study, it was suggested that the increased iNOS expressions with the increase in HSP90 expressions were due to the synergistic effect of these proteins in cytoprotection (Alsarhan, Amawi, Al-Mazari, Hurirah, & Alkhatib, 2020).

5.3. Testis cancer

Although the exact association between heat and testicular cancer is unknown, Socher et al. observed heat-induced, p53-mediated spermatogonia death (Socher, Yin, Dewolf, & Morgentaler, 1997). Failure of the germ cell HSPs may play a role in carcinogenesis (Richards, Hickey, Weber, & Master, 1996). Richards et al. recently proposed that changes in constitutive or induced expression of HSPs are linked to the sensitivity of testis tumor cells to heat and drugs (Richards, Hickman, & Masters, 1995). HSP70, HSP90, and HSP27 were investigated in the three human testis tumor cell lines 833K, GCT27, and GH. HSP70 and HSP90 had no linkage, however resistance was linked to a high constitutive level of HSP27 in this investigation. Furthermore, higher levels of HSP27 are linked to aggressive behavior and a shorter patient survival. As a result, reducing HSPs before to treatment appears to be a novel approach to improve drug sensitivity of tumor cells.

Many studies suggested that HSPs should be regarded as a prognostic factor first. More data is needed, however, to determine the specific importance of each heat shock protein expression for each form of urological cancer. In addition, these levels of expression should be compared to natural clinical outcomes as well as treatment sensitivity. Thus, it is suggested that influencing the activity of key heat shock proteins in testis cancers could be therapeutic, and the idea of treating individuals with vaccinations earlier in the disease phase could spur study (Lebret, Watson, & Fitzpatrick, 2003).

5.4. Other metabolic conditions

Prenatal and postnatal maternal malnutrition can result in metabolic syndrome and cardiovascular disease in adult offspring (Kereliuk, Brawerman, & Dolinsky, 2017). Changes in the mechanisms that control cell proliferation, apoptosis, and differentiation, on the other hand, can disrupt normal development under certain circumstances (Langley-Evans & McMullen, 2010), but cells and tissues adapt through a process known as developmental plasticity. Several studies in animals have hypothesized relationships between prenatal and early postnatal nutrition, birth weight, and postnatal reproductive development as explanations for variance in reproductive success found in adult animals in field situations (Martin, Blache, Miller, & Vercoe, 2010).

Studies in experimental animals have shown that maternal malnutrition during pregnancy and lactation leads to severe changes in the expression of HSP70 and HSP90 in testicular tissues of male offsprings. It has been reported that HSP90 expressions are significantly reduced in testicular tissue of male offsprings of both pregnancy and lactation and maternal undernutrition during pregnancy and lactation. Since malnutrition is known to directly induce oxidative stress and apoptosis in many tissues and

organs (Toledo, Perobelli, Pedrosa, Anselmo-Franci, & Kempinas, 2011), it is thought that decreased HSP90 expressions in this study indicate that malnutrition is related to other pathways leading to apoptosis. However, increased HSP70 expressions in testicular tissue in the study are both compatible with the literature and indicate the healing effect of HSP70 in testicular tissue by showing chaperone activity under oxidative stress conditions (Pedrana et al., 2021).

Male infertility can be caused by a variety of conditions, including infections, drugs, environmental pollutants, and metabolic abnormalities, which include impaired lipid metabolism (Oborna et al., 2010). Long-term intake of high-fat and high-calorie foods leads to obesity, and obesity and low fertility are public health issues that developed and developing countries are currently dealing with as a major concern (Cheng et al., 2020).

Several studies have shown the destructive effects of obesity on testicular tissue leading to decreased fertility in male individuals by histological, immunohistochemical and biochemical methods. Several of these studies investigated the expression levels of HSPs in spermatogenesis impaired by obesity. For example, in one study, experimental animals were fed high-fat foods for 24 weeks and their lipid metabolism was impaired. It has been shown that HSP60 expressions are significantly increased in both testicular tissue and cultured spermatogenic cells of experimental animals fed high-fat diets. It has been suggested that this increase in the expression of HSP60, one of the anti-apoptotic HSPs, emerges as a mechanism that protects cells from apoptosis.

6. Conclusion

In conclusion, further research is needed to determine the function of each HSP in the male reproductive system, as well as the importance of both structural and stress-induced expressions of HSPs in testis. Most of HSPs are only found in one type of testicular tissue cell, implying that these chaperones are important for their cytoprotection, proliferation, differentiation, and function. Blocking the expression or function of a protein and observing the pathological changes in the organism is a well-known approach of determining its function. Unfortunately, because some of the cells in the male reproductive system cannot be cultivated *in vitro*, basic techniques cannot be applied. Transgenic mice lacking testicular-specific HSPs, on the other hand, can be employed for this. Following the pathophysiology and healing process of a disease in experimental animals should provide helpful information about how HSP expression levels change under stress conditions. Furthermore, in future investigations, the expression patterns of HSPs under stress conditions should be properly described in this manner. The testicular specificity of HSPs, as well as their expression patterns under stress or during healing processes, provide insight into their roles.

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Chapter 9

TITLE: THE EFFECT OF *HLA* GENE RS9380343 POLYMORPHISM ON THE IMMUNE-ACTIVE HBV AND INACTIVE HBV PHASES

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INTRODUCTION

The hepatitis B virus (HBV) affects a lot of people. Liver failure, liver cirrhosis and cancer may develop in approximately 15% of infected people as a result of HBV infection. Approximately six million patients of acute HBV happen annually all over the world. The prevalence of HBV worldwide varies. HBV infection is epidemiologically classified as the lowest (<2%), low (2-4%), moderate (5-7%), high (>8%) endemicity regions (Ott, Stevens, Groeger, & Wiersma, 2012). Most of the patients are in Asian and African countries, where approximately 45% of the world population lives. 75% of infected people are in Asia and 12% in Africa (Gust, 1996). The serological positivity rate of HBV (including all contacted individuals) is 35-62% of the Chinese population (Yao, 1996), and 56-98% of the Sub-Saharan African population (Kew, 1996). Sub-Saharan Africa (5-7%) and most of Asia (5-7%) is located in the middle endemic region. It is the highest endemic region with >8% in the Western Pacific. The south of Europe, the Middle East, and Turkey is located in the low endemic region with a 2-4% (Kew, 1996; Ott et al., 2012).

The damage caused by HBV in the liver varies according to the immune response of the person. HBV can onset as an acute illness and get better or become chronic. Developing chronic HBV (CHB) risk is 90% in babies born to HBV e antigen (HBeAg) positive mothers, 25-30% in children under 5 years old, and less than 5% in adults (Beasley et al., 1983; Coursaget et al., 1987). HBV vaccine is a crucial preventive tool for new infections, but approximately four hundred million people suffer from CHB infection and it still causes approximately six hundred thousand deaths per year (O'Brien et al., 2011). Despite the achievement of the HBV vaccination in reducing this infection in recent years, the rate of vaccination unresponsiveness in healthy adults is approximately 10% (Lau et al., 2011; Vermehren et al., 2012). Yildiz et al. (2015) reported that the prevalence of HBV increases from west to east in our country. HBsAg positivity rate reaches 4% in Diyarbakır.

The liver's initial immune response to the HBV is nonspecific activation of the interferon system, natural killer cells (NK), and kupffer (liver macrophages) cells (Kazuhiro, Guidotti, Yasuhiko, & Chisari, 2000). After this nonspecific response, the immune response is directed to the specific response against the very important viral proteins. B lymphocytes take part in the humoral response, which is one of the two main arms of the immunity, and produce antibodies against HBV, while the other branch is the cellular arm of the immune system. Macrophages and T lymphocytes are involved in this arm (Delves & Roitt, 2000; Jung & Pape, 2002). Viral proteins are presented to T-cells by Kuffer cells and dendritic cells. Helper T cells (Th) polarize and divide into two. Th1 cells secrete interferon-gamma,

tumor necrosis factor-alpha (TNF-α), and interleukin-2 (IL-2), which helps cytotoxic T cells and macrophages inhibit intracellular pathogens. Th2 cells also secrete IL 4, 5, 6, and 10, stimulating B lymphocytes to produce antibodies and proliferate (Delves & Roitt, 2000; Jung & Pape, 2002). IL-2, IL-4, IL-6, IL-10, TNF-α, and interferon-gamma (INF-γ) are released from $CD4^+$ T cells. Released IFN-γ and interferon-alpha (IFN-α) stimulate human leukocyte antigen class I and II (HLA-I and HLA-II). HLA-I introduces the antigenic structures of HBV in hepatocytes to $CD8^+$ T cells on the hepatocyte surface (Delves & Roitt, 2000; Jung & Pape, 2002). The infected hepatocyte is then destroyed by Fas ligand, cytokines, and perforins. HLA-II presents antigenic structures of HBV such as plasma HBeAg and HBcAg to $CD4^+$ T cells on macrophages and sensitizes them. Immune-mediated elimination mechanisms may cause the end of the infection by eliminating infected cells, as well as cause chronic liver damage and hepatocellular cancer (Delves & Roitt, 2000; Jung & Pape, 2002). Because of this information, the role of HLA molecules in HBV elimination is very important.

"So what are these HLA molecules?" the HLA complex molecules are synthesized from about 40 of the approximately 200 genes on chromosome 6. Class I and class II molecules are synthesized from HLA genes, these molecules are structurally and functionally different (Klein & Sato, 2000). HLA class I proteins; it consists of alpha and beta chains. The chain of the HLA class I protein weighs 44-47 kD, while the β2 microglobulin chain is 12 kD. The chain has 5 domains. These areas are α1, α2, α3, transmembrane and cytoplasmic tail. The regions showing polymorphism are the α1 and α2 regions that form the peptide-binding groove. The α3 region is responsible for the interaction with the CD8 receptor on the cytotoxic T-cell. When the molecule is folded, the α1 and α2 regions form the peptide-binding groove. The β2 microglobulin chain is encoded over the β2 microglobulin gene located on the 15th chromosome outside the HLA locus and does not show polymorphism (Abbas & Lichtman, 2003). In the HLA Class I protein, the small beta chain is stable, but the amino acid sequence in specific regions of the alpha chain of the molecule located on the outside of the cell varies greatly. Every person generates 6 different alpha chains in their HLA Class I proteins. It has numerous variants in human society; it is among the most polymorphic proteins (Abbas & Lichtman, 2003; Nelson & Cox, 2005). Since individuals produce 6 different HLA Class I proteins, there is no possible two persons will have the same group. HLA-I molecules bind and present peptides resulting from proteolytic degradation and random protein conversion within the cell. HLA-I molecules present 8-11 amino acid long polypeptides to $CD8^+$ T-cells (Nelson & Cox, 2005).

HLA Class II proteins consist of the chain (32-34 kD) and the β chain

(29-32 kD). HLA Class II proteins contain areas of high variability near the amino terminal ends of both the alpha and beta chains. The regions showing polymorphism are the α1 and β1 regions. The β2 domain is responsible for binding to the CD4 receptor on the Th cell surface. HLA Class II molecules present 10-30 amino acid long polypeptides to $CD4^+$ T-cells. HLA Class II proteins are formed on the surface of several types of specialized cells (such as macrophages and B lymphocytes) for foreign antigens. All humans can generate as many as 12 variants, so it is unlikely that any two humans will have variants from the same group. HLA-II molecules bind and transport peptides originating from extracellular proteins digested by cells to the cell surface. HLA-II protein-peptide complexes bind to the T-cell receptors of Th cells. HLA-II molecules have a lot of polymorphic areas (Abbas & Lichtman, 2003; Nelson & Cox, 2005).

In infected cells, proteosomes constantly make their own peptides. Some of the peptides are picked up by special molecules called "transporter associated with antigen processing (TAP)". TAP 1 and TAP 2 proteins form a channel shape for the transport of peptides across the endoplasmic reticulum membrane (Klein & Sato, 2000; Parham, 1999). Peptides released by proteosomes bind to the cytosolic surface of the TAP molecule and are transported through this channel. HLA-I molecules are lined up on the luminal surface of the endoplasmic reticulum. These polypeptide chains are synthesized separately on ribosomes and bind to the cytoplasmic surface of the endoplasmic reticulum but do not assemble. These chains come to the luminal surface of the endoplasmic reticulum by special channels. Here, their premature folding is prevented by the chaperone molecule assembly (calnexin, calreticulin, endoplasmic reticulum p57, TAP-binding protein). After the chains are glycosylated, the α and β2 microglobulin chains come together non-covalently at the appropriate time. The HLA-I molecule is then immobilized with TAP-binding protein to peptide loading the TAP molecule. The appropriate peptide from the TAP channel to the waiting HLA-I molecule is attached to the peptide-binding groove of the HLA-I molecule. The complex formed by the TAP-binding protein and the TAP molecule is removed from the loaded peptide, and the peptide-loaded HLA class I molecule is on its way to the cell surface. Class I molecule resides in a fixed position on the plasma membrane with the transmembrane region of α chain (Klein & Sato, 2000; Parham, 1999).

HLA class II molecules, on the other hand, take up peptides via the endocytic protein processing pathway. Like the chains of the class I molecule, the class II chains are produced separately on the cytosolic surface of the endoplasmic reticulum and are then assembled and folded by chaperones and assembled on the luminal surface. Peptides to class II molecules are not loaded in the endoplasmic reticulum. Instead, it

associates with a constant chain produced in the endoplasmic reticulum. Part of the fixed chain acts as a stabilizer. With the closed membrane vesicle, the class II molecule and the fixed chain complex are transported to the cytoplasm region. This vesicle combines with the protein-loaded endosome to form the MHC class II compartment. Exogenous protein and fixed chain are degraded in this compartment (Klein & Sato, 2000; Parham, 1999). The class II HLA-DM molecule produced in the endoplasmic reticulum comes to this compartment and helps to load the peptides into the class II molecule. Thereafter, formed HLA-II complex is transported to the cell surface and peptide presented to CD4+ T cells. The process of peptide loading into HLA-I molecules occurs in most cells because there are always worn, damaged, and misfolded proteins. These proteins are degraded and replaced with new ones. Some proteins are broken down into peptides by enzymes in the cytosol. In contrast, the processing of exogenous proteins and peptide loading into HLA-II molecules is restricted to B cells, macrophages, and dendritic cells. Although there are HLA-I molecules loaded with exogenous peptides (bacterial and viral proteins) and class II molecules loaded with endogenous peptides (viral proteins), the mechanism has not been still explained (Klein & Sato, 2000; Parham, 1999). The result of protein processing is peptide-loaded HLA molecules on cell surfaces. Each cell synthesizes 100 to 300 thousand class I and class II molecules from each of its HLA genes because of each HLA molecule binds a peptide. Each uninfected cell has the HLA molecule to which it presents thousands of its peptides. Some of these peptides are presented in a few copies and most peptides are in 100 or more copies on the cell surface (Glas, Bogyo, Mcmaster, Gaczynska, & Ploegh, 1998; Rammensee, Bachmann, & Stevanovıc, 1997).

