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CONTENTS / İÇİNDEKİLER

CONTENTS / İÇİNDEKİLER

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1277673

Duzce Med J, 2023;25(2):103-114
Düzce Tıp Fak Derg, 2023;25(2):103-114

Post-Traumatic Stress Disorder After Natural Disasters: A Review

Doğal Afetler sonrası Travma Sonrası Stres Bozukluğu: Bir Gözden Geçirme

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ABSTRACT

Natural disasters, which are considered as one of the most important problems of recent times, affecting all humanity, have become a public health problem. Disasters have effects on both individuals and society in different dimensions, including health, economic, social, and psychological, and can have serious negative consequences. The number of individuals affected by natural disasters is increasing every year. While it is clear that natural disasters threaten human life and bodily health, little attention has been paid to their effects on mental health. Natural disasters threaten our psychological well-being in many ways, cause both short-term and long-term psychological distress and create a significant psychological burden. Post-traumatic stress disorder (PTSD) is a syndrome that occurs as a result of a serious threat or physical injury, a near-death experience, war-related trauma, sexual assault, interpersonal conflicts, child abuse, or a medical illness. When the literature is examined comprehensively, it has been determined that the incidence of PTSD after natural disasters is quite high. Causes of post-disaster PTSD include the nature of the trauma, its severity, loss of relatives and/or property, poor coping skills, displacement, and direct exposure to disaster. Studies have shown that both non-pharmacological and pharmacological treatments are effective on PTSD.
Keywords: Natural disasters; earthquake; flood; hurricane; post-traumatic stress disorder; treatment.

ÖZ

Son zamanların en önemli sorunları arasında değerlendirilen, tüm insanlığı etkileyen doğal afetler bir halk sağlığı sorunu haline gelmiştir. Afetlerin hem birey hem de toplum üzerinde sağlık, ekonomik, sosyal ve psikolojik olmak üzere farklı boyutlarda etkileri olmaktadır ve ciddi olumsuz sonuçlara ulaşabilmektedir. Her geçen yıl doğal afetlerden etkilenen birey sayısı da artmaktadır. Doğal afetlerin insan yaşamını ve beden sağlığını tehdit ettiği açık olmakla birlikte ruh sağlığı üzerindeki etkilerine çok az dikkat edilmiştir. Doğal afetler birçok yönden psikolojik iyi oluşumuzu tehdit etmekte hem kısa hem de uzun vadeli psikolojik sıkıntılara yol açabilmekte ve önemli bir psikolojik yük oluşturabilmektedir. Travma sonrası stres bozukluğu (TSSB) ciddi bir tehdit veya fiziksel yaralanma, ölüme yakın bir deneyim, savaşla ilgili travma, cinsel saldırı, kişilerarası çatışmalar, çocuk istismarı veya tıbbi bir hastalığın sonucu olarak ortaya çıkan bir sendromdur. Literatür kapsamlı olarak incelendiğinde doğal afetler sonrası TSSB görülme oranının oldukça fazla olduğu saptanmıştır. Doğal afet sonrası TSSB nedenleri arasında travmanın doğası, şiddeti, bireyin yakınını ve/veya mülkünü kaybetmesi, zayıf baş etme becerisi, yerinden edilmesi ve doğrudan afete maruz kalması gibi faktörler sayılabilir. Araştırmalar hem farmakolojik olmayan hem de farmakolojik tedavinin TSSB üzerinde etkili olduğunu göstermiştir.
Anahtar kelimeler: Doğal afetler; deprem; sel; kasırga; travma sonrası stres bozukluğu; tedavi.

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INTRODUCTION

Natural disasters are large-scale adverse events resulting from both geological and meteorological natural processes of the earth. Hurricanes, severe storms, earthquakes, volcanic eruptions, fires, floods, tsunamis, and drought are some examples of natural disasters. Natural disasters are often associated with death, trauma, and loss of housing. According to the International Disaster Database, the number of natural disaster events recorded in the world has increased rapidly after the middle of the 20th century (1). Although traumatic events have different characteristics, the main common feature is the psychological and physical effects on people. The difference between natural disasters and other traumatic events is that they create a mass trauma effect. While natural disasters affect large groups of people, they also negatively affect social services, communication, and social networks. The most important effects are that it causes physical and mental health problems in society (2). In this review, the effects of natural disasters on mental health and their relationship with post-traumatic stress disorder (PTSD) were discussed.

POST-TRAUMATIC STRESS DISORDER (PTSD)

Trauma is generally used for all kinds of events and situations that harm, hurt, and injure the mental and physical state of people in very different degrees. Trauma and trigger factor-related disorders were evaluated in a broad category within mental illnesses (3). According to version 5 of the Diagnostic and Statistical Manual of Mental Disorders (DSM), trauma and stress-related diseases; consists of acute stress disorder, PTSD, reactive attachment disorders, adjustment disorders, unrestricted social participation disorder, other defined trauma and stressor-related disorders, and unspecified stressor-related disorders (4).

History

PTSD is a syndrome that occurs after exposure to a traumatic event and manifests itself with symptoms of re-experiencing, avoidance, blunting, alienation, and hyperarousal. Throughout history, people have faced the risk of being exposed to trauma and traumatic events. The animal attacks that happened to our ancestors and the terrorist attacks that people are frequently exposed to probably produce similar psychological states. Considering all these, it can be said that PTSD has existed throughout human history (5). The first studies on PTSD were made as a result of the wars in the world. Even looking at the works written 2000 years ago, there are articles about war stress. One of the first examples that can be given to these works is the story of Herodotus's Marathon War in the fifth century in Ancient Greece. Ancient stories of war trauma are also found in the poem De Rerum Natura by Hippocrates and Lucretius. Later, PTSD flashbacks and nightmares of war experience can be found in the documents of the Hundred Years' War (1337-1453) between France and England (6). The place of trauma in psychology has been with the wars. The psychological problems experienced by the soldiers who were at the front during the war after the end of the war show that wars can affect people psychologically. Especially after the Franco-Prussian war in 1870, the psychological problems seen in soldiers returning from the front attracted the attention of people who specialize in mental health and made them concentrate on this issue. Delays were observed in the reactions of some of the soldiers returning from the front, they experienced the negative situations experienced at the front again, and they could no longer show interest in the activities they were interested in before the war. As a result of all these symptoms, it was recommended that these people be diagnosed with traumatic neurosis (7). PTSD has not just emerged as a result of wars. Difficult life conditions or other experiences also seem to cause traumatic symptoms. PTSD symptoms have historically been recognized under several different names. For example, in the late 1800s, a term referred to as "railway spine" was used to describe psychological reactions in people who witnessed or were exposed to railroad accidents (8). Bomb shock is a term used by British psychologist Charles Samuel Myers in the First World War to describe the outcome of traumatic experiences many soldiers suffered during the war. Bomb shock; is a notable term because it is a war-related disorder that includes overt and common psychiatric symptoms such as shyness, nightmares, and agitation. Soldiers diagnosed with bomb shock; reported a variety of somatic symptoms similar to combat fatigue, including heart palpitations, chest pain, tremors, fatigue, and even paralysis (9). During the First World War, British troops were subjected to many explosions, especially before the steel helmet was installed at the beginning of 1916. In fact, it is known that 60% of the deaths in the First World War were caused by explosions. Although there were soldiers who lost their lives in the face of the explosions, many soldiers survived. Soldiers who survived the explosions applied to health units for very different reasons. Memory loss, difficulty concentrating, hypersensitivity to noise, and tremor are the most common causes of admission. As the number of cases increased in the face of bomb shock, various explanations were tried to be made. Most of these explanations have been of organic origin. For example, a microscopic cerebral hemorrhage is caused by the jarring or harmful effects of an exploding bomb. On the other hand, Myers found that soldiers who were not very closely exposed to the explosion had similar symptoms (10). This finding proves the knowledge that witnessing the death or serious injury of his friends at the front can cause psychological symptoms (6).