In the HBV infection, after the hepatitis B virus activates the host's immune system, lymphocytic infiltrates predominantly activated by the adaptive immune system lead to portal inflammation, interface hepatitis, and lobular inflammation (Wong et al., 2013). With liver inflammation, the severity of the disease increases. According to the degree of fibrosis tissue, drug treatment is applied to the patients. However, some patients achieve HBV clearance by overcoming HBV infection spontaneously without using medication (Wong et al., 2013; Png et al., 2011; Croagh & Lubel., 2014). The phases of CHB infection are divided into 3 categories by the American Association of the Study of Liver Diseases (AASLD); immune tolerant phase, immune active phase and inactive phase (Terrault et al., 2018). CHB phases are defined according to HBV count, the e antigen of HBV (HBeAg) situation, and alanine amino transaminase level (Terrault et al., 2018; Sharifi, 2014). The initial phase of CHB is called the immune tolerant phase which has normal liver histology and alanine

aminotransferase (ALT) levels. In addition, these patients have HBeAg positivity and high HBV load. As the second phase, the immune active or the immune clearance phase that has HBeAg positive or negative but absolutely seen liver inflammation, high ALT levels and lower HBV load levels compared with the immune tolerant phase. As the final phase, the inactive HBV phase or immune control phase has HBeAg-negative, anti-HBeAg-positive, low HBV DNA load and normal ALT levels (Terrault et al., 2018; Sharifi, 2014; Aspinall, Hawkins, Fraser, Hutchinson, & Goldberg, 2011).

CHB phases or HBV clearance is depending on viral variations, age and sex of the host, genetic variants in the host-related immune system (Hu et al., 2012; Wong et al., 2013). One of these factors is polymorphisms in HLA genes. It is the first reported HLA DRB1*1302 allele associated with its protective effect against chronic HBV infection (Thursz et al., 1995). Subsequent studies have reported that other HLA genes are effective in HBV clearance (Ahn et al., 2000; Thio et al., 2000). The GWAS "Genome-Wide Association Study" studies, which started in the past years, continue to identify common genetic polymorphisms related to diseases. A two-stage GWAS study was performed to identify genetic variants that predispose to CHB (Kamatani et al., 2009). In this study, several polymorphisms in the HLA-DP gene were found to be associated with HBV risk. Kamatani et al. (2009) determined that 11 SNPs in the HLA-DP gene region were strongly associated with CHB in this GWAS study conducted in the Japanese population. These SNPs are; rs3077, rs9277535, rs3117222, rs3128917, rs9380343, rs10484569 and rs2281388.

The polymorphisms existed in the gene may influence HLA-DPB1 gene expression by interaction with cis-acting elements and transcription factors. Therefore, functional disorders may occur in the antigen presentation of HLA molecules. This study explored the HLA-DPB2 gene rs9380343 polymorphism in 116 patients with immune-active hepatitis B phase and in 115 individuals with inactive hepatitis B phase in a Turkish population. Additionally, this work is the first to survey the relationship between this polymorphism and HBV phases for the first time in the world.

MATERIALS AND METHODS

This work was ratified by the Cukurova University Ethics Committee in Turkey. The individuals in this study were recruited at Balcalı Hospital of Cukurova University. The participants signed an informed consent form respecting the usage of their blood specimens. This study realized according to the Helsinki declaration endorsed on the World Medical Association gathering in Edinburgh.

The both groups were obtained by the present AASLD guideline (Terrault et al., 2018). 116 patients with immune-active HBV phase included in the study group were detected as HBeAg positive or negative, definitely liver inflammation and damage, high ALT level, and low or high HBV DNA load. As a control group, 115 individuals with inactive chronic hepatitis B phase were detected HBeAg negative, anti-HBeAg positive, low HBV DNA levels, and regular ALT levels. The patients having co-infection with HCV, HDV, HIV and immunological diseases, and the immune tolerant phase excluded from this work. HLA variants of all patients were established with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Biochemical parameters and HBV DNA levels of all participants were established to analysis. The medical patterns of individuals were frozen at -80 ^{o}C until the experiment.

The isolation of genomic DNA, and the rs9380343 variants' analysis

All study subjects' genomic DNA was obtained from their blood samples according to the DNA-isolation kit's prospectus. The alleles of these variants were identified by PCR-RFLP. PCR-RFLP protocol in brief, PCR cycling conditions were used: 4 minutes at 95 °C, followed by 40 cycles at 94 °C, 60 °C for 58 seconds and 72 °C for 65 seconds, with the last extension at 72 °C for 10 minutes. The PCR band (a 155 bp fragment) was amplified by two sets of primers and was digested for over 12 hours with 5 units of restriction enzyme HaeIII at 37 °C. Afterward, the digested products were electrophoresed and monitored by UV. CC genotype had 134- and 21-bp bands, TC genotype had 155-, 134- and 21-bp bands, and TT genotype had a 155-bp band. For ensure quality control, the genotyping was done in the absence of disease situation information of the individuals and a 20% of random specimens of participants was determined twice by diverse experts, and reproducibility was 100 %.

Statistical analysis

The sufficient sample size and 80% statistic power was calculated with Quanto software using minor allele frequencies of these polymorphisms in HapMap, the disease's prevalence and the impact of minor allele on phenotype. All analysis was got by using the IBM SPSS software (USA). Comparisons in demographical variables between both groups were done the Student t-test or Mann-Whitney U test for continual variable, and chi-square test for categorical variables. Regression analysis with regulation for age and gender was got to establish risk determinant in statistic models between the groups. Analyzes were two-sided. P values < 0.05 were accepted significant.

RESULTS

The clinic data of the both groups were demonstrated in Table 1. The male gender in the immune active HBV phase and inactive chronic hepatitis B phase was 53.7% and 46.3%, respectively. Althougt male gender increased the immnu-active HBV risk, it was not statistically significant (OR=1.22 (0.92-1.63), P=0.15) (Table 1). There were differences between the other variables of the both groups were significant in respect to age, leukocyte, gamma glutamyl aminotransferase (GGT), aspartate aminotransferase (AST), albumin, hematocrit and HBV DNA level (Table 1).

Table 1. Distribution of selected characteristics in both groups.

Variable	Immune-active HBV group (%), n =116	Inactive HBV carriers group (%), n=115	*P* value
Age ‡	52.51 ± 13.30	45.28 ± 12.01	0.001§
Sex, n, (%)			0.13**
Male	80 (68.9)	69 (60)	
Female	36 (31.1)	46 (40)	
Drinker, (%)	6 (7.3)	4 (4.5)	0.44**
AST(U/L) †	27 (13-585)	23 (11-188)	0.001*
ALT (U/L) †	26 (8-1028)	23 (8-314)	0.18*
GGT (U/L) †	20 (5-242)	16 (6-480)	0.002*
Albumin (g/dL) ‡	3.92 ± 0.81	4.15 ± 0.45	0.01§
HCT(uL) ‡	40.09 ± 5.90	41.60 ± 5.35	0.03§
Leukocyte, ($x10^3$/ul) ‡	7.52±2.20	6.81±2.10	0.03§
HBV DNA, (IU/ml) †	2.10^5 ($200\text{-}1.7x10^8$)	$413(20\text{-}1.63x10^3)$	0.014*

*P values were estimated with *Mann-Whitney test*. **P values were estimated with *chi-square test*. §P values were estimated with *student t test*. †Median (min-max). ‡Mean±SD.

The rs9380343 polymorphism's allele and genotype distributions

The total allele distributions of rs9380343 polymorphism were 6.9% and 93.1% for T and C, respectively. The genotype frequencies were similar in the each groups (for TC genotype; 13.9% vs 13.8). Furthermore, a relationship was not determined for these polymorphism's allele and genotype distribitions in the both groups (Table 2). When genotype frequencies analyzed in respect to gender groups, TC heterozygote was more in the males than females (15.4% vs 11%, P=0.35) (Table 3). Additionally, TT homozygous genotype was not found in either group. Therefore, we were unable to perform further genotype modeling analyses. Furthermore, a significant different was not determined between the two groups by allele and genotype frequencies of this polymorphism in gender groups (Table 3).

Table 2. The relationship between rs9380343 polymorphism and the immune-active HBV risk

***HLA* gene**	**Immune-active HBV, n =116, (%)**	**Inactive HBV carriers, n =115, (%)**	**P-value**	**OR (95% CI)**
rs9380343				
Allele frequency, n (%)				
C	216 (93.1)	214 (93)		1.00 (Reference)
T	16 (6.9)	16 (7)	0.98*	0.99 (0.48-2.03)
Genotype frequency, n (%)				
CC	100 (86.2)	99 (86.1)		1.00 (Reference)
TC	16 (13.8)	16 (13.9)	0.84**	1.10 (0.49-2.35)
TT	0	0	-	-

*P values were estimated with *chi-square test* analysis. *P values were calculated with *regression* analysis. Adjustments were performed for age, sex, and drinking. TT homozygous genotype was not found in either group. Therefore, we were unable to perform further genotype modeling analyses.

The relationship between rs9380343 variant and immune-active HBV risk

To explore whether increased risk of immune-active HBV by this variant, we implemented regression analysis with regulation for age and drinking between both groups in males and females (Table 3). In females, the T allele and TC genotype of rs9380343 polymorphism was not statistically significant although it increased 2.69- and 2.82-fold immune active HBV risk, respectively (P=0.16 and P=0.17) (Table 3). Contrary, the T allele and the TC genotype of rs9380343 polymorphism did not statistically significant in males (Table 3). When we perform a risk analysis of total allele frequency regardless of gender, carrying the TC risk genotype of the rs9380343 polymorphism increases the risk of immune-active HBV by 1.10 times, but it is not statistically significant, either (Table 2). Similarly, the T risk allele of the rs9380343 polymorphism appeared to be protective allele in the total allele frequency analysis, but it was not found to be significant, either (Table 2).

Table 3. Analysis of rs9380343 polymorphism and immune-active HBV risk by the gender.

rs9380343	Allele/Genotype	Immune-active HBV, N (%)	Inactive-HBV carriers, N (%)	P values	OR (95% CI)
Male, N (%)		80 (53.7)	69 (46.3)		
Allele frequency					
	C	150 (93.8)	125 (90.6)	--	1.00 (Reference)
	T	10 (6.2)	13 (9.4)	0.31*	0.64 (0.27-1.51)
Genotype frequency					
	CC	70 (87.5)	56 (82.2)	0.28*	-
	TC	10 (12.5)	13 (18.8)	0.25**	0.57 (0.22-1.47)
	TT	0	0	-	-
Female, N (%)		36 (43.9)	46 (56.1)		
Allele frequency					
	C	66 (91.7)	89 (96.7)	--	1.00 (Reference)
	T	6 (8.3)	3 (3.3)	0.16*	2.69 (0.65-11.18)
Genotype frequency					
	CC	30 (83.3)	43 (93.5)	0.15*	1.00 (Reference)
	TC	6 (16.7)	3 (6.5)	0.17**	2.82 (0.64-12.38)
	TT	0	0	-	-
Total		Males (149)	Females (82)		
	CC	126 (84.6)	73 (89)	0.35*	
	TC	23 (15.4)	9 (11)		
	TT	0	0		

*P values were estimated with chi-square test. **P values were calculated by regression analysis. Adjustment were done by age and drinking.

DISCUSSION

The current study surveyed the impact of the HLA gene polymorphism rs9380343 on the risk of immune-active HBV phase predisposition using a case-control group for the first time in a Turkish population which is a Caucasian population. There are only three studies have associated rs9380343 polymorphism with chronic HBV infection, that one of these studies is the article I previously investigated in terms of chronic HBV infection and HBV spontaneous clearance (Akgöllü, 2019). Another study investigated the relationship between this polymorphism with chronicity risk of HBV infection that explored using a healthy control group (248 persons), an HBV spontaneous clearance group (571 persons) and 521 HBV patients (Guo et al., 2011). Thirdly, a study from Japan conducted the Genome-Wide Association Study (GWAS) survey by recruiting 786 Japanese HBV patients and 2,201 healthy controls (Kamatani et al., 2009).

Guo et al. (2011) reported that in the persons with the TC genotype of rs9380343 polymorphism had higher the developing risk of CHB infection compared to persons with the CC genotypes when the CHB group was compared with the healthy control and HBV spontaneous clearance groups (TC vs CC, for the HBV group vs healthy group: OR=1.81 (1.39-2.35), P<0.0001 and for the HBV group vs HBV spontaneous clearance: OR= 1.84 (1.39-2.44), P<0.0001, respectively). Similarly, when the chronic HBV with healthy control and HBV spontanues clearance groups compared to each other in the same study, it was reported that the persons with TT genotype had 2.65-fold and 3.12-fold higher the developing risk of CHB infection than in those with CC genotype (CC vs TT, OR=2.65 (1.84-3.82), P<0.0001; OR=3.12 (2.0-4.73), P<0.0001, respectively) (Guo et al., 2011). Kamatani et al. (2009) declared that the T allele of the rs9380343 polymorphism is a risk allele for chronicity of HBV infection and is statistically highly significant.

In this work, total allele distributions of these variants in the control group which is inactive HBV phase were got 93% and 7% for rs9380343 C and T, respectively. The frequency of T allele in the Asian population was found to be quite high compared to the Caucasian population (29.6% vs 6%) (Guo et al., 2011; Akgöllü, 2019). In the current study, the allele distribution of rs9380343 polymorphism was not statistically significant between HBV phase groups. In the current study, the individuals having TC genotype of rs9380343 polymorphism had a 1.10-fold higher immune-active HBV risk than the persons who haven't got TC genotype, but not significant (P=0.84 OR=1.10 (0.49-2.35)). In the studies reported from China and Japan, the persons having T allele of this polymorphism were highly significant in respect to CHB infection risk (Guo et al., 2011; Kamatani et al., 2009). The results of these studies are in contrast to the current study. Moreover, in my

previous research, which included 238 chronic HBV patients and 238 HBV spontaneously cleared individuals; I determined that individuals with the T allele had a 1.88-fold higher risk of CHB infection than those having other allele (P=0.038). I also determined that the having TC genotype had a 2.23-fold greater risk of developing CHB infection than those without this genotype (P=0.017) (Akgöllü, 2019).

The current study also analyzed an association between this polymorphism with immune-active-HBV risk according to gender groups, it didn't find any statistically significant albeit the T risk allele of rs9380343 increased immune-active HBV risk in females (OR=2.69 (0.65-11.18), P=0.16). Similarly, males were not significant, either (OR=0.64 (0.27-1.51), P=0.31). Moreover, in the genotype analysis, while the TC genotype of this polymorphism 2.82-fold increased to immune-active HBV risk in females, it isn't appears to be effect in males. However, it could not reach statistical significance in both gender groups. There is only one study investigating this relationship in terms of gender, and that is my previous research. The risk of chronicity of HBV infection was increased in male individuals with T allele or TC genotype, and it was statistically significant (P=0.006 and P=0.008, respectively) (Akgöllü, 2019). There is no other study investigating this polymorphism in the immune-active HBV and inactive HBV phases of hepatitis B infection. Therefore, it is not possible to make a comparison.

Some studies declared that the expression of HLA-DP genes was found to be effected by single nucleotide polymorphisms. As known, the variants within the 3' UTR of a gene can impact mRNA expression via binding of transcription factors or regulation by microRNAs (O'Brien et al., 2011). Thereupon, O'Brien et al. (2011) reported that some important SNPs reduced mRNA expression of HLA-DP genes in healthy liver biopsy samples of 651 individuals of European descent. However, they didn't determine a significant relationship between rs9380343 polymorphism and HLA-DP gene expression level. Although this polymorphism does not cause amino acid changes, two GWAS studies reported that the rs9380343 polymorphism plays a prominent role in the chronicity of HBV infection (Guo et al., 2011; Kamatani et al., 2009). However, the mechanism of how this polymorphism affects has not been clarified yet. Except for HBV infection, no another study has investigated the relationship between this polymorphism and diseases.

The current study has several limitations:

a) The all participants included in the current study were in from Adana and surrounding provinces, whence, the present work does not representative all patients with immune-active and inactive HBV in the

Caucasian population.

b) Since the number of samples decreases in the relationship analysis with rs9380343 polymorphism in terms of gender, the statistical power decreases. For this reason, it is imperative to work with a larger sample number in Caucasian population.

c) This study only investigated the relationship of rs9380343 polymorphism with immune-active and inactive HBV phases. Therefore, the relationship of others polymorphisms in the *HLA-DP* gene with HBV phases has not been established.

In conclusion, this study is the first to show the relationship between HBV infection stages and *HLA-DPB2* gene rs9380343 polymorphism in Turkey and all over the world. The results of this study demonstrated that there is no effect of rs9380343 polymorphism on the immune-active HBV and inactive HBV phases. However, it would be appropriate to conduct this research with a larger sample to confirm this result in the same case-control groups. It is also important to carry out transcription, post-transcription and post-translation studies to elucidate the mechanisms of action of these and similar polymorphisms in liver diseases.

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Chapter 10

"THE FEAR OF COVID-19 FAMILIAL INFECTION SCALE" TURKISH VALIDITY AND RELIABILITY STUDY

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1.INTRODUCTION

Following the World Health Organization (WHO) report of pneumonia cases with unknown cause in Wuhan, China on December 31, 2019; a novel coronavirus (COVID-19) was identified as the cause by Chinese authorities (WHO, 2020). 81% of COVID-19 cases are reported to have asymptomatic clinic, while severe disease (for exp. dyspnoea, hypoxia or more than 50% lung involvement in images within 24-48 hours) is reported in 14% and clinical disease (for exp. shock, respiratory failure or multiorgan dysfunction) is reported in %5 (Wu & McGoogan, 2020). The disease is transmitted by droplets emitted by coughing and sneezing and from the surfaces patients touch (by touching the eye, mouth and nose mucosa with hands) (Ministry of Health, 2020). It has been reported that the virus is spread with a high speed and as of October 2020, the number of infected individuals is 35.347.404 and the number of deceased individuals is 1.039.406 (WHO, 2020). While mentioning some common psychosocial effects caused by deadly epidemics, Strong stated that fear, panic, stigmatization, moral debates and call for action characterised the initial reactions. He reported that the psychology of the epidemic transformed into a new collective epidemic that showed a rapid spread from person to person. He also reported that it included at least three different kinds of psycho-social epidemic: the epidemic of fear, the epidemic of explanation and the epidemic of proposed actions. In the epidemic of fear, the basic worry of individuals includes their suspicions about themselves, their families and loved ones catching the disease (Strong, 1990).

Healthcare professionals fight on the front lines against COVID-19 infection and they are evaluated as in very high risk group in terms of the risk of COVID-19 infection (Sakaoğlu, Orbatu, Emiroğlu, & Çakır, 2020). During this pandemic period, one of the main factors affecting stress levels among healthcare professionals is being afraid of getting infected with COVID-19. It is especially the fear of transmitting the virus to family or friends (Ahorsu et al., 2020). Maunder et al. reported that healthcare professionals were worried about transmitting the infection to their children, about stigmatization by their friends and about the care of their children if they get hospitalized or quarantined (Maunder et al., 2003). When other studied conducted are examined, it can be seen that an important source of fear was not only catching the disease, but at the same time the fear of transmitting to other family members and having family members who are afraid of getting infected by them (Chan et al., 2005; Goulia, Mantas, Dimitroula, Mantis, & Hyphantis, 2010).

"The Fear of COVID-19 Familial Infection Scale" was developed by Mayer et al. in 2020. It is a highly reliable and valid scale and it includes 6 items developed to evaluate health professionals' fears of infecting the

virus to their families (spouses and children) and relatives and to evaluate whether they had families and relatives who were afraid of being infected by them during the COVID-19 epidemic (Mayer, Etgar, Shiffman, & Lurie, 2020). No scales were found which evaluated health professionals' fears of infecting the virus and having families and relatives who were afraid of being infected by them. In epidemics with a high contagiousness and life threatening risk such as COVID-19, the fear and anxiety of individuals about themselves and their relatives getting infected are transferred from the individual dimension to the social dimension (Doğan & Düzel, 2020). For this reason, it is thought that translating this scale into Turkish will enable assessing the fear levels of healthcare professionals and providing supportive programs and trainings in accordance with the needs of healthcare professionals.

2.MATERIAL and METHOD

2.1.Study Design: The study used methodological design.

2.2.Population and Sample of the Study: The study was conducted between September 10 and 30, 2020 with healthcare professionals who were married and who had children. The questionnaire form created via docs.google.com/forms was sent to healthcare professionals online (e-mail, whatsapp). It is stated in literature that adaptation studies should be tested with a sample which is at least 5-10 times the number of items (Seçer, 2015). For this reason, the study was completed with 352 healthcare professionals who volunteered to participate and who answered the questionnaires within the specified dates.

2.3.Data Collection Instruments

"Personal Information Form" and "The Fear of COVID-19 Familial Infection Scale (FCFI)" were used in the study to collect data online from the participants.

2.3.1.Personal Information Form: There are 8 questions in the form which was prepared by the researchers to find out the socio-demographic features (age, gender, level of education, profession, financial situation, number of children, state of caring for COVID-19 patients and the state of having COVID-19) of the participants.

2.3.2.The Fear of COVID-19 Familial Infection Scale (FCFI): It was developed by Mayer et al. (2020) to find out individuals' fear of familial COVID-19 infection (Mayer et al., 2020). The 6-item and 5 Likert type scale is scored as "1-strongly disagree" and "5-strongly agree". It has two sub-dimensions: The first sub-dimension "Fear of infecting others (FIO)" includes three items (1,2,3). The second sub-dimension "Perception of Others' fear of being infected by me (POF)" includes three

items (4,5,6). The lowest score one can get from each question is 1, while the highest is 5. The minimum possible total scale score is 1, while the maximum possible score is 30 and fear of familial infection increases as score increases (Mayer et al., 2020). In this study, mean values were used to measure the score of each subscale.

2.4.Data Analysis: SPSS 17.0 and LISREL 8.8 package program were used to analyze the study data. Personal Information Form was evaluated with numbers and percentages. For validity, Barlett Tests, Kaiser-Meyer-Olkin Index (KMO), Exploratory Factor Analysis, Confirmatory Factor Analysis and Principal Component Analysis were used for the determination of content and construct validity. In terms of reliability, internal consistency and homogeneity were found by Cronbach's a Coefficient, Pearson Correlation analysis and item-total score correlation.

2.5. Ethical Considerations: Before the starting the study, written permission was taken from scale developers. The study was conducted in accordance with Declaration of Helsinki. Ethics Committee approval of a foundation university (2020/08 numbered) was taken for the study.

2.6. Psychometric Assessment of the Scale

2.6.1.Validity

2.6.1.1. Language validity:

Translating a scale into another language causes an inevitable change in the essence of the scale in terms of differences in expression and conceptualization. While adapting a scale to a new culture, it is very important to examine scale items carefully, to make necessary transformations for meaning in the translated language and to standardize for individuals who use the translated language in order to minimize the differences (Çapık, Gözüm, & Aksayan, 2018). By paying attention to the aforementioned issues, the scale was translated into Turkish. First the researchers and then two faculty members translated the scale. The resulting Turkish form was edited and reviewed. Following this procedure, the process of back translation into English was done by a linguist who knew both cultures and both languages well. The original scale and Turkish translation were reviewed to evaluate any changes in meanings of the items. The translations which best expressed each item were presented to the opinions of 7 experts.

2.6.1.2.Content validity: Content Validity Index-CVI was used for testing whether the language and culture were equivalent, for proving item content validity and for evaluating expert opinions (Çapık et al., 2018). For each of the items, experts needed to choose the suitable expression among from "4=completely suitable", "3=very suitable", "2=suitable but

the items need small changes" and "1=not suitable" for scoring the items between 1 and 4. Content validity index (CVI) was found as 0.98 with Davis technique. >0.80 content validity indicates item adequacy. Thus, the scale's content validity was found as statistically significant (Çapık et al., 2018) and no items were deleted.

2.6.1.3.Construct validity

Factor analysis is the method commonly used for testing construct validity. It is a procedure performed for the assessment of whether it is possible to group scale items under different dimensions and it has two types as Exploratory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA). ETA is used to identify under how many sub-dimensions the items in the measurement tool can be grouped. For the confirmation of this construct, CFA is calculated (Güngör, 2016; Seçer, 2015). As a measurement method to measure sample adequacy, KMO and Bartlett's test are conducted before construct validity analysis (Seçer, 2015). While A KMO level of <0.50 cannot be accepted, a value between .80 and .90 is good and a value of >0.90 is very good (Çokluk, Şekercioğlu, & Büyüköztürk, 2016).

Factor analysis was tested with Principal Component Analysis and Varimax rotation. The view that items should have at least 0.30 factor load and items below this value should be deleted was applied (Seçer, 2015). EFA was followed with CFA to support the findings of the scale sub-dimensions. CFA showed that $X2/sd$ ratio of ≤5, RMSEA value of ≤0.07 and GFI, CFI, IFI values >0.90 were lower limits of the model's data fit index (Çapik, 2014).

2.6.2.Reliability

It is suggested to use Cronbach's α internal consistency coefficient to analyze homogeneity and internal consistency of scale items. Higher values show that items are homogeneous and they test elements of the same characteristics (Güngör, 2016; Özdamar, 2017). ≥0.70 Cronbach's alpha reliability means that the measurement tool can be used in studies (Güngör, 2016; Özdamar, 2017).

The association between item scores and the total score is analyzed with item-total correlation coefficients. The suggestion that acceptable coefficient should be ≥0.30 was applied in item selection (Büyüköztürk, 2017).

Test-retest means reaching consistent results in repeated measurements of the test. Correlation analysis is used to evaluate the results of both tests. A correlation coefficient closer to 1 indicates that the test has better test-retest reliability (Tavşanel, 2019). 2 weeks later, the scale was given to 50 patients for test-retest reliability (Karasar, 2016; Tavşanel, 2019).

3.RESULTS

Analysis of the participants' demographic features showed that the participants had a mean age of 33.86 ±7.08, 72.7% were female, 67.9% were undergraduates, 62.8% were nurses, 50.6% had one child and 75.9% had higher income than expense. It was found that 62.2% of the nurses were not providing care to COVID-19 patients and 98.3% did not have COVID-19 (Table 1).

Table 1. Demographic Characteristics of the Participants

Mean Age (Mean ±SD)		33.86 ±7.082	
		n	%
Gender	Female	256	72.7
	Male	96	27.3
Level of Education	High School	22	6.3
	Associate degree	53	15.1
	Undergraduate	239	67.9
	Post graduate	38	10.8
Profession	Doctor	17	4.8
	Nurse	221	62.8
	Midwife	53	15.1
	Other*	61	17.4
Level of income	Income<expense	78	22.2
	Income=expense	7	2.0
	Income>expense	267	75.9
Number of children	1	178	50.6
	2	126	35.8
	3	4	12.8
	4 and more	3	0.9
The state of providing care to COVID-19 patients	Yes	133	37.8
	No	219	62.2
The state of having COVID-19	Yes	6	1.7
	No	346	98.3

*** Pharmacist, Paramedic, Emergency medical technician, Radiology technician**

3.1.Validity

Barlett's Test of Sphericity analysis showed that KMO value was 0.80 and X^2 value was 1523.46. The results were significant at p=0.000 level (Table 2). This result showed that the sufficiency and suitability of sample size for factor analysis (Capik, 2014).

Table 2. Results of the Kaiser–Meyer–Olkin measure of sampling adequacy and Bartlett's test of Sphericity

Test		Results	
Kaiser–Meyer–Olkin measure of sampling adequacy		0.80	p < 0.001
Bartlett's test	Approx. Chi-square	1523.46	
	df	15	
	Sig.	0.00	

Varimax rotation technique was employed for factor analysis. Values ranging between 0.76 and 0.90 were found for factor load values in the EFA conducted for the validity of FCFI and it was found that 83.12% of the total variance was explained (Table 3). As a result, 6-item FCFI with 2-dimension was obtained.

Table 3. Mean scores, item-total correlation coefficients, factor loads, alpha coefficients and FCFI variance explained

Item load	Factor load	Mean (SD)	Corrected Item-total Correlations	Cronbach's Alpha if Item Deleted
1	0.90	4.55 (0.91)	0.69	0.86
2	0.89	4.45 (0.95)	0.71	0.85
3	0.88	4.50 (0.88)	0.73	0.85
4	0.88	3.65 (1.18)	0.66	0.86
5	0.89	3.68 (0.21)	0.71	0.85
6	0.76	4.05 (0.96+)	0.66	0.86
% Variance Explained				Total = 83.12
Cronbach's a				Total =0.88 f1:0.92 f2:0.86

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In Table 4, confirmatory factor analysis (CFA) fit index values of FCFI were found as X^2=12.51, df= 7 ($p<0.05$), X^2/df=1.78, RMSEA=0.047, CFI= 1.00, RMR= 0.028, SRMR= 0.031,GFI= 0.99, AGFI= 0.96 and NFI= 0.99 and it was found that the model fit was acceptable (Capik, 2014). CFA Path Diagram of FCFI after DFA model can be seen in Figure 1.

EFA and CFA showed that Turkish form of FCFI with 6 items and 2 sub-dimensions was confirmed without any changes on the original scale form. All the results obtained show that the scale has a high validity in Turkish culture.

Table 4. Confirmatory Factor Analysis Results

Fit criteria	Found	Appropriate	Acceptable
x^2/df	1.78	<2	<5
RMSEA	0.047	<0.05	<0.08
CFI	1.00	>0.95	>0.90
RMR	0.028	<0.05	<0.08
SRMR	0.031	<0.05	<0.08
GFI	0.99	>0.95	>0.90
AGFI	0.96	>0.95	>0.90
NFI	0.99	>0.95	>0.90

RMSEA : Root Mean Square Error of Approximation; CFI : Comparative Fit Index; RMR :Root Mean Square Residual ; SRMR : Standardized Root Mean Square Residual; NFI: Normed Fit Index; GFI: Goodness of Fit Index; AGFI: Adjusted Goodness of Fit Index

3.2.Reliability

In the analyses conducted to test scale reliability, the data were reapplied to 50 individuals from the sample on whom EFA was conducted and the pretest-posttest correlation was found as 0.92. This value obtained indicated that the scale has a high external reliability and a stable structure (Tavşanel, 2019).