PTSD in DSM Diagnostic Systems

DSM-I includes the diagnosis of "Great Stress Reaction", which requires the individual to be exposed to extreme stress in the face of traumatic events such as war or natural disaster; flood, earthquake, explosion, etc. (11). In one study, war sounds were played to three groups of men. These 3 groups consist of; men who have never been in combat, men who have been in combat but show no signs of psychiatric disorders, and war veterans with symptoms specific to war syndrome. While the sounds were played, physiological response measurements such as EEG, pulse, and respiratory rate were taken. The group, which had no war experience, gave mild, directive responses to the sounds of war. War veterans in good mental health showed mild to pronounced physiological and behavioral responses, while war syndrome veterans showed behavioral disturbances so marked that it was impossible

to record physiological responses. These findings support the diagnosis of Major Stress Reaction (12). In 1968, DSM-II was published by the American Psychiatric Association (APA). For unclear reasons, the diagnosis of Major Stress Reaction was removed from DSMII and no diagnosis was included for pathological reactions after trauma experience (11). The Major Stress Reaction diagnosis was replaced by the Transient Situational Disorders diagnosis category. This disorder encompasses a broader set of stressors and underlines the necessity for the traumatic experience to be extremely unbearable or unusual. Although war-related traumas are grouped under this diagnosis, trauma-related psychiatric disorders are rarely included. In case the post-war symptoms persisted, the soldiers were diagnosed with Anxiety Neurosis, a personality disorder (9). Included in the DSM-III (1980), PTSD was named as Post-Vietnamese Syndrome or Delayed Stress Syndrome, first described in veterans returning to the United States after the Vietnam War. However, its international acceptance is not swift or uncontroversial. Recognition of this disorder has been slower in the United Kingdom, where it was initially thought to be specific to the United States and Vietnam veterans (10). PTSD was first included in the DSM-III under the name Anxiety Disorders. The presence of an obvious event that may cause significant distress to almost everyone is required for a diagnosis of PTSD to be made. While DSM-III reinstated the category for responses to extreme stress, it avoided creating a list of specific types of trauma and instead mandated "the presence of a recognizable stressor that would produce significantly distressing symptoms in almost everyone". The inclusion of PTSD in the DSM-III established a remarkably sustainable and productive period in the scientific study of psychological trauma. PTSD serves as a unifying structure. It has allowed different groups of clinical researchers focusing on seemingly different types of trauma, such as war, sexual assault, and natural disaster, to recognize commonalities in their work on the fundamental and traumatic aspects of psychological trauma (13). The DSM-IV definition of PTSD stressor is quite different from previous versions. The definition is divided into two. The first section shows the frequency of appropriate stressors; the second part stipulates that "the person's response must include intense fear, helplessness, or horror". This two-part definition emphasizes the objective characteristics of trauma and is instead based on the principle that people may perceive and react differently to similar events. The transition from the objective nature of the stressor to the subjective experience of the victim is evident not only in the added subjective component but also in the stressor (14). According to the DSM-IV, to be diagnosed with PTSD, a person must experience, witness, or encounter an event that is significantly life-threatening, involving injury or threats to physical integrity, and the experience must provoke a subjective response of fear, helplessness, and horror (15).

Differences in PTSD Classification in DSM-5

DSM-5 was released in May 2013. The definition of PTSD has undergone significant changes in this version. The most significant of the changes include moving the diagnosis of PTSD from Anxiety Disorders to a separate section for Trauma and Stress-Related Disorders. Considering the reason for the change, it was evaluated as having common features of trauma-related diseases and increasing the reliability of diagnosis (16). In addition, "Reactive Attachment Disorder of Infancy or Young Childhood" was taken from the "Disorders Usually First Diagnosed in Infancy, Childhood, or Adolescence" and placed in the "Trauma and Trigger-Related Disorders" section of the DSM-5. In addition, "Limitless Social Participation Disorder", which is a determinant in "Reactive Attachment Disorder of Infancy or Young Childhood" in DSM-IV-TR, has been transformed into a separate disorder with the name "Limitless Social Participation Disorder" in DSM-5 (17).

PTSD After Natural Disasters

Since the existence of humanity, disasters are one of the events that affect social life the most. Disasters are natural-origin events that occur unexpectedly, interrupt normal social life, cause negative social and economic effects, cannot be prevented, and cannot be overcome with current opportunities and resources (18). The World Health Organization (WHO) defines a disaster as "an unexpected, sudden ecological phenomenon that exceeds the capabilities and capacity of the institution, disrupts normal functioning, and requires external assistance". The International Federation of Red Crescent and Red Cross (IFRC) defines it as "a situation where an accident, natural event or man-made event that develops as a result of sudden or long processes other than armed conflict poses a significant and major threat to human life, health, property or the environment, and the functioning of society is seriously impaired". Disasters are classified as natural disasters and man-made disasters. Natural disasters are earthquakes, volcanic eruptions, floods, droughts, heavy rains, frost, snow, and storms, and man-made disasters are chemical, biological, radiological, and nuclear accidents, wars, migrations, terrorist incidents, and fires (19). Worldwide, about 400 natural disasters occur annually. In the last two decades, natural disasters have negatively affected approximately 800 million people and caused 3 million deaths (20). Disasters are an important public health problem because they cause heavy loss of life and property, interrupt society and health services, the timing is uncertain, have heavy economic burdens, disrupt psycho-social well-being, and cause serious health problems in the future (21).

Witnessing a natural disaster is an experience that deeply affects people, resulting in anxiety and stress (22). These symptoms, which seem like a normal reaction at first, can become a serious mental health problem when the level of stress and anxiety experienced doesn’t decrease over time (23). Although disasters have been around since the existence of humanity, the assessment of their psychiatric effects is relatively new. Because meeting the socio-economic needs such as shelter and nutrition after the disaster has been a priority for both the donors and the victims. Providing post-disaster psychiatric help and drawing attention to this field entered the agenda of the WHO after 1995 (24). Natural disasters not only cause problems such as property loss, infrastructure damage, and resource destruction but also cause secondary psychological disturbances. In post-disaster screening studies, the most common mental illness was found to be PTSD. Major Depressive Disorder takes second place. It