In addition, the internal reliability of the scale was found as 0.88 for the whole scale. Internal consistency coefficient was 0.92 for Fear of infecting others sub-dimension and 0.86 for Perception of Others' fear of being infected by me sub-dimension (Table 3). These values show that the 6-item scale had a high internal consistency (Çokluk et al., 2016; Özdamar, 2017). All of the item-total correlation coefficients in the scale were higher than 0.30 (0.66-0.73) (Table 3)

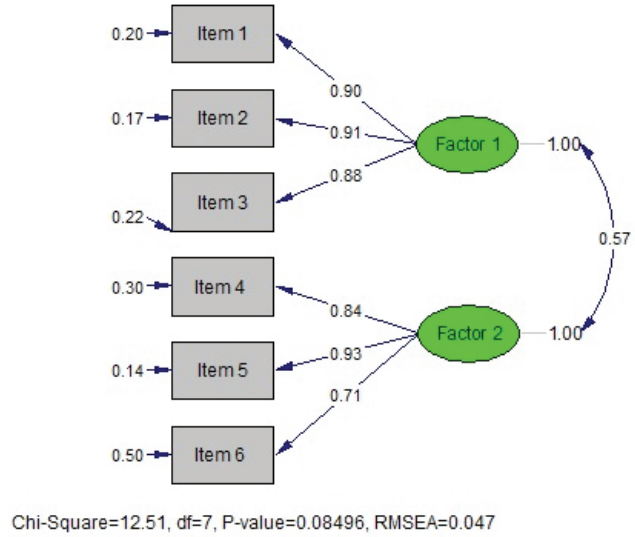

Figure 1. PATH Diagram Regarding the Factor Structure of the Scale

4. DISCUSSION

It was found that there were no specific scales were found in Turkey assessing the COVID-19 familial infection fear of healthcare professionals. Therefore, "The Fear of COVID-19 Familial Infection Scale" (Mayer et al., 2020) was adapted and its reliability and validity was tested. This section discusses the findings of FCFI consisting of 6 items and two sub-dimensions.

4.1.Validity

In the present study, construct validity of Turkish version of FCFI was tested with EFA and CFA. Before the analysis of construct validity, KMO value and Barlett Sphericity Test values were calculated to find out whether sample size was suitable. KMO value was calculated as 0.80, Barlett Sphericity Test was calculated as $x2$=1523.46, and df: 780; p=0.000. It is stated in literature that KMO value should be at least 50 and above, while Barlett Sphericity Test value should be statistically significant (Çokluk et al., 2016). Thus, the number of data was confirmed to be sufficient for factor analysis.

In Turkish adaptation of the scale, 83.1% of total variance was explained. In the original scale, the rate of total variance explained was found as 69.4% (Mayer et al., 2020). In line with these results, it was confirmed that similar to the original scale, FCFI included two sub-dimensions and the factor structure was sufficient.

In this study, item factor loads were between 0.76 and 0.90. Original scale by Mayer et al. (2020) had factor loads between 0.73 and 0.86 (Mayer et al., 2020). It is stated in literature that the acceptable value for factor loads can be as low as 30 (Büyüköztürk, 2017; Seçer, 2015). In line with these results, no items were deleted because there were no items with <0.30 factor load.

The index values calculated to examine the model fit were $X2$=12.51, df= 7 ($p<0.05$), $X2$/df=1.78, RMSEA=0.047, CFI = 1.00, RMR = 0.028, SRMR = 0.031,GFI= 0.99, AGFI= 0.96 and NFI = 0.99. These values showed the model was acceptable as it was (Çapik, 2014). CFA, which confirmed the exploratory factor analysis, confirmed the two dimensional structure of the scale as well.

4.2.Reliability

Total Cronbach's a coefficient was 0.98, while it was 0.92 for FIO and 0.82 for POF sub-dimensions. In the original scale by Mayer et al. (2020), it was found as 0.79 for FIO sub-dimension and as 0.75 for POF sub-dimension (Mayer et al., 2020). In literature, it is stated that the measurement instrument is sufficient for use in researches when Cronbach's a reliability is 0.70 and above, while it is stated to have high reliability when Cronbach's a reliability is 0.80 and above (Özdamar, 2017; Tavşanel, 2019). These results show that FCFI in healthcare professionals had high internal consistency and high reliability.

In the study, item-total correlation coefficients were between 0.66 and 0.73. In literature, the acceptable value for item selection is ≥0.30. An item is considered to be effective and sufficient to measure the targeted behaviour when it has a high correlation coefficient (Büyüköztürk, 2017; Özdamar, 2017). This result shows that the scale has high reliability.

FCFI was applied to 50 individuals with an interval of 2 weeks for retest analysis. The statistically significant correlation found was high and positive ($p<0.001$). The result shows that the scale has high consistency over time and indicates that it is possible to get reliable results for more than one application.

5. CONCLUSION

It was concluded that original scale and the present study had consistent result. The scale's two factor structure was confirmed. High cronbach's α internal consistency coefficient, item-total correlation and test-retest analysis were found. These results show FCFI is a valid and reliable instrument in measuring the fear of familial COVID-19 infection in healthcare professionals.

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Chapter 11

EVALUATION OF CHILDREN'S USAGE OF EMERGENCY DEPARTMENTS IN TURKEY

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1. Background

Emergency Departments (EDs) provide immediate and emergency care for patients with life-threatening medical conditions, trauma or injuries – they should not treat minor illnesses or provide primary care (Chamberlain & Carraccio, 1994; Chande *et al.*, 1996; Liu *et al.*, 1999). However, the American College of Emergency Physicians (1990) stated that increasing visits with medically non-urgent conditions is something which greatly concerns healthcare professionals and administrators because of the medical and economic implications.

The demand for EDs increasing, and this pressure is significant concern (Amiel *et al.*, 2014). This becomes an increasing problem for patients, healthcare staff and ED services. EDs are visited annually by approximately 4 million children in England (Hemingway and Redsell, 2011). It is estimated that approximately 25% of the UK child population receives treatment in EDs every year (Hendry *et al.*, 2005). A significant proportion of these are children with minor illness (Phillips & Robson, 1992). A UK-based patient survey at EDs found that more than half of the participants who were triaged presented with a minor illness and could have been treated elsewhere (Coleman *et al.*, 2001). Many parents bring their children with minor illness to an ED rather than their GP or other primary care services. The usage of EDs by patients with minor illness is an important and still unresolved problem causing a burden to health services (Lega & Mengoni, 2008). This unnecessary participation in an ED might affect quality of care, workload, resource utilization and cost. Sands *et al.* (2011) identified the most common medical presenting problems of children aged 0-15 years as those shown in Table 1 below. Also, a study by Armon *et al.* (2001), which was conducted ten years earlier than that of Sands *et al*. (2011), identified almost the same common medical problems.

Table 1: Presenting problems of medical attendees aged 0-15 years. (Prepared on the basis of Sands et al.'s (2011) study)

Presenting problem	**Percentage (%)**	**Rank**
Breathing difficulty	(20.13)	1
Febrile illness	(14.14)	2
Diarrhoea, vomiting	(13.97)	3
Rash	(8.60)	4
Cough	(6.74)	5
Abdominal pain	(6.54)	6
Seizure	(6.30)	7
Other	(5.20)	8
Ingestion	(4.11)	9
Headache	(2.20)	10

EDs have some advantages which affect their usage. These advantages of the ED services might affect parents' decision to use an ED. For instance, researchers have identified four main functions served by the ED: addressing an urgent medical care, providing care which can only be given at a hospital ED, serving when other regular services are unavailable, and delivering care on a regular basis (Beland *et al.*, 1998; Nadel, 1993). The ED services might be used as an alternative service by patients who are not satisfied with the care which they are receiving at their primary care services, such as problems over scheduling appointments and long waiting times (Hunt *et al.*, 1996; Kellermann, 1991; Murphy, 1998b; Shipman *et al.*, 1997). Issues such as these might lead parents to use an ED for minor illness.

Olshaker (2009) reported that more than 90% of ED directors claimed that overcrowding causes several problems: unnecessary occupancy of ED beds, long waits, diversion of ambulances and dissatisfaction of both patients and ED staff. Hendry *et al.* (2005) argued that most children presenting with a minor illness can be appropriately managed in primary care rather than an ED. Moreover, Myers (1982) suggested that GPs can manage and treat minor illness. All these problems have led to research on this topic.

A number of studies have found a relationship between overcrowding and patients' negative experiences of ED and therefore reporting reduced satisfaction (Gilligan *et al.*, 2007; Timm *et al.*, 2008; Weiss *et al.*, 2005). Increases in the usage of the ED have also been associated with increased waiting times and greater mortality (Pines & Hollander, 2008; Royal College of Emergency Medicine, 2015). Overcrowding may also affect the performance and reputation of health organisations (Royal College of Emergency Medicine, 2015).

2. Decision-Making of Parents

Padgett and Brodsky (1992) identified three stages of decision-making regarding ED use; problem recognition, decision to seek help, and decision to use an ED. However, there is no rigorous evidence to support this model. In addition, decision-making has both cognitive and emotional components (Beresford and Sloper, 2008). Therefore, parents might not be able to make a logical decision due to their own psychology.

Exploring the decision-making process leading to visits to an ED is important for a better understanding of the reasons behind parents' decisions about children with minor illness. The decision-making of parents of children with minor illness in choosing an ED rather than a GP is complex (Murphy, 1998a). Parents' decisions regarding whether to visit EDs include family income level, expectations developed at community

level, level of urgency, travel time, and child health factors (Graham *et al.*, 2010). However, there are other determinants, which affect the parents' decisions, such as parents' psychology, lack of knowledge regarding child care, access issues, and reassurance needs.

3. Resources Utilisation

Usage of hospital resources not only increases costs, but could also affect the quality of emergency care if inappropriate use of EDs continues and EDs become overcrowded (Ding *et al.*, 2006; Halfon *et al.*, 1996; Martin, 2000). Time has shown that the increase in the number of patients who use an ED for non-urgent conditions affects the cost, the quality of EDs, service staff and the quality of healthcare delivery (Phelps *et al.*, 2000). There is therefore a need to investigate how to reduce the number of parents who attend an ED inappropriately. Attendance at an ED for minor illness and non-urgent care can be reduced (Lega & Mengoni, 2008). In addition, there are three main reasons which affect the decision-making process regarding attending an ED. These reasons are that better care is received at an ED, the urgency of patients' complaints, and immediacy (Northington *et al.*, 2005). Changes in access to, timing and contents of the care/services provided by GPs could encourage patients to shift from EDs to GPs and other primary care services (Butun *et al.*, 2019; Lega & Mengoni, 2008). Therefore, it is important to develop further understanding of why parents of children with minor illness choose to attend an ED instead of a GP or other primary care services.

4. Reasons for ED use

One systematic review by Morrison *et al.* (2013) explored the relationship between health literacy and paediatric emergency department utilisation. Morrison *et al*. (2013) found that approximately one in three parents seeking care for their children at an ED had low health literacy levels and limited ability to understand and make a decision for their children. The authors suggested that there is a relationship between low health literacy and increased usage of ED services (Morrison *et al*., 2013). Neill *et al.* (2014) argued that levels of knowledge and experience of childhood illness affect parents' ability to assess the severity of their children's illness and therefore increase the likelihood of them seeking consultation.

Previous negative experiences with a GP might affect parents' decision to visit an ED instead of a GP (Neill *et al.*, 2014). Palmer *et al*. (2005) reported that some patients believed that GP services could not meet their needs for various reasons such as that A&E is more appropriate than their GP, they were referred by a GP, no GP appointment was available, it was out of office hours, the non-availability of other healthcare services, accessibility issues and already having visited a GP without a

good outcome. Hence, they came directly to an ED instead of a GP. All these factors might increase the tendency to attend an ED, even for minor conditions. Therefore, there is a need to identify which problems with GP lead the parents to attend an ED, by exploring the determinants that affect their decision, in order to enhance the understanding of parental decision-making, so as to use the ED.

Dissatisfaction with the GP is another reason given for visiting an ED. Palmer *et al*. (2005) found that 55% of patients (48) cited doctors' (meaning GPs') behaviour as 'offhand', 'dismissive', 'rude', 'arrogant', 'abrupt' or 'unsympathetic'. Some patients were not satisfied after receiving treatment by an inexperienced GP who was unable to perform a procedure successfully (Murphy, 1998a; Palmer *et al.*, 2005).

Difficulties with getting an appointment from a GP is another issue that leads parents to visit an ED; one of the reasons given for visiting an ED is that no appointment with a GP was available (Stanley *et al.*, 2007). Some participants in Neill *et al.* (2014) study explained, that after losing trust in their GP, they sought access to other available services, when they were unable to get an appointment with their GP. The unavailability or absence of a GP affect the patients' decision over attending an ED with minor illness (Wood & Cliff, 1986). Difficulties over getting an appointment led the parents to use an ED. All these problems with their GP might increase the number of parents of the children with minor illness, who attend an ED. It is important to investigate the ways in which the GPs can give appointments to as many patients as possible. Therefore, these issues with the GP, from the parents' perceptive, with their narratives, should be looked into, along with the explanations and ideas, so as to gain a deeper understanding of the phenomenon.

In addition, EDs have some advantages compared with other primary care services from a parental perspective, and some parents therefore chose to attend an ED because of those advantages. For instance, some parents believe that their child will receive better treatment from more experienced doctors when attending an ED (Hendry *et al.*, 2005). The accessibility of an ED is another reason for patients to present at an ED. ED services are open twenty-four hours a day, so some patients see this as a guarantee of receiving treatment as opposed to trying to contact their GP (Wood & Cliff, 1986). Some patients said that their GP was too far away and that the ED was the nearer service (Murphy, 1998a). Shearer *et al*. (2015) identified the reasons for choosing an ED as its proximity to the patient's home, they expected to be seen quickly, and they expected to receive quality care. Also, prior experience is another reason given for choosing an ED. Shearer *et al*. (2015) claimed that patients who have had positive experience with an ED would be more likely to reuse that service. On the other hand, patients who

have had negative experience with an ED would be less likely to revisit that service (Shearer *et al.*, 2015).

5. Emergency departments in Turkey

Turkish citizens are able to visit and access EDs directly. EDs in Turkey were free at point of care for all patients until 2012. In 2012, the Turkish government introduced a small co-payment (8 Turkish Lira, £1.70 in 2018 prices) for those who were triaged as non-urgent (green-zone). The aim of this initiative was to reduce the number of non-urgent ED visits (Bektemur *et al.*, 2015). EDs are still free of charge for those who are triaged as semi-urgent (yellow-zone) and urgent (red-zone) (Bektemur *et al.*, 2015). Healthcare staff categorise patients on the electronic system after discharging them and decide the best triage category. The payment is subsequently included in their next prescription costs at the pharmacy. Consequently, patients may not be aware of their triage category or the subsequent fee for those triaged as green-zone.

Triage was described by Crouch *et al.* (2017) as identifying the urgency of care in ED settings. EDs in Turkey use a triage system which has three categories: green-zone (non-urgent conditions), yellow-zone (potentially urgent, semi-urgent) and red-zone (urgent conditions). Patients are grouped into one of these three categories and the green-zone category distinguishes patients with non-urgent conditions from others (Ministry of Health Turkey, 2015). Table 2 shows the triage codes and provides examples of the conditions. In Turkey, ED doctors are responsible for the triage of patients attending and they run the triage system. The doctors can therefore decide whether cases are urgent, potentially urgent or non-urgent.

Table 2. Triage system in the EDs of Turkey

Triage Categories	**Description of the categories**	**Examples of cases**
Green-zone code (Non-urgent)	Patients who have arrived at the hospital on foot and those observed as having minor health problems which are stable in general and could be dealt with by providing outpatient care Patients in this category are admitted to the green-zone area within two hours	1) Moderate level of pain which does not cause high risk 2) Any minor symptoms in a patient whose general condition and vital signs are stable

Yellow-zone code (Semi-urgent)	Probability of life-threatening conditions, risk of losing any organs, and conditions which have a high morbidity rate Conditions which continue for a long time and have the potential to be serious Patients in this category are admitted to the yellow-zone area within one hour	1) Simple haemorrhages 2) Chest injuries with no chest or respiratory problems 3) Persistent vomiting 4) Vomiting and diarrhoea without symptoms of dehydration 5) Patients with severe abdominal pain
Red-zone code (Urgent)	Life-threatening conditions Conditions which need urgent medical attention Patients in this category are immediately admitted to the red-zone area	1) Cardiac arrest 2) Respiratory arrest 3) Trauma 4) Vascular disorder

A description of a patient's journey through an ED in Turkey is shown in Figure 1. This is the usual process for ED patients. Urgent patients are seen by ED staff immediately after arrival at the ED.

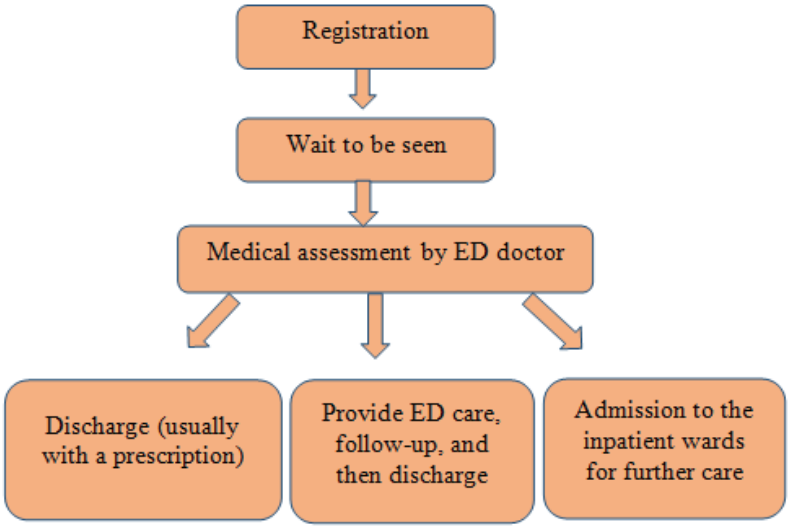

Figure 1. A patient's journey through an ED in Turkey

6. Trends in using EDs

Pitts *et al.* (2008) stated that demands on EDs in the US have been increasing. The number of ED visits in the US increased by 30% from 94.8 million to 123 million between 1998 and 2008 (Hsia *et al.*, 2011).

EDs in the US were visited by approximately 131 million people in 2011 (Healthcare Cost and Utilization Project, 2014) and 141 million in 2014 (Centers for Disease Control and Prevention, 2014), demonstrating a steady increase in the number of ED visits over this time period.

In terms of paediatric visits, EDs in the US are visited by approximately 25 million children annually, with estimates of the proportion of these visits for non-urgent conditions between 37% and 60% (Brousseau *et al.*, 2007). Even though many families in the US have a primary care provider, some parents use the ED for non-urgent care on a regular basis (Zandieh *et al.*, 2009). Halfon *et al.* (1996) found that over three years, 11,420 (3.6%) of 312,255 patients revisited the ED a second time on the same day. These non-urgent ED visits and revisits contribute to overcrowding in the ED and decrease the effectiveness of the ED.

In 2017-2018, the number of ED visits in England was reported as approximately 23.8 million in all types of ED services and 16 million of these were in major EDs referred to as Type 1 ED services (National Health Service, 2018). Approximately 40% of ED visits in England were considered non-urgent (Ismail *et al.*, 2013). In paediatric populations, EDs were visited annually by approximately 4.3 million children in England in 2017-2018 (National Health Service, 2018). Further statistics published by NHS England show that the number of ED visits increased by approximately 13% over the ten-year period from 2004-2005 to 2015-2016 (NHS England, 2017). The number of ED visits in England increased by 5.5%, which is equal to 2,210 more visits daily in 2016 compared with 2015 (Baker, 2017). In addition, there were one million ED visits in Wales in 2017 (Stats Wales, 2018), 1.34 million in Scotland in 2016 (Baker, 2017) and approximately 823,000 in Northern Ireland in 2017-2018 (Department of Health, 2018). In all four UK countries, approximately 19 million people visited the ED in 2017/18, which is equivalent to 292 visits per 1,000 people.

Further statistics available from high-income countries show that there were approximately 7.5 million ED visits in Australia in 2015-2016 and an increase of 3.8% on average each year between 2011-2012 and 2015-2016 (Australian Institute of Health and Welfare, 2016). Of these 7.5 million ED visits, 9.5% were considered to be non-urgent (Australian Institute of Health and Welfare, 2016). In Belgium, there were approximately 3.2 million ED visits per year and this had increased by 0.2 million from 2009 to 2012 (Belgian Health Care Knowledge Centre, 2016). According to a study conducted in twelve EDs across Belgium, approximately 40% of 1,873 ED visits were regarded as non-urgent presentations (Benahmed *et al.*, 2012). In Singapore, there were approximately one million ED visits in 2017 (Ministry of Health Singapore, 2017). In Italy, approximately 57%

of 134,358 ED visits over a two-year period were considered inappropriate visits after evaluation by triage staff (Vedovetto *et al.*, 2014).

7. Comparison of the use of EDs in Turkey with high-income and middle-income countries

The healthcare systems and EDs in middle-income countries could have more problems than those in high-income countries, especially in terms of cost, staffing level, patient safety, and cultural and systemic influences on the utilisation of healthcare services. The priorities of countries may differ and middle-income countries may not yet be aware of this multi-faceted problematic issue. Despite the presence of this problem in Turkish EDs, the issue has not attracted enough attention. However, the overcrowding of EDs in Turkey recently appeared in media coverage in 2018 and the Ministry of Health is now aware of this problem (Ministry of Health Turkey, 2018). The Turkish government has highlighted the need to reduce the number of ED visits and increase the capacity of EDs (Presidency of the Republic of Turkey, 2018).

The usage of ED services by non-urgent patients is on the increase in Turkey. The population of Turkey is approximately 80 million, but 90 million and 104 million visits were made to EDs in Turkey in 2013 and 2014 respectively (Eray, 2015), equating to 1.2 – 1.4 visits per person per year. Further data in relation to ED use in Turkey was requested and obtained from the Ministry of Health Turkey by email as these statistics are not currently available to the public. The statistical data obtained show that EDs in Turkey received approximately 111 million visits in 2015, 118 million in 2016, and 124 million in 2017, which equates to 1.2 - 1.5 visits per person per year (Personal Communication, 2018). In addition, according to the Turkish Ministry of Health (2014), 43% of ED patients were considered non-urgent. The ED services in Turkey are much more frequently accessed compared with those in high-income countries. For example, research has shown that ED services were used by 131 million people per year in the US (population 325 million), and by 19 million people in the UK (population 65 million) (Eray, 2015). However, annual ED visits in Turkey exceed the total population and represent 1550 visits per 1000-population. The EDs in Turkey are faced with a high volume of patients, fewer healthcare staff per capita, and higher rates of staff exposure to violence (Erenler *et al.*, 2014). Erenler *et al*. (2014) provided statistical data about the number of ED visits and showed that EDs in Turkey are three to four times more overcrowded than those in high-income countries. Table 3 shows a comparison between seven countries in terms of their population, annual ED visits and the number of visits per 1000-population. Table 3 and Figure 2 show that the number of ED visits per annum in Turkey is greater than its population.

Table 3. ED visits per 1000-population per year across seven countries

Country (type of healthcare service)	**Annual number of ED visits** (reference year)	**Number of ED attendances per 1000-population per year**	**Source**
United States (no universal healthcare service)	141 million (2014)	433	Centers for Disease Control and Prevention (2014)
United Kingdom (universal health care service)	19 million (only Type 1 EDs) (2016)	292	Baker (2017); (Stats Wales, 2018); (Department of Health, 2018)
Australia (universal health care service)	7.5 million (2015-2016)	312	Australian Institute of Health and Welfare (2016)
Belgium (universal health care service)	3.2 million (2012)	290	Belgian Health Care Knowledge Centre (2016)
Singapore (universal health care service)	1 million (2017)	180	Ministry of Health Singapore (2017)
Malaysia (universal health care service)	9.3 million (2013)	315	Sivasampu *et al.* (2015)
Turkey (universal health care service)	90 million (2013) 104 million (2014) 111 million (2015)* 118 million (2016)* 124 million (2017)*	1125 1300 1375 1475 1550	Eray (2015); Ministry of Health Turkey*

*The data for 2015, 2016 and 2017 were obtained from Ministry of Health Turkey by email on request as these are currently not available to the public.

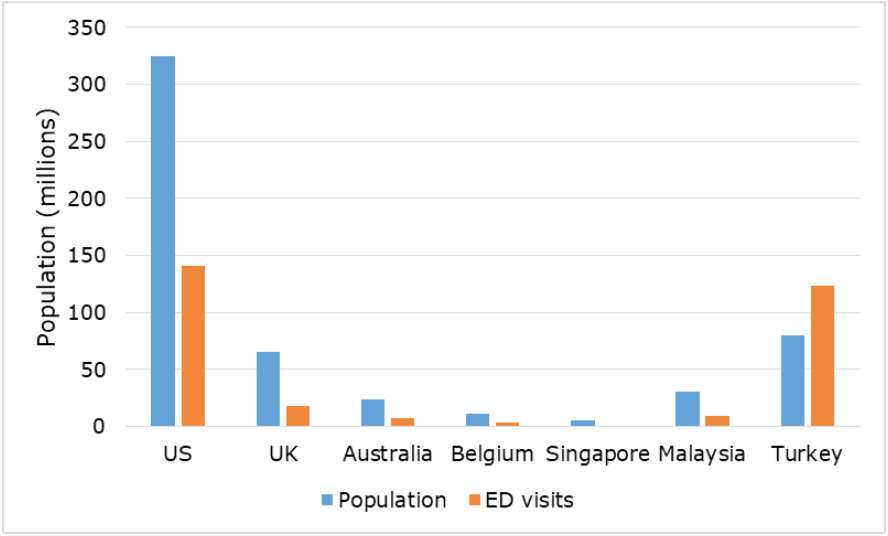

Figure 2. Population and number of ED visits per annum

Figure 2 shows the ratio of ED visits per 1000-population. Whereas Australia, Belgium, Malaysia, Singapore, the US and the UK ranged from 180 to 433 ED visits per 1000-population, Turkey reported a ratio of 1550 visits per 1000-population which shows that the number of ED visits was more than the population. These statistics show that there is a large demand for ED services in Turkey compared with the US, the UK and other high-income countries.

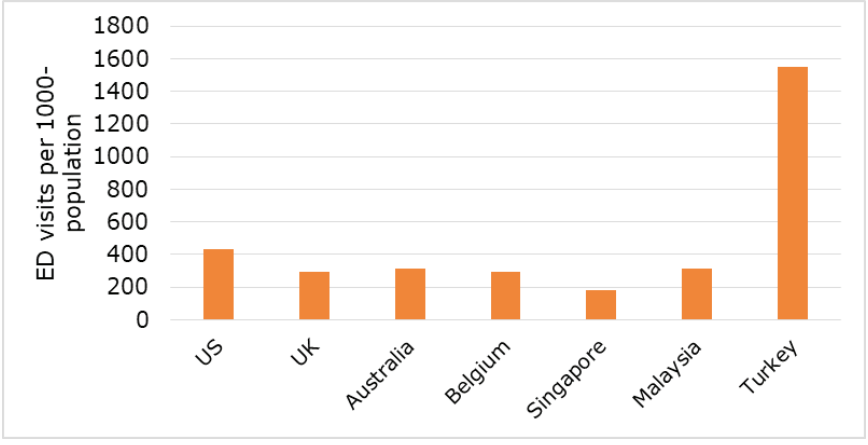

Figure 3. International comparison of ED visits per 1000-population

Morley *et al*. (2018) found that in Australia, those who live in areas of lower socio-economic status were more likely to use an ED than those who live in areas of higher socio-economic status. This could be similar at the

country level, with less-developed countries impacting on the healthcare system, as well as people's habits of using the healthcare services.

Further statistics have shown that a total of 312,255 patients were admitted to one ED in Turkey over a three-year period and 11,420 (3.6%) of them revisited the ED within twenty-four hours (Incesu *et al.*, 2016). These readmissions to the ED contribute to overcrowding.

Family health centres in Turkey are well equipped but this might not be well known by communities, especially when and in what conditions they should be visited. This could be a result of the low level of health literacy in the community or the ineffective introduction of this new way of delivering healthcare services to the public. Countries which have developed primary care systems can help to prevent those with a minor illness or a less urgent condition from attending an ED. Figure 3 shows that ED use in Turkey is significantly higher than in high-income countries. Possible explanations for this high rate of use could be not using the primary care services as the point of first contact or the unavailability of primary care services out of hours.

8. Conclusion

Taking into consideration all the factors discussed in this chapter and their implications for the EDs and the healthcare system, it is clearly seen that there is high pressure on Eds and on the professionals working in EDs. Moreover, governments spend huge budgets meeting the needs of patients who genuinely need ED services. Better understanding of the rationale of parents' attending an ED and presenting with children with minor illness might identify opportunities to reduce the number of parents who have tendency to seek to use ED services for children with minor illness.

This chapter concludes that there is a high demand for ED services and this caused many negative consequences on ED services, ED staff, and patients. Using the ED in Turkey is approximately four times more than high-income countries. Therefore, there is a need to reduce clinically unnecessary ED visits.