can also be seen in diseases such as alcohol use disorder and Generalized Anxiety Disorder. In addition, psychological effects such as suicidal ideation/attempt, and alcohol and nicotine addiction may occur in people who are exposed to a major disaster (25). Although the order and level of post-disaster mental illnesses change, the relationship between disaster and psychology seems to make its importance felt in every period. After natural disasters, there is an increase in PTSD, major depressive disorder, sleep disorders, generalized anxiety disorder, substance use disorder, suicide, and grief reactions (1). PTSD, on the other hand, is one of the most frequently observed and studied mental health disorders together with natural disasters (26). Although natural disasters affect many people at the same time, according to the World Mental Health Survey, the prevalence of traumatic stress symptoms after a natural disaster is around 3.8% (27). In their study of individuals affected by the Van-Erciş earthquake, they reported the rate of PTSD as 35.5%. In addition, it has been reported that the loss of family members or a relative, housing and health problems after the disaster, unemployment, temporary relocation, financial difficulties, and language problems affect the development of PTSD (28). It has been reported that 90.2% of children who experienced an earthquake in Bingöl are in the risk group for the diagnosis of PTSD (29). Cénat and Derivois (30) reported that 36.7% of those affected by the Haiti earthquake experienced PTSD and 25.9% had depression. In addition, it was stated that being a disaster woman, old age, youth (18-24 years old), low education level and unemployment variables were associated factors for PTSD and depression. In the study conducted by Salloum and Overstreet (31) after Hurricane Katrina, it was reported that 53% of the students were at risk for PTSD and 40% for depression. A study of flood victims in Indonesia showed that 52% of the participants experienced PTSD, and 98.3% re-experienced PTSD symptoms (32). In a study conducted in China, it was reported that the prevalence of PTSD among flood victims was 9.2% (33). In a systematic review and meta-analysis study conducted in 2015, the incidence of PTSD was found to be 15.74% in post-flood disaster victims (34). The results of a meta-analysis study showed that the prevalence of PTSD in children in the first, second, third, and fourth 6 months after an earthquake and a flood was 19.2%, 30%, 24.4%, and 20.4%, respectively (35). The results of another review study showed that the prevalence of PTSD among healthcare workers during coronavirus disease 2019 (COVID-19) was 13.52% (36). Few studies have examined PTSD after natural disasters, especially among first responders, firefighters, and police officers (37). On top of that, researchers have found that the prevalence of PTSD is high in first responders. To give an example from the studies, PTSD was found in 21% of the firefighters who responded to the 1999 Chi-Chi earthquake in Taiwan (38). In 2005, 22% of firefighters who responded to Hurricane Katrina developed PTSD in the subsequent process (39,40).

Risk Factors for the Development of PTSD after Natural Disasters

When the risk factors for the development of PTSD after natural disasters are evaluated, some factors come to the fore. Age, gender, race-ethnicity, economic resources, education level, employment status, personality traits, level of coping with the problem, emotional regulation, prior exposure to trauma, pre-existing mental illness, biological vulnerability, and social support are the most significant risk factors for the development of PTSD. Young age and female gender are the most prominent risk factors for the development of PTSD (2,41). Gender is another factor that can affect the prevalence of PTSD in victims of floods and other disasters. According to studies, women who survived the earthquake had higher PTSD than men (42). Women are at higher risk of suffering from PTSD after disasters (43-45). It showed that people who lost their property after the flood or were not supported by their families, such as widowed or divorced women, experienced more stress and had higher PTSD scores (46,47). Being an ethnic minority has also been found to predict an increased prevalence of symptoms and risk of disorders after natural disasters (48). In addition, black individuals were found to be more risky in terms of PTSD (41). In the wake of Hurricane Harvey in 2017, a recent study found that black individuals and younger people reported higher levels of post-traumatic stress symptoms (49). Regarding the economic component of resilience, lower socioeconomic status has been reported to be consistently associated with greater post-disaster distress (1,50). The loss of one's own private resources after a natural disaster can affect the individual both materially and spiritually, thus affecting the individual's self-perception and self-confidence (51). It has been determined that many survivors of disasters have adaptation problems in the life process, daily life, and work life of individuals due to the chronic stress they have experienced during the recovery period following the disaster (52). The factors that cause this chronic stress can be attributed to job loss or resource depletion, which leads to socioeconomic distress in the post-disaster period. In a large survey of Hurricane Katrina survivors, an increase in other mental health disorders, especially PTSD, was found in individuals. In addition, the suicide rate doubled from 3-6% (52). In addition, it was determined that 27% of PTSD cases and 47% of suicidal tendencies occur in the future (52). The economic hardship that the disaster survivors will experience arises in the process of individuals being unemployed after the disaster, unable to pay their current debts, and rebuilding the destroyed or damaged property. The prevalence of major depressive disorder in survivors of Hurricane Katrina 2 years later was found to be higher than the data determined in the general population and after other natural disasters, and it was associated with the resulting economic difficulties (53). The educational status of disaster victims is always emphasized as an important factor affecting post-disaster resilience and mental health outcomes (41,54). It has been reported that countries with high income and education level experience fewer losses than countries with low income and low education levels (55). Education can have positive outcomes by influencing perceptions of risk, increasing the knowledge and skills needed to face disasters, and influencing successful access to resources (41). While addressing the increased risk for personality traits, the most common trait associated with post-disaster psychopathology is neuroticism (2,41,56). There is a growing body of literature documenting a close

relationship between neuroticism and PTSD and depressive symptoms (57). In a study conducted on children who survived the Wenchuan earthquake, which killed approximately 90,000 people in China, it was found that older children with neurotic personality traits and a high propensity for the trauma experienced a longer duration of PTSD symptoms (58). Another study of Chinese survivors of the Wenchuan earthquake revealed similar findings in adults. As a result of this study, both neuroticism and psychoticism were found to be positively associated with the morbidity of long-term PTSD (59). Similar results were found in different cultures. A study of firefighters after the devastating and severe fires in Greece in August 2007 found that an increase in neuroticism scores was significantly associated with an increased likelihood of having PTSD after the disaster (60). Inadequacies in coping and problem-solving skills are also risk factors for the development of PTSD (61). One of the negative coping mechanisms after natural disasters is substance use. In New Orleans, the hospitalization rate for substance abuse disorders increased from 7.13 per 1,000 before Hurricane Katrina to 9.65 per 1,000 after Hurricane Katrina. This data shows that substance use disorders may accompany PTSD (62). Individuals who survive multiple traumatic events often experience long-term and short-term problems that affect their lives (63). The level of resilience to trauma is found to be lower in individuals who have been repeatedly exposed to natural disasters and trauma (64). Studies have found that the pre-existing distress increases mental disorders and reduces resilience after natural disasters (41). The literature also suggests that a pre-existing diagnosis of anxiety disorder reduces post-disaster resilience and predisposes participants to other mental health disorders (64,65). Research by Xie et al. (66) shows that the cumulative effect of exposure to two earthquakes on mental health problems is more serious than one earthquake. Disasters often involve communities not directly exposed to trauma, such as those who have lost family members, friends, or colleagues or lost property, were forced to relocate, or were exposed through the media. This raises two critical points about the burden and nature of post-disaster psychopathology. First, the mental health consequences of such events among those indirectly exposed to a disaster may be just as deteriorating as those directly exposed to or close to the disaster epicenter (40). Most of the people interviewed in the post-9/11 national surveys reported that they were indirectly exposed to the attacks, mostly through TV broadcasts (67). Studies have found that religious coping, a secure relationship with God, and belief in the meaning of life are inversely related to PTSD. People with high religious coping have a lower risk of major depressive disorder and a higher quality of life (68). A similar study with Hurricane Katrina survivors found that negative religious coping in the context of perception of punishment was positively associated with acute stress disorder symptoms, while in the context of perception of abandonment, it was associated with higher functional impairment (69). A study of Pakistani earthquake survivors found that negative religious coping in the context of perceiving punishment was associated with higher symptom levels and negative emotions (70). Having a previous mental illness increases the risk of developing PTSD in the post-disaster period (2,41,54). In a study investigating the psychological responses of firefighters working in disasters after the severe forest fires in Greece, it was found that people with symptoms such as insomnia and depressive symptoms in the pre-event period were associated with the risk of developing PTSD (60). A study of Hurricane Katrina survivors found that those with pre-disaster mental illness were associated with an increased risk of PTSD symptoms (71). A study conducted after a severe earthquake and tsunami in Chile in 2010 found that pre-disaster PTSD was significantly associated with an increase in post-disaster death thoughts and suicide attempts in individuals (72). It has been shown that social support plays an important role in the context of outcomes in the post-disaster period (1). In individuals who experienced the Alberta forest fire, social support from their environment was found to be associated with the post-disaster recovery process (73). It has also been documented that perceived spousal support in postpartum mothers after the Iowa flood, objective stress on depression buffered, and reduced depression levels. This situation also draws attention to the importance of social support in the post-disaster process (41). Another study of Alberta wildfire survivors found that spiritual resources such as positive perspectives, feelings of faith and hope, compassion, and gratitude shared in their spiritual community contributed to increased resilience that helped them support other families and communities in the wake of the disaster (74). Following the collapse of the Buffalo Creek dam in West Virginia, personal trauma was found to be more strongly associated with reductions in perceptions of family support, while the loss in the community was more strongly associated with decreases in perceptions of extra-familial support and social involvement (75). Biological and genetic resilience factors are growing areas of study, demonstrating the potential to find better treatments and identify individuals within the population who may be predisposed to a higher probability of psychopathology (2,41,58). In terms of PTSD in children who survived the Wenchuan earthquake, it has been reported that N-methyl-d-aspartate (NMDA) receptors at the molecular level are an important component of learning and memory. The TrkB (rs920776) gene, which produces the single transmembrane receptor TrkB, modulates NMDA receptor activity through brain-derived neurotrophic factor (BDNF). This ensures that survivors are protected from PTSD. In addition, in the same study, G72 genes (rs3916966, rs3918341) and CNTF genes were also found to be protective factors in PTSD (58). In a study examining the effects of prenatal maternal stress during the Quebec ice storm in 1998, the BDNF (rs6265) and COMT (rs6480) genotypes were found associated with hippocampal volume in their offspring (76). Current findings provide some evidence for gene-environment interactions following a natural disaster.

Clinical Symptoms of PTSD after Natural Disasters

PTSD causes many functional disorders, especially in occupational and social areas. It is characterized by sudden thoughts, nightmares, re-experiencing the traumatic event, avoidance of traumatic events, hypervigilance (sensory sensitivity), and sleep disturbances. Symptoms usually begin within three months of the early traumatic event, sometimes years later, in which case it is referred to as

PTSD with Delayed Onset. Symptoms must last more than a month and be severe enough to suggest PTSD. The clinical features of PTSD are grouped under four main headings: The first group includes findings related to increased arousal. The state of arousal, which is the first response to stress, manifests itself as "increased arousal" in PTSD. They are the most common symptoms of PTSD (77). Continual anxiety, insomnia, and concentration disorder are also included under this heading. Sleep-related disorders are common in PTSD and are associated with an increased risk of suicidal ideation, attempt, and related death (78). It has been shown that adrenergic stimulation is increased in the examinations performed in the state of increased arousal. The tachycardia and increase in tone seen in patients can also be explained by the increase in adrenergic stimulation (79). The second cluster of symptoms is about re-experiencing the traumatic event. These can be in the form of thoughts, perceptions, or dreams. In these cases, the person may re-perceive the sounds or smells associated with the event (80). Sometimes, the person can relive the moment of trauma by experiencing dissociative flashbacks. The incidence of dissociative symptoms in individuals with PTSD is reported to be 8-13%. This can take seconds, sometimes minutes, or even hours. During this period, the person is buried in memories of the moment of trauma, but it is important that the person does not have impaired consciousness and then returns to the present moment (79). The third group is related to avoidance behavior. The person is aware of the difficulty in controlling himself in the face of events in the outer world and withdraws into his inner world. Situations such as avoiding places that remind of the event, avoiding talking about the event or moving away from the spoken environment, and decreasing in activity and interests appear as a result of avoidance behavior (80). The fourth cluster of symptoms is the changes in cognition and mood. Memory impairment can also be seen in these individuals. Dissociative amnesia is more common in various traumatic events such as war genocides and sexual and physical abuse. People experiencing depersonalization say that they watch themselves like a movie. They say that they watch themselves from afar and sometimes they can feel unrealistic. Patients describe the derealization state as "a distant and foggy world" (80).

MASS GRIEF AFTER NATURAL DISASTERS

In addition to all these psychological and physiological reactions, disasters also bring the phenomenon of loss and mourning. In cases where life is in danger, a relative is lost, or financial means are lost, people exhibit certain attitudes and behaviors. All of these emotional, behavioral, and intellectual reactions are mourning reactions (81). Although the concept of mourning is known as the mental state experienced after the loss of a loved one, the loss of anything to which a commitment has been developed is also the cause of the mourning process. The way mourning reactions are expressed is shaped according to the environment and culture. The grieving process is a normal process that occurs after the trauma and it is expected not to exceed six months to two years on average. However, the mourning phenomenon that exceeds this period can be a harbinger of a psychopathological condition. Especially as a result of major disasters; the advanced process of death and fear of losing loved ones paves the way for the formation of complicated and pathological mourning (81). Providing adequate psychological support, especially to individuals in the grieving process, is very important in the fulfillment of post-traumatic psychological support and grief counseling services. When examining the literature, there is a field of research on the concepts of loss and mourning that goes back to the article "Mourning and Melancholia" published by Freud in 1922. In this work, Freud examined the analysis of grief and the investigation of the phenomenon of mourning in the inner life. Freud mostly showed an evaluation and approach on the individual's inner world and the process of spiritual struggle of the mourning phenomenon. After this work of Freud, many researchers started to work on grief and traumas in his theory. The most well-known theoretical model in the post-disaster traumas and grief process is Kübler-Ros's Five-Stage Theory of Grief (82). According to the perspective of this theory, individuals grieving after a post-disaster loss go through the following process, respectively:

- Denial and isolation.
- Anger process.
- The bargaining process.
- The process of experiencing depression.
- Acceptance process.

The reactions given during this process are:

- Emotional reactions: Depression, hopelessness, anxiety, guilt, anger, loneliness.
- Behavioral responses: crying, withdrawal, and burnout.
- Cognitive reactions: Constantly thinking about the deceased, low self-esteem, thoughts of helplessness, difficulty concentrating, and denial.
- Physiological reactions: Substance use, loss of appetite, fatigue, and somatic complaints.

In the first stage, individuals do not want to accept death and loss and develop different defense mechanisms. This situation leads to the denial of the event and the rejection process by causing inhibition. In the second stage, when it is understood that death cannot be denied, the individual now experiences an anger process. In the next stage, the helpless individual tries to bargain with God in return for whatever he has to give. Failure in this process brings the individual to depression in the next stage, and this experience may be the beginning of the psychopathological process. In the last stage, the acceptance and normalization of life continue and the lost individual will no longer come back. However, individuals who develop obsessions at any stage of this process may experience a transition to psychosomatic and psychopathological diseases. In this context, post-traumatic psychological counseling services and grief counseling by experts constitute an important area of treatment (81,83). In addition to all these, an important problem is that inexperienced individuals who provide post-disaster support also show similar traumatic and mourning reactions as individuals who experienced disasters. After the traumatic events, those who are directly exposed to the event, as well as those who witness the situation, relatives of the victims, and people who take part in the relief efforts may also show signs of traumatic stress.

The reactions of these groups are referred to as secondary traumatic stress or indirect traumatization in the literature. Considering the sources of stress that aid workers face due to their work, it is thought that this group may experience stress reactions similar to those directly exposed to traumatic events. It is important for the healthy functioning of the process that well-equipped mental health workers provide the necessary psychological support in this field (84). The loss of a loved one during a natural disaster is especially traumatic and distressing given that death often happens suddenly and unexpectedly (85). Pathological grief rates, as high as 40-50%, have been reported among those who have experienced traumatic loss after a disaster (86-89). Among the factors affecting the course of traumatic grief are the way the news is covered on TV, social media, the prevalence of trauma, how quickly post-traumatic help arrives, and how the deceased is buried.