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Chapter 12

META-ANALYSIS FOR DETERMINING THE HIGH FEAR LEVEL SCORE OF THE FCV-19S SCALE

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1. Introduction

The novel coronavirus (2019-nCoV), which emerged in Wuhan, China in December 2019, created an inescapable and unavoidable situation and rapidly spread to the other provinces of China and from there to the whole world.(Shigemura, Ursano, Morganstein, Kurosawa, & Benedek, 2020) Currently, there are approximately 145 million confirmed COVID-19 cases and over 3 million deaths in the world. Considering that approximately 800 million people are protected by vaccination, approximately 7 billion people are still at risk worldwide.("World Health Organization (WHO). Coronavirus (COVID-19) Dashboard," (2021 accessed April 15 2021)) Quarantine measures or curfews are still implemented in many countries, based on important issues, such as the number of individuals at risk, hospital capacities, and the economy. Social isolation and loneliness, which are among the disadvantages of the implemented quarantine measures or curfews, negatively affect the mental health of individuals, leading to an increase in alcohol and drug use, self-harm, and even suicides.(Mamun & Griffiths, 2020; Ornell, Schuch, Sordi, & Kessler, 2020) In addition, it is reported that quarantine measures lead to a significant increase in domestic violence, child abuse, and sexual assault cases.(Elemo, Satici, & Griffiths, 2020)

It is a fact that infectious diseases lead todrastic emotional states, such as anger, grief, and sadness.(Reynolds et al., 2008)However, fear appears as a characteristic feature when compared with other emotional states.(Rubin & Wessely, 2020) It has been reported that fear disrupts rational thinking (reasoning) and mental health and also exacerbates pre-existing mental health disorders or leads to excessive anxiety.(Sakib et al., 2020)This fear and state of panicmay cause physical problems, such as palpitations, chest tightness, and insomnia.(Dong & Zheng, 2020) In addition to affecting mental health, fear due to the pandemic also leads to anxiety in individuals that they will infect their own relatives.(Schimmenti, Billieux, & Starcevic, 2020)These findings demonstrate the importance of accurately predicting and controlling the effects of the fear of the pandemic on individuals.

Estimating the fear of COVID-19 correctly and initiating psychiatric interventions if deemed necessary based on the results is essential primarily for the health of individuals and secondarily for public health. In this respect, some scales were developed to determine the level of fear.(Ahorsu et al., 2020; Arpaci, Karataş, & Baloğlu, 2020; Lee, 2020; Taylor et al., 2020)"Fear of COVID-19 Scale (FCV-19S)" scale, which is developed by Ahorsu et al., has been translated into over 20 languages and achieved very successful results. It is a very practical scale in terms of its implementation. A minimum of 7 and a maximum of 35 points can be achieved in the 5-point Likert scale (1: strongly disagree; 5: strongly agree), which consists of 7

items. It is reported that the level of fear increases with an increase in the obtained scalescores.(Ahorsu et al., 2020) In this regard, it has been noted that there are very few studies which aims to determine the cut-off value for the total scale score.(Doshi, Karunakar, Sukhabogi, Prasanna, & Mahajan, 2020; Nikopoulou et al., 2020) Therefore, the purpose of this study is to determine the mean value for each item of the scale based on a meta-analysis and subsequently, to determine the score corresponding to high fear level with these mean values.

2.Materials & Methods

2.1. Protocol and registration

This protocol follows the recommendations established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.(Moher, Liberati, Tetzlaff, & Altman, 2009) However, since it was a single-author study, International Prospective Register of Systematic Reviews (PROSPERO) or Cochrane Library registration could not be created.

2.2. Literature search and selection

A comprehensive systematic literature search was conducted using online databases, including PubMed, Google Scholar, ScienceDirect, MedRvix, Web of Science, and Springer to identify all FCV-19S studies from December 2019 to April 2021.Our search terms were “FCV-19S,” “The Fear of COVID-19 Scale,” “Fear of COVID-19 Scale,” and “COVID-19 Scale”. The review processes and the flow of these processes are presented in the PRISMA flowchart (Figure 1).

For the meta-analysis, we included studies which were conducted on individuals of age >18 (or mean age (± 2 standard deviation> 18)), studies which reported the mean and standard deviation for each item, and studies which were published before April 1, 2021. Studies which reported mean item values as per gender or age groups, studies which did not fully meet the required statistical arguments for meta-analysis, opinion articles and letters which did not report original data, and studies which reported cases with incomplete information were excluded.

2.3. Assessment of methodological quality

The Agency for Healthcare Research and Quality (AHRQ) scale was used to assess the quality of the cross-sectional studies included in the analysis.(Zeng et al., 2015) The AHRQ checklist includes 11 items which include the options “Yes,” “No,” or “Unclear”. “No” or “Unclear” answers are scored “0” and those answered “Yes” are scored “1”. The included studies were classified into the categories based on the total score (good

= 8–11, fair: 4–7, and poor: 0–3).(Pequeno, Cabral, Marchioni, Lima, & Lyra, 2020)

2.4. Statistical approach

Studies included in the meta-analysis were described using the number of observations, mean and standard deviation of items, and quality score statistics. For studies that did not precisely report age groups in the eligibility criteria, the final decision was made after performing the necessary analysis.

Meta-analysis was conducted using the R v3.6.1 packages "meta v4.10-0" and "metafor v2.1-0." The overall mean obtained from studies reporting a single mean was calculated using the inverse variance method for pooling. This process was performed by taking the log transformation of the individual mean in each study to minimize errors.(Higgins, White, & Anzures-Cabrera, 2008)All meta-analyses were performed using random effect models. Cochran's Q, I^2,and H^2 statistics were used to determine the heterogeneity between studies. Publication bias was evaluated by visual inspection of the asymmetry of funnel plots.In case publication bias was indicated, we performed the trim-and-fill method to assess the possible effect of publication bias.(Duval & Tweedie, 2000) Forest plots were used for visual representation of the results.

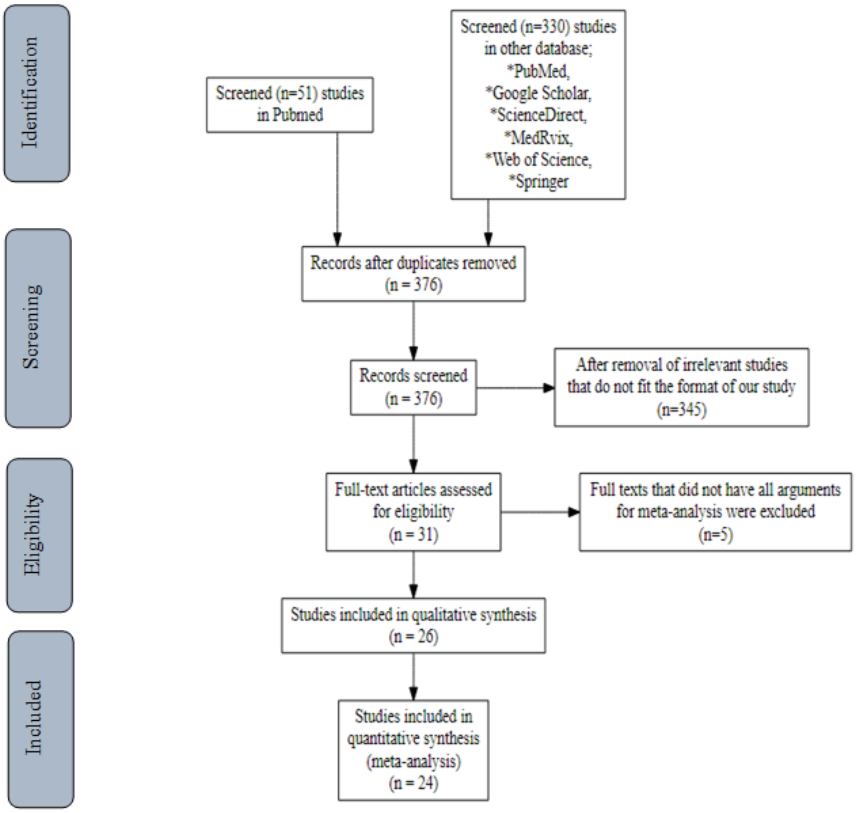

Figure 1: *Study selection and characteristics.*

3. Results

The meta-analysis was conducted with a total of 24 studies (20 orginal article, 1 review article, 3 brief report) consisting 44804 individuals, all of which were cross-sectional (online).(Alyami, Henning, Krägeloh, & Alyami, 2020; Cavalheiro & Sticca, 2020; Caycho-Rodríguez et al., 2021; Caycho-Rodríguez et al., 2020; Chang, Hou, Pakpour, Lin, & Griffiths, 2020; Doshi et al., 2020; Elemo et al., 2020; R. C. Giordani, Giolo, Muhl, & Zanoni da Silva, 2020; R. C. F. Giordani, Zanoni da Silva, Muhl, & Giolo, 2020; Magano, Vidal, Sousa, Dinis, & Leite, 2021; Mahmood, Jafree, & Qureshi, 2020; Martínez-Lorca, Martínez-Lorca, Criado-Álvarez, Armesilla, & Latorre, 2020; Pang et al., 2020; Perz, Lang, & Harrington, 2020; Rahman et al., 2021; Sakib et al., 2020; Satici, Gocet-Tekin, Deniz, & Satici, 2020; Soares, Afonso, Martins, Pakpour, & Rosa, 2021; Soraci et al., 2020; Stănculescu, 2021; Tzur Bitan et al., 2020; Warren et al., 2021; Winter et al., 2020; Zolotov, Reznik, Bender, & Isralowitz, 2020) However, since there were seven different results in one study and two

different results in another, our resultsreflected that of a meta-analysis of 31 studies instead of 24(Table I). The minimum age of all participants was 18. The AHRQ score of the studies was a minimum of 5, a maximum of 10, and an average of 6.6.

For item 1, the values of I^2= 99.7% and H^2 = 308were found in the calculations made with trim fill. However, crude calculations (Q = 4332.2, $p < 0.001$, I^2 = 99.4%, and H^2 = 180.6) yielded results with a higher performance. Using the random effect model,the mean value for Item 1 was found to be 3.19 [95% CI:3.05–3.33](Figure 2A).For Item 2, it was found that the mean value was 3.33 [95% CI: 3.25–3.42] using a random effect model, and with the trim fill method we obtained Q = 2456.9, $p <0.001$, I^2 = 98.5%, and H^2 = 68.2 (Figure 2B). Based on the crude results for Item 3 (Q = 3427, $p < 0.001$, I^2 = 99.3%, and H^2 = 149.1), the mean value was found to be 1.76 [95% CI: 1.65–1.88] using the random effect model (Figure 2C). Based on the results of the trim fill method for Item 4 (Q = 3692.6, $p < 0.001$, I^2 = 99.2%, and H^2 = 119), the mean value was found to be 2.93 [95% CI: 2.81–3.06] using a random effect model (Figure 2D). Based on the crude results for Item 5 (Q = 4458.7, $p < 0.001$, I^2 = 99.5%, and H^2 = 185.7), the mean value was found to be 3.00 [95% CI: 2.86–3.15] using a random effect model (Figure 2E). Based on the crude results for Item 6 (Q = 1436.2, $p < 0.001$, I^2 = 98.0%, and H^2 = 65.2), the mean value was found to be 1.81 [95% CI: 1.72–1.90] using a random effect model (Figure 2F). Based on the crude results for Item 7 (Q = 1436.26, $p < 0.001$, I^2= 98.0%, and H^2 = 55.8), the mean value was found to be 2.09 [95% CI: 2.00–2.17] using a random effect model (Figure 2G).

While analyzing each item, some studies were excluded from the analysis as they were of an extreme value based on the funnel plot. Overall, the excluded studies were Study ID: 6, 8, 14, 24, and study 1 and 7 by Caycho-Rodríguez et al.

Based on the combination of all these results, we obtained a total score of 18.11 for the seven items of FCV-19S. Therefore, the fact that the total score obtained from FCV-19S is above 18.11 indicates the fear of COVID-19 is higher than the average.

Table I: Statistics of studies included in meta-analysis

Study ID	Author	N	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Quality[a]
1	Satici et al.	1304	3.5 (1)	3.6 (1.1)	1.8 (0.9)	3.1 (1.3)	3.7 (1.1)	2 (1)	2.4 (1.2)	6
2	Bitan et al.	649	3 (1.2)	3.2 (1.1)	1.6 (0.7)	2.2 (1.1)	2.9 (1.1)	1.7 (0.9)	1.8 (0.9)	6
3	Alyami et al.	639	3 (1.2)	3.3 (1.1)	1.7 (0.9)	2.4 (1.2)	3 (1.2)	1.7 (0.9)	1.9 (1.1)	6
4	Sakib et al.	8550	3.6 (1)	3.5 (1.1)	2.5 (1.1)	2.9 (1.2)	3.5 (1.1)	2.4 (1.1)	2.9 (1.2)	6
5	Magano et al.	1122	3.2 (1.1)	3 (1.1)	1.7 (0.8)	2.7 (1.2)	2.8 (1.1)	1.8 (0.9)	2 (1.1)	7
6	Elena Stănculescu	809	2.6 (1.2)	2.9 (1.3)	1.8 (1)	1.7 (1)	2.4 (1.2)	1.4 (0.8)	1.5 (0.9)	6
7	Martínez-Lorca et al.	606	2.9 (1.1)	3 (1.2)	1.7 (1)	2.4 (1.3)	3 (1.3)	1.8 (1)	2 (1.1)	5
8	Elemo et al.	307	4.2 (0.9)	4 (1)	2.3 (1)	2.9 (1.2)	3.2 (1.2)	2.2 (1.1)	2.5 (1.1)	7
9	Pang et al.	228	3.5 (1.1)	3.2 (1.2)	1.8 (1)	3.4 (1.2)	2.8 (1.2)	1.8 (1)	2 (1.1)	5
10	Perz et al.	237	3.2 (1.2)	3 (1.3)	1.9 (1.1)	2.7 (1.4)	3.1 (1.4)	2 (1.1)	2.3 (1.3)	5
11	Winter et al.	1397	3.1 (1.7)	2.9 (1.7)	1.6 (1)	2.2 (1.5)	2.7 (1.7)	1.6 (1.1)	1.6 (1.1)	7
		1023	3.6 (1.7)	3.5 (1.8)	1.7 (1)	2.4 (1.6)	3.3 (1.8)	1.9 (1.2)	1.9 (1.3)	
12	Mahmood et al.	501	3.1 (1.1)	3.3 (1.1)	2 (0.9)	2.5 (1.1)	3.4 (1.1)	2 (0.9)	2.3 (1.1)	6
13	Cavalheiro et al.	354	2.8 (1.3)	3 (1.3)	1.5 (0.8)	2.5 (1.3)	2.7 (1.2)	1.6 (0.9)	1.7 (1.1)	6
14	Caycho-Rodríguez et al.	1291	2.6 (1.3)	2.7 (1.4)	1.3 (0.8)	1.7 (1.2)	2.2 (1.3)	1.3 (0.8)	1.5 (1)	7
15	Chang et al.	400	3 (1.3)	2.8 (1.3)	2.4 (1.1)	2.8 (1.3)	2.7 (1.2)	2.4 (1.1)	2.4 (1.6)	9
16	Doshi et al.	1499	3.2 (1.1)	3.1 (1.1)	2.2 (1)	2.5 (1.2)	2.9 (1.2)	2 (1)	2.1 (1)	10
17	Soares et al.	1203	2.8 (0.3)	3.1 (0.3)	1.4 (0.2)	2.6 (0.4)	2.7 (0.3)	1.5 (0.4)	1.9 (0.3)	7
18	Rahman et al.	516	3.4 (1.2)	3.2 (1.3)	1.9 (1.2)	2.7 (1.4)	3.3 (1.3)	1.9 (1.1)	2 (1.2)	8
		1605	2.6 (1.3)	2.7 (1.4)	1.3 (0.8)	1.8 (1.2)	2.3 (1.3)	1.3 (0.8)	1.5 (1)	
		311	2.9 (1.3)	3.1 (1.4)	1.5 (1)	2.4 (1.5)	2.4 (1.4)	1.6 (1)	1.9 (1.2)	
		739	3.2 (1.3)	3.1 (1.3)	1.9 (1.2)	3 (1.4)	2.6 (1.3)	1.9 (1.2)	2.2 (1.3)	
19	Caycho-Rodríguez et al.	326	3.1 (1.4)	3 (1.5)	1.8 (1.2)	3 (1.5)	2.6 (1.4)	1.8 (1.2)	2.2 (1.4)	8
		850	3 (1.3)	3 (1.4)	1.7 (1.1)	2.8 (1.5)	2.6 (1.4)	1.7 (1.1)	2.1 (1.3)	
		258	3 (1.3)	3.2 (1.4)	1.6 (1.1)	2.4 (1.4)	2.5 (1.4)	1.6 (1)	1.9 (1.2)	
		370	2.2 (1.2)	2.6 (1.4)	1.2 (0.7)	1.6 (1.1)	1.9 (1.2)	1.2 (0.6)	1.4 (0.9)	
20	Soraci et al.	249	3.4 (1.1)	2.9 (1.3)	1.5 (0.9)	2.4 (1.3)	2.9 (1.3)	1.6 (0.9)	2.1 (1.3)	7
21	Zolotov et al.	370	3.1 (1)	3 (1.2)	1.3 (0.7)	1.6 (0.8)	2.9 (1.2)	1.4 (0.8)	1.6 (0.9)	5
22	Giordani et al.	7430	3.6 (1)	3.5 (1)	2 (0.9)	3.3 (1.1)	3.3 (1.1)	2 (1)	2.2 (1.1)	7
23	Giordani et al.	4638	3.6 (1)	3.5 (1)	1.9 (0.9)	3.2 (1.1)	3.3 (1.1)	2 (0.9)	2.2 (1)	6
24	Warren et al.	5023	4.3 (1.8)	4 (1.8)	2.4 (1.6)	3.3 (1.9)	3.7 (1.9)	2.5 (1.6)	2.4 (1.6)	8