TREATMENT APPROACHES

Secondary symptoms such as involuntary symptoms, negative mood, dissociation, avoidance, and arousal, which are frequently observed in the PTSD clinic, affecting important areas of life such as family and work, and psychiatric comorbidity constitute the treatment goals. Treatment options are classified as pharmacotherapy and psychotherapy. The UK-based National Institute for Health and Clinical Practice (NICE) recommends drug therapy as a second-line treatment for PTSD. It is recommended to give priority to psychotherapy in mild cases and to carry out psychotherapy and pharmacotherapy together in moderate and severe cases.

The importance of early interventions and treatment is to ensure the safety and stability of individuals, as well as to prevent distress reactions and risky behaviors and to preserve functionality. This also reduces the severity of PTSD symptoms. The primary goal of early intervention is to reduce the progression of symptoms to a psychiatric disorder. When psychiatric disorders do occur, early diagnosis and treatment are the goals. Goals also include:

- Recognizing the neuropsychiatric symptoms of the related disorder and referring them to relevant medical professionals.
- To treat acute symptoms and restore functionality with treatment including psychotherapy and pharmacotherapy with biopsychosocial strategic planning.
- Distinguish between "normal" and pathological responses in survivors.
- Provide grief and loss treatment.
- Early recognition and treatment of psychiatric disorders.
- Management of relapses of psychiatric disorders in at-risk and socially unsupported groups.
- To provide long-term management of negative psychosocial consequences of natural disasters such as bereavement, financial loss, property loss, and unemployment.

Pharmacotherapy

The first goal of treatment is to prevent the development of PTSD by initiating post-traumatic treatment at an early stage. The general principle is to treat the leading symptom cluster and accompanying conditions, if any. Reduction of avoidance and hyperarousal symptoms, regulation of destructive behaviors, control of uncontrollable impulses, treatment of accompanying psychotic or dissociative symptoms, and anxiety or depressive symptoms should be considered as general treatment principles. An appropriate intervention immediately after the trauma experience ensures that the traumatic event is processed appropriately in the mind and recorded in the memory without its threatening effect. However, when not intervened, the traumatic experience continues to continue for years in risky individuals. Studies emphasize the importance of intervention time, and this time period is considered as 'golden hours' in some studies. Hypoactivation of the post-traumatic HPA axis is associated with traumatic sensory memory formation. Therefore, HPA hypoactivation is interpreted as a risk factor for PTSD. For this reason, it has been reported that the use of benzodiazepines, especially in the early period, increases the symptoms and this drug group constitutes a risk factor for PTSD. On the other hand, it has been reported in animal experiments that cortisol administration immediately after trauma reduces the risk of PTSD (90). In a study, it was reported that the administration of beta-blockers immediately after trauma has a protective role in the development of PTSD (91). Although the treatment option depends in part on the clinical presentation, antidepressants are the leading treatment option. It includes selective serotonin reuptake inhibitors (SSRIs) as the first choice as a pharmacotherapy approach. Tricyclic antidepressants, mood stabilizers, and atypical antipsychotic, beta blocker drugs are other treatment options. If there is no adequate response from drug therapy, the dose of the drug should be increased at recommended intervals. If there is no response from the treatment, it is recommended to switch to another class of antidepressant or to add an antipsychotic to the treatment. When SSRIs are used, they can cause or exacerbate insomnia and agitation. The duration of maintenance therapy is 6-12 months in acute PTSD patients, and 12-24 months in chronic PTSD patients, with more than 75% improvement in symptoms. In patients with residual symptoms, drug therapy should be at least 24 months (92).

Psychosocial Treatments

Among the psychotherapy approaches, it has been reported that the two most studied approaches in the literature and shown to be more effective than others are cognitive-behavioral therapies and eye movement desensitization and reprocessing EMDR. Behavioral approaches in the treatment of PTSD were generally made by Foa et al. (93). According to this model, both cognitive and emotional processes are important, and the cognitive-affective fear construct is central to PTSD. Even though the fearful life is wanted to be bestowed in the uninvited re-experiencing process, it does not reach the whole process because it is irresistible. For this reason, avoidance behaviors increase, and the interpretation that the person is helpless and always vulnerable appears. In behavioral treatment, it is aimed to experiencing traumatic stimuli and images, activating the fear structure associated with the traumatic experience, and learning that the images are no longer dangerous (93). In line with the theories of Foa et al. (93), they developed Prolonged Exposure Therapy, which predicts a three-stage change consisting of exposure to fear-triggering stimuli, repeated and prolonged exposure, and reduction in anxiety levels (94).

Different researchers have also emphasized the importance of cognitive factors in the pathogen of PTSD. It has been shown that when the person is exposed to a triggering stimulus that is not objectively harmful during therapy, cognitive restructuring, and fear memory are regulated and avoidances are reduced, ultimately reducing anxiety. One therapy method that has been demonstrated in placebo-controlled studies is EMDR therapy. Although the exact mechanism of action is not known, it has been stated that exposure to traumatic moments causes a deterioration by making neural changes, and the balance that is disturbed by two-way eye movements is re-established. In the so-called third-wave therapies, it is planned to observe the symptoms of PTSD rather than focus on them, to accept their existence voluntarily, and to focus on the value areas that the individual attaches importance to (93). Mindfulness is one factor that has also been found to be associated with positive post-disaster psychiatric outcomes (57,94,95). Mindfulness training following a disaster has been shown to provide modest benefits for improving psychological well-being (96).

New Developments

New promising pharmacological agents for PTSD have been clinically experienced. The most recent of new drugs are corticotropin-releasing hormone antagonists and those that affect the endocannabinoid system. CB1 receptor-mediated endocannabinoid agents have proven helpful in forgetting bad memories in animal model studies. Increased levels of anandamide (an endogenous cannabinoid) in the amygdala regulate short-term fear extinction (97-99). High CB1 receptor availability in those with PTSD has been shown to correspond to lower circulating anandamide levels in the blood. These findings support the possibility that cannabinoids such as tetrahydrocannabinol may be an alternative treatment agent in the treatment of PTSD. Another molecule of interest is ketamine. Ketamine is a non-competitive antagonist of the NMDA receptor that affects learning and memory. A rapid reduction in PTSD symptoms has been demonstrated following the intravenous administration of ketamine (100). Drugs that block dopamine reuptake and are associated with NMDA receptor action, such as 3,4-methylenedioxy-methamphetamine (MDMA), have also received attention. Given the potential for abuse of these compounds, it is important to determine whether their use in the treatment of PTSD outweighs the potential risks (101,102). In addition, NPY agonists are an important development for the treatment of PTSD (103). Transcranial magnetic stimulation, deep brain stimulation, and the use of new neurofeedback techniques are other alternative treatment modalities for PTSD (104,105). Another new treatment has been the use of the glycine receptor agonist d-cycloserine in combination with prolonged exposure psychotherapy to accelerate the elimination of fear conditioning (106).

CONCLUSION

The incidence of PTSD after a natural disaster is not to be underestimated. If the traumatic stress symptoms that occur after a traumatic event last longer than a month, this suggests that this situation may become chronic. For this reason, due to the nature of natural disasters, if the destruction and destruction encountered is high, it will take more than a month to return to daily life after the disaster and to return to the old state of well-being. The potential risk for PTSD also increases when we consider that it takes much longer to recover after the most frightening disasters such as earthquakes, and the time required for debris removal and reconstruction is months or even years. Early detection and intervention programs are necessary for planning PTSD management.

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Genotype and Allele Frequencies of Irritable Bowel Syndrome (IBS)-associated Single Nucleotide Polymorphisms among Malays in Malaysia

Malezya'daki Malaylar Arasında İrritabl Bağırsak Sendromu (IBS) ile ilişkili Tek Nükleotid Polimorfizmlerinin Genotip ve Alel Frekansları

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ABSTRACT

Aim: Irritable bowel syndrome (IBS) defined by chronic or recurrent abdominal pain or discomfort and changes in bowel habits, is the most common functional gastrointestinal disorder. Studies proved that polymorphisms in the genes were one of the key roles in the underlying IBS. This study aimed to investigate the genotypes and allele frequencies of the IBS-associated single nucleotide polymorphism (SNP) from the genes GNB3 (rs54443) and SCN5A (rs8015124) in unrelated, healthy Malays of Malaysia.
Material and Methods: The genomic DNA of 404 subjects was set to nested, multiplex, and allele-specific PCR to determine the aforementioned SNPs. The PCR results were validated through the Sanger sequencing analysis.
Results: Malays possessed a slightly higher frequency of wild (C) than mutant (T) alleles in the rs5443 with 56.3 vs 43.7%. However, the frequencies of the alleles were equivalent in the subset of Malay females (C-50%, T-50%). For rs1805124, only 18.6% of Malays carried the mutant allele G with less than 10 subjects being homozygous mutant GG carriers. Concurrently, the Hardy-Weinberg equilibrium of the SNPs in the study was not deviated.
Conclusion: IBS is a common gastrointestinal problem that has significantly reduced the life quality of oneself and become an economic burden to societies. Though the mutant alleles were rather low, the IBS-associated polymorphisms, rs5443 and rs1805124 were noted to be commonly present in the Malays. Further research on the local IBS patients is recommended to affirm the association of rs5443 and rs1805124 polymorphisms and the syndrome.
Keywords: Genetic population; irritable bowel syndrome; Malay; rs5443; rs1805124.

ÖZ

Amaç: Kronik veya tekrarlayan karın ağrısı veya rahatsızlığı ve bağırsak alışkanlıklarında değişiklik ile tanımlanan irritabl bağırsak sendromu (irritable bowel syndrome, IBS), en sık görülen fonksiyonel gastrointestinal bozukluktur. Çalışmalar, genlerdeki polimorfizmlerin, IBS'de altta yatan anahtar rollerden biri olduğunu kanıtlamıştır. Bu çalışmada, Malezya'nın ilişkisiz, sağlıklı Malaylarında, GNB3 (rs54443) ve SCN5A (rs8015124) genlerinden, IBS ile ilişkili tek nükleotid polimorfizminin (single nucleotide polymorphism, SNP) genotiplerinin ve alel frekanslarının araştırılması amaçlanmıştır.
Gereç ve Yöntemler: Toplam 404 katılımcının genomik DNA'sı, yukarıda belirtilen SNP'leri belirlemek için yuvalanmış, multipleks ve alele özgü polimeraz zincir reaksiyonu (polymerase chain reaction, PCR) ile analiz edildi. PCR sonuçları, Sanger sıralama analizi ile doğrulandı.
Bulgular: Malaylar, rs5443'te %56,3'e karşı %43,7 ile mutant (T) alellerinden biraz daha yüksek bir yabanıl (C) alel frekansına sahipti. Bununla birlikte, alellerin frekansları Malay kadınlar alt grubunda eşitti (C-%50, T-%50). rs1805124 için, Malayların sadece %18,6'sı mutant alel G'yi taşıyordu ve 10'dan az katılımcı homozigot mutant GG taşıyıcılarıydı. Aynı zamanda, çalışmadaki SNP Hardy-Weinberg dengesinden de sapmamıştı.
Sonuç: İBS, kişinin yaşam kalitesini önemli ölçüde düşüren ve toplumlara ekonomik yük haline gelen yaygın bir gastrointestinal sorunudur. Mutant alellerin oldukça düşük olmasına rağmen, IBS ile ilişkili polimorfizmlerin, rs5443 ve rs1805124'ün Malaylarda yaygın olarak bulunduğu kaydedildi. rs5443 ve rs1805124 polimorfizmleri ile sendromun ilişkisini doğrulamak için yerel IBS hastaları üzerinde daha fazla araştırma yapılması önerilir.
Anahtar kelimeler: Genetik popülasyon; irritabl bağırsak sendromu; Malay; rs5443; rs1805124.

INTRODUCTION

Irritable bowel syndrome (IBS) which defines by the presence of chronic or recurrent abdominal pain or discomfort and changes in bowel habits, is the most common functional gastrointestinal disorder (FGID) in the world (1,2). The syndrome has affected global populations variously with a prevalence between 7-21% and significantly reduced the life quality of the patients and become an economic burden to the region (1). Other than the host factors of pathophysiological, environmental, and psychological, numerous findings have proved that genetic background also has set up a key role in the underlying of IBS (3-5). The mutations or polymorphisms in the gene might implicate the inflammation in the intestinal, alter the cytokine response, and increase permeability or microbiome which eventually leads to the IBS (3). Several genetic association studies have identified the single nucleotide polymorphism (SNP) from the genes of G-protein subunit (GNβ3); rs5443 and sodium channel protein type 5 subunit alpha (SCN5A); rs1805124 to be significantly associated with the etiology of IBS in different ethnic groups in the world (1,6,7).

GNβ3 is a gene located in chromosome 12p13 that encodes guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3 which is responsible for various functions such as ion channel, hormones, and contraction and acts as a 'molecular switch' in the pathways of signal transduction (7). Due to its crucial role in many cell mechanisms, genetic abnormalities in G protein subunits have led to a higher chance of the etiology of a wide range of clinical problems including the IBS (6). A synonymous SNP in the gene, rs5443 (C825T) in exon 10 caused an exchange of amino acid cytosine by thymidine at position 825 and led to the deletion of 41 amino acids coding protein in the sequence (8,9). This resulted in an increase of intracellular signal transduction between receptors and effectors which caused the motoric or sensory abnormalities of the GI tracts and pathophysiologic mechanisms of the IBS. The rs5443 has given rise to three possible genotypes; CC, CT, and TT, where the T allele is associated with alternative splicing and formatting of a truncated but functionally active splice variant (10,11). Studies performed among South Korean showed that the TT genotype was associated with constipation-predominant IBS (12,13).

The gene of SCN5A resides on chromosome 3p22.2 in the human genome, spanning more than 101kbp and consisting of 29 exons. The SCN5A-encoded $Na_v1.5$ Na^+ channel is expressed in interstitial cells of Cajal and smooth muscle in the circular layer of the human intestine (14,15). The polymorphism in the SCN5A which interrupt the smooth muscle electrical waves and mechanical sensitivity suggesting that the event is a potential pathogenic to the IBS. Researchers have reported that the polymorphisms in the SCN5A gene namely rs1805124 (histidine-558-to-arginine, H558R) that influence the $Na_V1.5$ function would contribute to the IBS pathophysiology (12,13). The SNP was caused by a T to C transition in the sequence which exhibited the TT, TC, or CC genotype variants. It was reported that individuals with this gain-of-function SNP of SCN5A exhibited a higher risk of IBS (16). A genome-wide association study on SCN5A genetic polymorphisms in a few ethnics also revealed a significant link between its variants and the IBS (17).

Though many reports have indicated the prevalence of GNβ3 and SCN5A gene polymorphisms; rs5443 and rs1805124 in other populations around the globe, the data on the SNPs in the Southeast Asia populations are yet very limited. Such data is absolutely crucial in order to investigate the genetic risk, in particular ethnic towards the IBS susceptibility. Therefore, in this study, we will investigate the genotypes and allele frequencies of the rs54443 and rs8015124 among unrelated healthy Malays of Malaysia. The results from this study can be initiated as a database and a risk predictor on the IBS for Southeast Asia people especially Malays in future genetic association studies.