Values for each item are expressed in mean (sd).[a]:Articles are classified into the categories based on the total score: good˜8-11, fair˜4-7, and poor˜0-3 in AHRQ quality scoring.

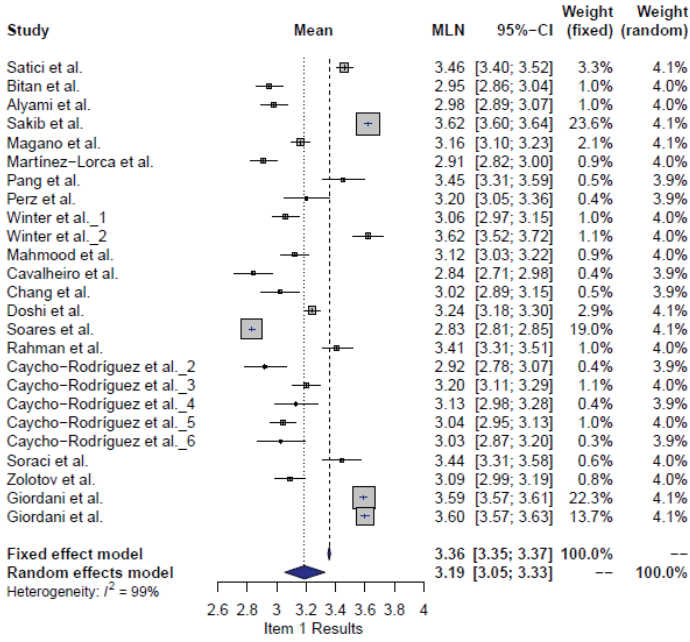

Figure 2A: *Results of Item 1*

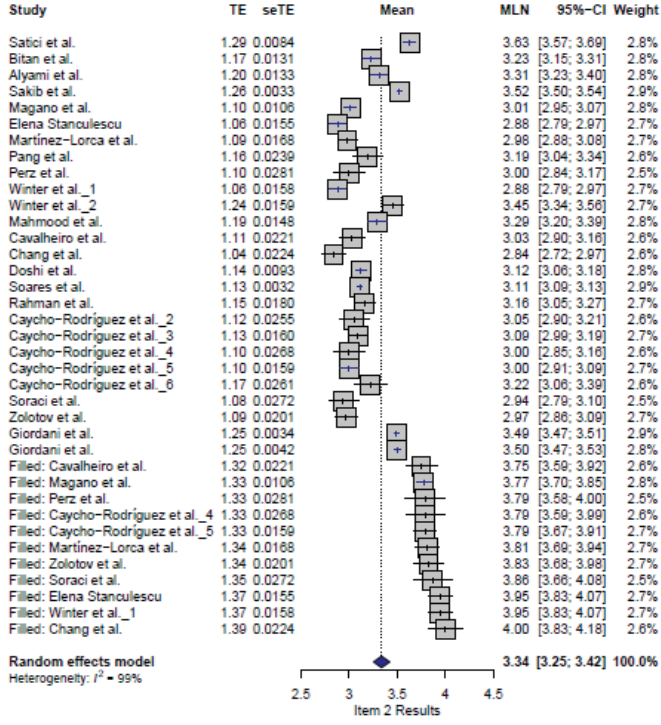

Figure 2B: *Results of Item 2*

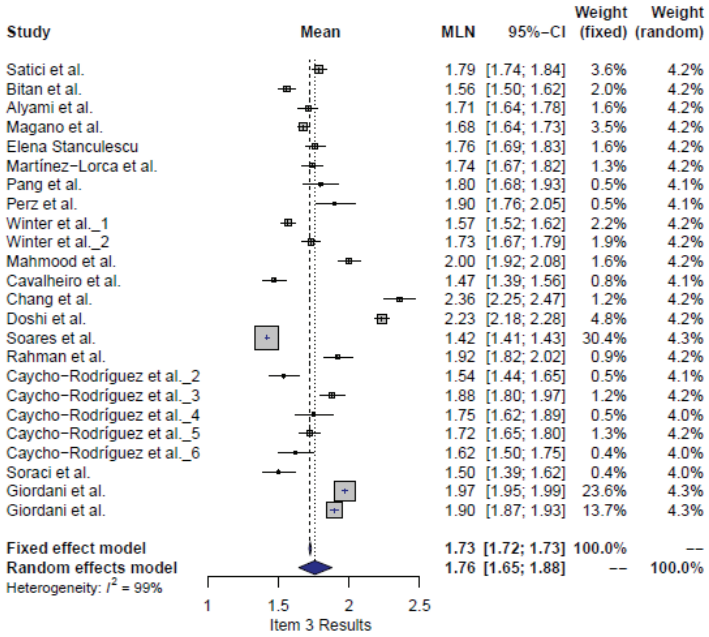

Figure 2C: *Results of Item 3*

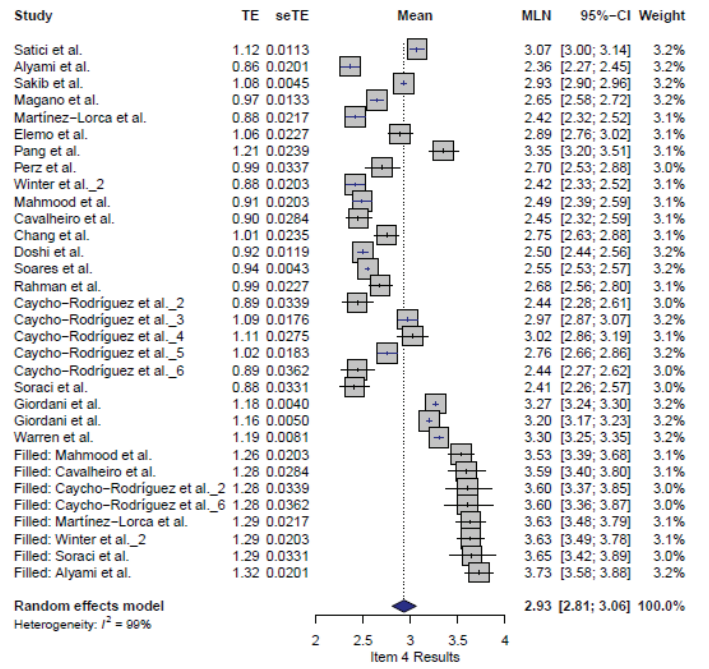

Figure 2D: *Results of Item 4*

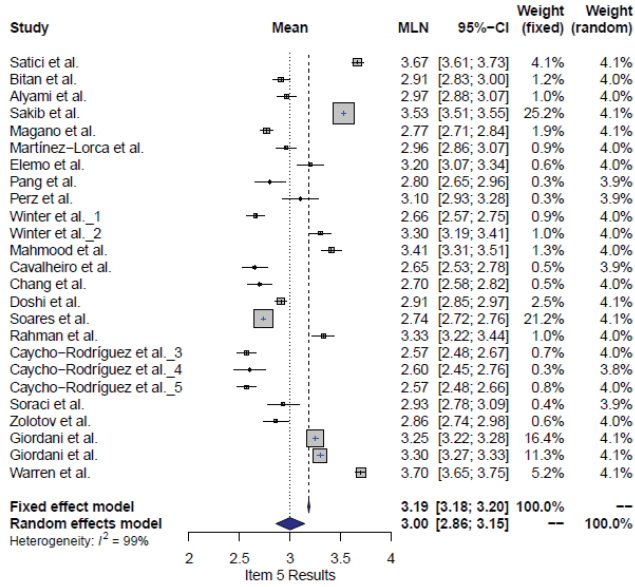

Figure 2E: Results of Item 5

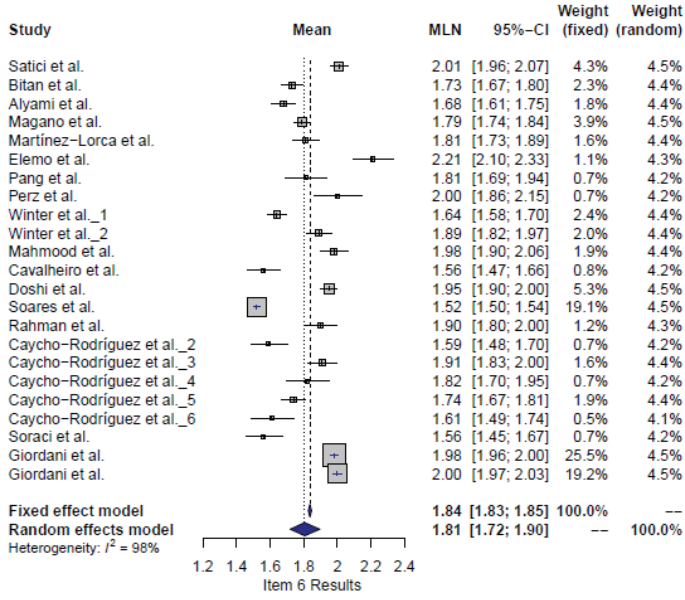

Figure 2F: *Results of Item 6*

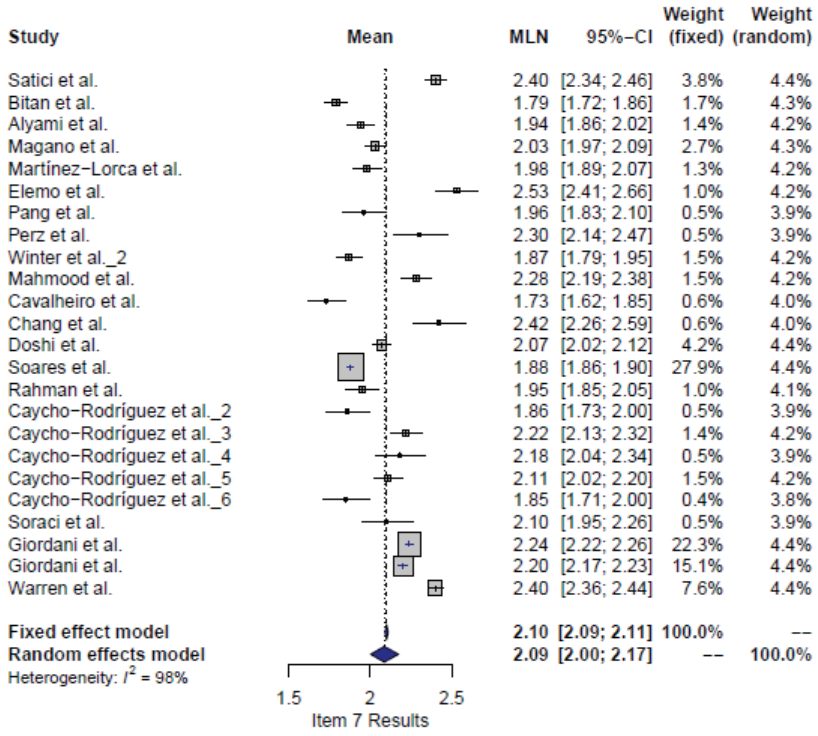

Figure 2G: *Results of Item 7*

4. Discussion

Recently, we are observing subsequent waves of the pandemic; many countries are experiencing the third wave, whereas some are experiencing the fourth. Approximately 55 thousand cases are identified in Turkey per day, and approximately four million cases worldwide have been identified last week.("T.C. Ministry of Health. Republic of Turkey Ministry Covid-19 Information Page," (2021 accessed April 15 2021); "World Health Organization (WHO). Coronavirus (COVID-19) Dashboard," (2021 accessed April 15 2021)) All these issues lead to anxiety and panic, which increases the fear of COVID-19. Therefore, determining the level of fear analyzed in the study has become crucial. As a result we performeda meta-analysis consisting 44804 individuals in order to determine the potential danger level of the score obtained from the FCV-19S.The mean values obtained were 3.19 for Item 1, 3.33 for Item 2, 2.93 for Item 4, 3.00 for Item 5, 1.81 for Item 6, and 2.09 for Item 7, and the total scale score was 18.11.

The FCV-19S was translated into many languages; however, it was reported that the minimum score of the scale was 7 points and the maximum score was 35 points.The level of fear increased as the score obtained from the scale increased, and a clear index could not be reported. (Ahorsu et al., 2020) A small number of studies to determine the index have been carried out in only one place. Hence, it was deemed necessary to perform an extensive analysis for the scale in question. Therefore, we attempted to determine the critical value for each item and finally for the total scale score.