MATERIAL AND METHODS

Ethics and General information

This is a comparative and observational genotyping population study that involved the largest major ethnicity in Malaysia, the Malay. Subjects were 404 healthy, unrelated Malays (328 male, 76 female) who obtained and informed consent from a previous study, Development of Ethno-pharmacogenetics Relatedness and Personalized Medicine project (Grant no. 1001/PSK/8620013). The subjects were considered healthy as they were all blood donors including not having any critical illnesses, normal pulse and blood pressure, and unmedicated diabetes, anemia, or hypertension. This research was admitted by Universiti Sultan Zainal Abidin (UniSZA) Human Research Ethics Committee (UHREC), Terengganu, Malaysia (Reference no: UniSZA.C/2/UHREC/628-2/73, Date: 19.02.2019), and the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM), Kelantan, Malaysia (Reference no: USM/JEPeM/19020149, Date: 08.04.2019).

Genotyping

All genomic DNA was extracted from 200 µL of -20º C EDTA whole blood using QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the protocol. A two-step polymerase chain reaction (PCR) combining nested, multiplex, and allele-specific techniques was performed to determine the GNβ3 and SCN5A SNPs, rs5443 and rs1805124. These PCR techniques were used to ensure that the sensitivity, robustness, and reproducibility of the method would achieve 100% and the false-positive result could be avoided. The list of primers used in this study was shown in Table 1. The 1st PCR thermal cycler was begun with pre-denaturation at 95º C for five min followed by 30 cycles of denaturation (95º C) and annealing (65º C) for 30 sec, the extension (72º C) for 1 min and the final extension (72º C) for 5 min. For 2nd PCR thermal cycler, cycles were reduced to 20 while the time of extension to 30 sec. The annealing temperature was increased to 68º C. The DNA yield and the PCRs products were assessed using a non-mutagenic staining reagent on the 1.5% agarose gel electrophoresis. The gel image was viewed under UV light and analyzed through a digital imaging analysis system (Alphaimager, CA). Sanger sequencing was performed on the selected samples for result validation using an Applied Biosystems 3130xl Genetic

Analyzer (Applied Biosystems, USA) according to the manufacturer's recommendations. Gene polymorphism examination was performed via Chromas.

Statistical Analysis

Data of the SNPs from this study were analyzed using descriptive analysis. The observed genotype distributions were presented in count and percentage. The percentage of allele frequencies in the ethnic was deliberated from the figures. Hardy-Weinberg equilibrium (HWE) was calculated by comparing the observed and expected genotype frequencies using the chi-square test with one degree of freedom to determine possible deviation in the Malay and the genders. A p-value of <0.05 was considered significant. All statistical analyses were performed using IBM SPSS v.20 for Windows.

RESULTS

Participants' demographics, the genotype distributions, and allele frequencies of IBS-associated SNPs, rs5443 and rs1805124, were summarized in Table 2. The age range of subjects was 19-55 with a mean of 29.0±9.8 years (the mean age was 30.0±1.11 years for males, and 26.0±7.9 years for females). The majority of the subjects were male (n=328). The determination of wild type, heterozygous mutant, and homozygous mutant variants of all subjects was based on the amplification of the sequence from the 2nd PCR. Each DNA sample was subjected in parallel in two wells consisting of the wild and variant type primers set to determine their variant. For example, as can be seen in Figure 1B, the genotype of rs5443 and rs1805124 for S1 was pronounced as homozygous wild type since only the amplifications of wild type sequence occurred where the band only appeared in the wild primer well. Meanwhile, S4 possessed a heterozygous mutant genotype in rs5443 after the amplifications appeared in both wells but become homozygous mutant for rs1805124 due to the amplification that solely appeared in the mutant variant well as observed.

Table 2 exhibited the genotype distributions and allele frequencies of rs5443 and rs1805124 polymorphisms in the Malay population of Malaysia. The HWE for both SNPs did not deviate either in total or the gender subsets since their p-values were more than 0.05 suggesting no unexpected genetic drift or sampling bias occurred.

Table 1. List of primers used to the genotype-specific site of GNβ3 and SCN5A genes in the 1st PCR followed by the amplifications of rs5443 and rs1805124 in the 2nd PCR

SNP	1st PCR		2nd PCR	
	Sequence	Product size	Sequence	Product size
rs5443	*FW_GNB3* CTG ATC CCT GAC CCA CTT GC *RV_GNB3* AGT CCG AAA TGG GAG CTG A	349 bp	*FW_rs5443C (Wild type)* TCA TCT GCG GCA TCA CGT C**C** *FW_rs5443T (Variant type)* TCA TCT GCG GCA TCA CGT C**T** *RV_GNB3* AGT CCG AAA TGG GAG CTG A	208 bp
rs8105124	*FW_SCN5A* GGG TGC TCT AGC ATC ACA GG *RV_SCN5A* GAT GAA AAC AGC ACA GCG GG	245 bp	*FW_SCN5A* GGG TGC TCT AGC ATC ACA GG *RV_rs1805124A (Wild type)* GGA GAG CGA GAG CCA CC**A** *RV_rs1805124G (Variant type)* GGA GAG CGA GAG CCA CC**G**	225 bp

SNP: single nucleotide polymorphism, PCR: polymerase chain reaction, FW: forward, RV: reverse. The diluted amplicons of 1st PCR were subjected to the 2nd PCR. Every sample would underwent two sets of primers in the 2nd PCR, wild and variant types to determine the genotype variant. Band would appear on the gel only when the amplification took placed.

Table 2. Genotype distributions and allele frequencies of rs5443 and rs1805124 polymorphisms among Malays

	All (n=404)		Male (n=328)		Female (n=76)	
Age (years), mean±SD	29.0±9.8		30.0±1.1		26.0±7.9	
	rs5443	**rs1805124**	**rs5443**	**rs1805124**	**rs5443**	**rs1805124**
Observed genotype, n (%)						
WT	130 (32.2)	269 (66.6)	109 (33.2)	231 (70.4)	21 (27.6)	38 (50.0)
HM	195 (48.3)	120 (29.7)	161 (49.1)	88 (26.8)	34 (44.8)	32 (42.1)
MT	79 (19.5)	15 (3.7)	58 (17.7)	9 (2.8)	21 (27.6)	6 (7.9)
Allele frequency, (%)						
Wild	C- 56.3	A- 81.4	C- 57.8	A- 83.8	C- 50.0	A- 71.0
Mutant	T- 43.7	G- 18.6	T- 42.2	G- 16.2	T- 50.0	G- 29.0
Predicted genotype, n (%)						
WT	128 (31.7)	268 (66.3)	110 (33.4)	230 (70.2)	19 (25.0)	38 (50.0)
HM	199 (49.3)	122 (30.2)	160 (48.7)	89 (27.2)	38 (50.0)	31 (40.8)
MT	77 (19.0)	14 (3.5)	59 (17.9)	8 (2.6)	19 (25.0)	7 (9.2)
p	0.702	0.723	0.913	0.860	0.360	0.837

SD: standard deviation, SNP: single nucleotide polymorphism, WT: wild type, HM: heterozygous mutant, MT: mutant type

According to the table, the genotypes of rs5443; CC, CT, and TT, and rs1805124; AA, AG, and GG were found existed either in male or female Malays. For rs5443, the allele frequencies seemed to be slightly higher in the wild, C-56.3% (n=325) than in the mutant, T-43.7% (n=247) of Malays (p=0.702). However, Malay females derived equal frequencies (both n=55) for both alleles in rs5443, C-50.0%, T-50.0% (p=0.360). Meanwhile, the frequency of rs1805124 mutant allele G was low (18.6%, n=135, p=0.723) among Malays, which was found at 16.2% in the male subset (n=97, p=0.860) and 29.0% in the female subset (n=38, p=0.837). To the best of our knowledge, this study was the first to publish on the polymorphisms of rs5443 and rs1805124 among Malaysian ethnicities.