Statistics such as a single mean, ratio, or correlation may sometimes avoid many uncertainties. Thus Lyssenko et al. examined the dissociation in psychiatric disorders in their study and determined the mean dissociation score in dissociative disorders followed by posttraumatic stress disorder, borderline personality disorder, and conversion disorder using the mean values obtained from previous meta-analyses.(Lyssenko et al., 2018) In another study, Meseguer-Henarejos et al. determined the mean of internal consistency and intra and interrater reliability for the Berg Balance scale in clinical, nonclinical, and mixed populations.(Meseguer-Henarejos, Rubio-Aparicio, López-Pina, Carles-Hernández, & Gómez-Conesa, 2019) Tomaszewski et al. used a meta-analysis for a single mean to avoid variation in morphometric measurements of sciatic nerves and found that they could manage concerns in this area.(Tomaszewski et al., 2016) Li et al. performed a single rate meta-analysis for studies which included clinical data, clinical symptoms, and laboratory tests for COVID-19 and found results that could help clinical-epidemic prevention and control of the disease.(Li et al., 2020)

In order to determine the cut-off value for the FCV-19S, a cross-sectional (online) study on 538 individuals was conducted by Nikopoulou et al.(Nikopoulou et al., 2020) In the mentioned study, it was reported that a total scale score of 16.15 and above was significant in terms of anxiety, health anxiety, and posttraumatic stress symptoms. The cut-off score was calculated using receiver operating characteristic curve by categorizing the scores obtained from the Generalized Anxiety Disorder 7-item (GAD-7) and the Short Health Anxiety Inventory (SHAI) scale.(Salkovskis, Rimes, Warwick, & Clark, 2002; Spitzer, Kroenke, Williams, & Löwe, 2006) Therefore, the risks of FCV-19S scale were reported using the risks in these scales (cut-off point of 10 for GAD scale and cut-off point of 18 for SHAI). However, in the present study, the value that could pose a risk (18.11) was determined using only the FCV-19S scale scores.

Doshi et al. conducted a study with 1499 people to determine the cut-off score for FCV-19S (Doshi et al., 2020) In the mentioned study, the mean score obtained from the scale was used for determining the score,

and the cut-off value was found to be 18 ± 5.68. The score from the present study is consistent with the study by Doshi et al.

Anxiety disorders, which become more pronounced in times of contagious pandemics and crises, negatively affect the mental health of individuals.(Pappas, Kiriaze, Giannakis, & Falagas, 2009) Compared to other scales, in addition to directly determining the fear of COVID-19, the FCV-19Scan help alleviate the fear of individuals by emphasizing a negative relationship in the context of resilience, happiness, and fear using a positive psychology approach and can help reduce the stigma, anxiety, and stress associated with COVID-19(Ahorsu et al., 2020; Stănculescu, 2021).

5. Conclusion

The COVID-19 virus, which has caused a pandemic, infects new people every day, and some of these people die as a result. This results in fear for all humanity. Therefore, it is necessary to determine the level of fear and situations that may lead to danger. Based on the results of our study, scores above 18.11 on the FCV-19S scale indicate that the level of fear is high.

Limitations

The first limitation was the high I^2 value. This may have resulted due to the useof results from many different countries for performing the meta-analysis. However, some methods were used to prevent this, e.g.,a few studies, especially those with an extreme value, were excluded from the analysis. Another limitation was that fear scores were not based on gender and age groups. The final limitation was that the PROSPERO/Cochrane record could not be created, since this was a single-author study.

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Chapter 13

PROTON PUMP INHIBITORS AND THE OSSEOINTEGRATION

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Introductıon

Dental implants are accepted as one of the most important innovations in dentistry. Although the replacement of missing teeth is an effective and predictable treatment method in patients with full or partial dental loss as it provides, comfort, aesthetics, function and stability, failures may still occur (Chrcanovic et al., 2017).

The survival of osseointegrated dental implants depends on the success of osseointegration, which is the direct structural and functional connection between the live bone and the dental implant surface. Therefore, bone metabolism, which includes bone formation and re-modelling, plays a very important role in the success of osseointegration. Several factors are thought to affect bone metabolism and therefore, osseointegration. In addition to the implant properties and local factors, it has been suggested that age, gender, smoking, systemic diseases, and drugs used could affect bone metabolism and osseointegration (Wu et al., 2017).

The concept of osseointegration

The scientific basis of current implantology was formed by Per-Ingvar Brånemark. In the 1950s, in studies conducted by Brånemark of the microcirculation in rabbit bones, it was discovered that chambers made of titanium fused permanently with the bone. The fusion of live bone with implants with a layer of titanium oxide is so great that they cannot be separated from each other. Therefore, Brånemark used the term "osseointegration" to describe this stable fixation between titanium and bone tissue (Guglielmotti et al., 2019; Brånemark, 2005).

Osseointegration was initially described as the direct structural and functional bond between the regular live bone and the weight-bearing implant surface. When there is direct contact between the implant and the bone, the implant is said to be accepted as osseointegrated when there is no progressive movement. Although the term of osseointegration was initially used for titianium metal implants, it is now used for all biomaterials that have the capacity for osseointegration, such as ceramics (Guglielmotti et al., 2019).

Brånemark defined osseointegration again in different ways from different viewpoints:

From the perspective of the patient, the implant, which does not show loosening and does not cause inflammation or pain under functional forces, provides stable support for the prosthesis.

In terms of macroscopic and microscopic biology, it provides a direct structural and functional bond, without showing deformation under normal

physiological forces or stimulating rejection mechanisms. Under light microscope examination, no connective tissue or fibrous tissue should be found between the implant surface and the newly formed bone.

In terms of macroscopic biomechanics, bone in the region which is osseointegrated should not show an increasing or changing amount of movement compared to bone marrow under different functional loading. The deformation shown by an implant to a certain severity of loading throughout the lifetime of an individual means that the implant is osseointegrated at that rate.

In a microscopic biophysical aspect, there is coverage of most of the implant surface by mineralised bone tissue under light and electron microscopy and therefore no other material which can impair the bond can come between the bone/bone marrow- implant bond (Brånemark et al.2001; Aydın, 2017).

When a defect occurs in the existing bone matrix, direct bone healing or the osseointegration process are activated. After surgical placement of the implant into the prepared implant bed, a series of vascular and immunological events occur, followed by the formation of new bone over time.

After the onset of osseointegration, the process continues in 3 steps;

1. The formation of woven bone

2. Bone deposition formed from lamellar and parallel fibres

3. Bone remodelling (Aydın, 2017).

Local factors affecting osseointegration

a. Bone quality, density, and amount

b. Implant design, shape, and surface properties

c. Properties of the implant material and sterility

d. Prevention of excess heat during drilling

e. Dimensions (vertical and horizontal) of the alveolar crest

f. Prevention of migration of fibrous tissue

g. Provision of primary stabilisation (Newman et al.,2011)

Systemic factors affecting osseointegration

a. Age: various changes associated with ageing seen in the trabecular structure of bone can have a negative effect on osseointegration (Aydın, 2017).

b. Smoking: smoking can have a negative effect on wound healing following intra-oral surgical procedures, and it has been reported that it can increase the risk of surrounding bone loss and implant failure (Aydın, 2017)

c. Systemic diseases and drugs used

- Diabetes mellitus
- Osteoporosis
- HIV
- Cardiovascular diseases and anti-hypertensive drugs
- Neurologial diseases
- Hypothyroidism
- Rheumatoid arthritis
- SSRI drugs
- Proton pump inhibitors (PPI) (Aghaloo et al., 2019)

PPIs

By inhibiting the proton pump (H+/K+ ATPase) functions, PPIs suppress stomach acidity. PPIs are used for the prevention and treatment of acid-related gastrointestinal diseases such as oesophageal, duodenal, and gastric ulcers, ulcers related to the use of non-steroid anti-inflammatory drugs (NSAID), stress-related gastritis, dyspepsia, gastro-oesophageal reflux disease (GERD), and Zollinger-Ellison syndrome. They are also used in combination with antibiotics to eliminate Helicobacter pylori, a bacterium causing stomach and duodenum ulcers of acid origin (Wu et al., 2017; Aghaloo et al., 2019).

It has been said that long-term use of PPIs has some potentially harmful effects. It is thought in particular that the long-term inhibition of acid secretion has potential effects on the absorption of vitamins and minerals. With the inhibition of stomach acid secretion, the absorption of several nutrients, drugs and vitamins could be affected associated with low pH, primarily vitamin B12, calcium, iron, and magnesium (Aghaloo et al.,2019; Özdemir&Okuroğlu, 2015).

The effect of PPIs on bone and calcium metabolism:

Calcium is a mineral found in bone and absorption occurs in the small intestine dependent on pH. By impairing intestinal calcium absorption, PPIs can cause serum and urine calcium levels to fall, and therefore, PPIs could impair bone metabolism. The acidic environment in the gastrointestinal

system facilitates the expression of ionised calcium from undissolved calcium salts. In this sense, PPIs decrease the calcium present by increasing the pH in the small intestine. This results in a negative calcium balance leading to bone mineral loss and can affect bone health and osseointegrated dental implants (Wu et al., 2017; O'Connell et al., 2005).

The pH balance impaired by the use of PPIs affects calcium absorption. Calcium malabsorption and low amounts of calcium intake have been shown to be among the causes of secondary hyperparathyroidism. Varying calcium levels associated with hypergastrenemia can have a negative effect on bone metabolism by inducing hyperplasia and hypertrophy of parathyroid glands. This can cause an increase in parathyroid hormone (PTH) levels. A continuously increasing PTH level related to the calcium serum concentration may cause losses in bone strength and quality (Yang et al., 2006; Wu et al., 2017).

Specifically, PPIs suppress gastric acid secretion by selectively and irreversibly inhibiting the H1/K1 ATPase found in the membrane of parietal stomach cells. In addition to the digestive system, H1/K1 ATPase can also be found in other tissues such as bone. Proton pump osteoclasts have also been found to be located in plasma membrane. It has been reported that osteoclast activity can be inhibited by PPIs having an effect on the vacuolar H+-ATPase (V-ATPase) of osteoclasts. Another effect of PPIs on bone metabolism is the decrease in osteoclastic differentiation mediated by osteoblastic cells. Furthermore, PPIs can show an effect on osteoblastic matrix mineralisation by inhibiting phosphoethanol-amine/phosphocholine phosphatase (PHOSPHO1) in bone matrix vesicles and tissue non-specific alkaline phosphatase (ALP) (Wu et al., 2017; Roberts et al., 2007).

By decreasing endosteal width growth and increasing osteoid width, and proportionally decreasing the bone mineral content of the bone mass, PPIs prevent bone formation and impair bone mineralisation. This is most likely related to PPIs reducing the expression of bone formation markers such as bone morphogenetic protein (BMP) 22,, BMP-4 and cysteine rich protein (CRR)-61 (Histing et al., 2012). Systemic PPI intake may cause a reduction in density, weight, cortical thickness, and mineral content of bone, in addition to the biomechanical properties (Altay et al, 2019). In an experimental study of the long-term use of PPIs, the mechanical properties of rat femurs were not seen to change, but in the rats applied with 300 mol/kg/day omeprazole, bone demineralisation was observed in the rat femurs and this suggested a susceptibility to bone fractures (Yanagihara et al., 2015).

The relationship between PPI use and bone metabolism has been

accepted by the USA Food and Drug Administration (FDA). This shows that bone mineral density (BMD) is affected by PPIs affecting calcium homeostasis and impairing calcium absorption. The effect of PPIs on BMD is a matter of debate. In some studies, no difference has been observed in BMD between subjects using and not using PPIs, while others have shown a significant decrease in BMD and trabecular volume BMD in those using PPIs (Maggio et al., 2014; Wu et al., 2017).

Some studies have stated that there could be an association between long-term PPI use and an increased risk of fracture (Ito & Jensen, 2010; Yang et al., 2006; Targownik et al., 2008; Vestergaard et al., 2006). In a meta-analysis by Zhou et al, it was reported that long-term PPI use increased the risk of fracture in any region of the body by 33%, increased hip fracture risk by 26%, and shoulder fracture risk by 58% (Zhou et al., 2016). Other studies have determined an increased risk of fracture in postmenopausal females using PPIs for more than one year (Lewis et al., 2014; Kim et al., 2020). In contrast to the theory related to osteoclasts, there are also studies which have reported that PPIs increase bone density by reducing bone resorption, could prevent osteoporosis, and will not increase the risk of fracture (Targownik et al., 2017; Hoff et al., 2020).

The effects of PPIs on osseointegration

Despite the negative effects of PPIs on bone, there are few studies related to the effects on osseointegration and dental implants. It is known that there are rates of widepread and long-term-use of PPIs in patients planned to receive implants. To obtain the highest level of current information on this subject, it is of great importance that the most recent literature is evaluated.

There is a direct association between implant success and the contact between bone and implant. In an in-vivo study by Al Subaie et al, the postoperative administration of systemic omeprazole in rats was reported to reduce osseointegration by approximately 50%, and compared with the control group, the rats treated with omeprazole had lower peri-implant bone volume/tissue volume and a lower percentage of bone-implant contact (Al Subaie et al., 2016).

In another study, the effect on osseointegration of different levels of omeprazole was evaluated, and no statistically significant difference was determined between the groups in biochemical and biomechanical terms (Tekin et al., 2021).

In a cohort study by Wu et al, the failure rates were evaluated of 1733 osseointegrated dental implants in 799 patients. The failure rates were found to be 6.8% in patients using PPIs and 3.2% in those not using PPIs.

In another study of 3599 implants in 999 patients, the failure rates were found to be 12% in those using and 4.5% in those not using PPIs. From these findings it has been reported that PPI use could be associated with the risk of failure of osseointegrated dental implants (Wu et al., 2017; Chrcanovic et al., 2017).

In a study that examined the association between systemic PPI intake and early implant failure, the implant failure rate of patients using PPIs was found to be 4.9-fold higher than that of patients not taking PPIs, and it was therefore concluded that PPI use could be associated with early implant failure (Altay et al.,2019).

In the International Team Implantology (ITI) Consensus Report published in 2018 (Jung et al., 2018) it was stated that;

1) The limited evidence related to the effects of short and long-term drug use on dental implant treatment outcomes shows that there may be an association between the intake of some drugs that affect bone metabolism and the implant failure rate.

2) The increase in the implant failure rate with the use of PPIs is statistically significant.

Conclusion

Dental implants are of great importance in dentistry in respect of being able to provide function, aesthetics, and comfort, lost because of missing teeth. There are several local and systemic factors that affect the osseointegration and early and late success of dental implants. The use of systemic drugs is evaluated as one of these factors.

PPIs are one of the most widely prescribed drugs throughout the world. It is predicted that the long-term use of PPIs can affect bone metabolism through various mechanisms, and this negative effect can be proven in many studies that have been conducted on bone metabolism. However, very few studies have examined the effects of PPIs on the osseointegration and success of dental implants.

In conclusion, the changes that can occur in calcium and bone metabolism and in the bone structure of patients using PPIs should be evaluated as risk factors than can negatively affect the osseointegration and success of dental implants. When these risks are taken into consideration, the need for long-term use of PPIs should be questioned, and patients with long-term use must be evaluated in respect of the risk and must be followed up.

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