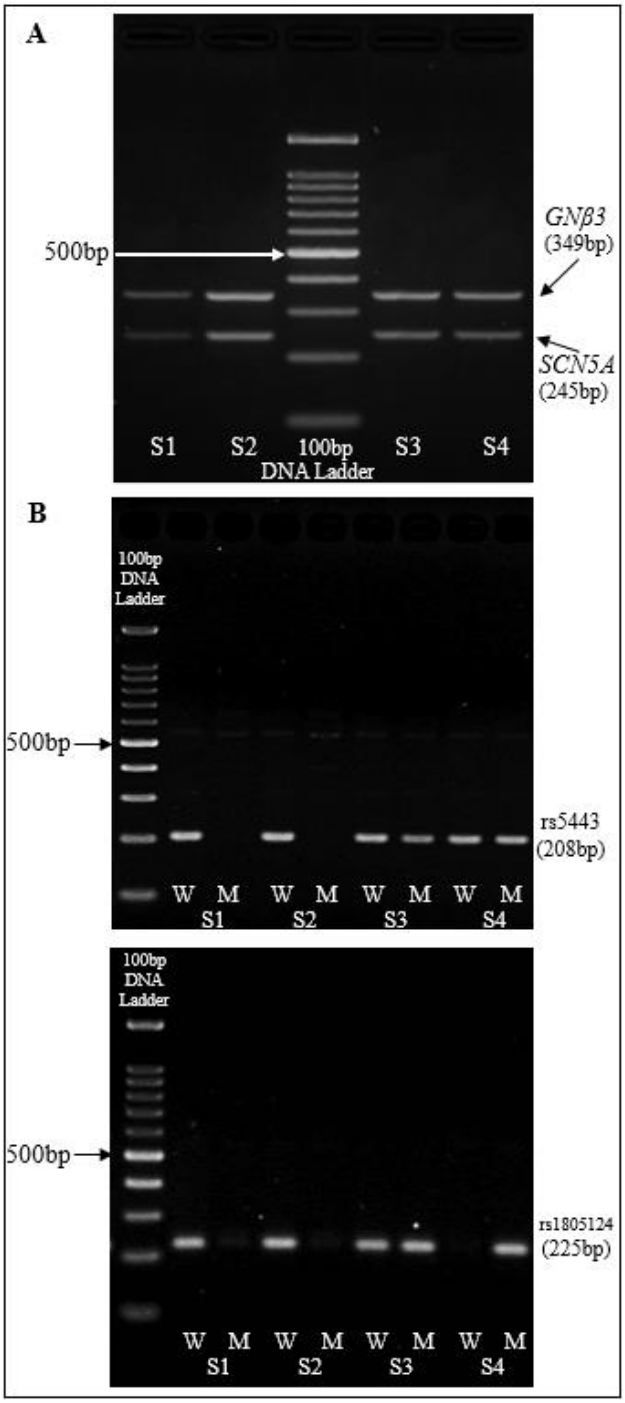

Figure 1. Band amplifications on the 1.5% gel electrophoresis from 1st and 2nd PCRs. **A)** The amplifications of GNβ3 and SCN5A interest regions from the 1st PCR. **B)** The amplifications of rs5443 and rs801524 sequences from the 2nd PCR.
Both SNPs were applying the same thermal condition, therefore they could be run simultaneously in a thermal cycler. W: well of wild type primer set, M: well of variant type primer set, Sn: subject number

DISCUSSION

This study has successfully genotyped the genes and their SNPs which susceptible to the IBS; GNβ3 (rs5443) and SCN5A (rs8015124) on 404 DNA samples of unrelated, healthy Malay of Malaysia. Tables 3 and 4 display the comparisons of the genotype and allele frequencies for rs5443 and rs1805124 in Malay from this study and other healthy ethnicities around the world. The data tabulated in the tables were depicted from the control group of comparatives, case-controlled studies related to the SNPs. According to the Table 3, the Quilombo and African-American dominated T allele more than C at the frequencies of 62.4% (n=184) and 71.6% (n=86) accordingly (18,19). The findings were aligned with the data from 1000 Genomes (1000genomes.org), Gambian Genome Variation (international genome.org/), and ALFA Allele Frequency (ncbi.nlm.nih.gov/snp/rs5443) projects where populations with African ancestors exhibited the T allele frequency higher with more than 70%.

The populations of Korean and Han Chinese reported similar prevalence of genotype and allele frequencies of rs5443 in two separate studies with CT genotype being the most common (Korean - 12,13 and Han Chinese - 20,21). On the other hand, Japanese are among the individuals who appeared to have comparable distributions of C and T allele frequencies in the populations (22), with C-50.7% (n=493) and T-49.3% (n=483), besides the Koreans (12,13) with average C-50.5% (n=441), T-49.6% (n=435). Meanwhile, Greek was the least to consist the frequency of mutant allele T among the populations, 13.8% (n=44) with only less than 5% (n=6) of them possessing TT in the individual (23). Notably, none of the ethnic groups from the 1000 Genomes or HapMap (ftp://ftp.ncbi.nlm.nih.gov/hapmap) or ALFA Allele Frequency databases has recorded the mutant allele T frequency as lowest as that observed in the Greek. Though the substitution of C to T in the rs5443 was pronounced to distract the intracellular signal transduction in the GI tract, the SNP may manifest a unique attribution in an ethnic or in the context of population gene heredity. However, more studies should be performed to solidify the findings.

According to the data presented in the Table 4, Taiwanese individuals exhibited the lowest frequency with a prevalence of only 2.6% (n=7) of the rs1805124 mutant allele, G (24). While Vietnamese population demonstrated the lowest occurrence of this allele among the populations in the ALFA Allele Frequency database, with a frequency of 8.7% (n=53). Ancient Sardinian also exhibited the least of mutant allele frequency as indicated by the database, 7.0% (n=2). However, the total subjects of the ethnic were very limited (n=30) making its statistical power of study reduced which could potentially affect the reliability and generalizability of the data. Simons Genome Diversity Project (ncbi.nlm.nih.gov/bioproject/PRJNA586841) documented that the African ancestors derived the highest allele mutant with 60.6% (n=131). Nevertheless, to the

best of our knowledge, till to date, no study has reported on the association of FGIDs or IBS prevalence and rs1805124 polymorphism in the population. On average, the ethnic groups displayed in the table seemed to exhibit lower frequencies (less than 23.5%) of the mutant allele rs1805124, G, compared to mutant allele rs5443, T, in the Table 3.

The polymorphisms of rs5433 and rs1805124 were also been documented to have significant correlations with the etiology of pathophysiology and clinical manifestations of functional dyspepsia (12,13,23), obesity (11,21,25), atrial fibrillation (26-28), sudden cardiac death (29-31) and many more. Due to the concern in the increasing cases of critical medicinal illnesses including the IBS, the SNP genotyping study is crucial to predict the DNA risk in the ethnic and find proper solutions for the health management and treatment inter-individually. However, compared to those monogenous hereditary diseases, it is undeniable that other non-genetic factors also play roles in causing related diseases in the individual which was not evaluated in this study. Furthermore, more than one gene has involved in IBS cases making the genetic factor is still

Table 3. Comparisons of genotype and allele frequencies of rs5443 in Malays and other reported healthy ethnics worldwide

Ethnic	n (M/F)	Age (years), mean±SD	Observed genotype, n (%)			Allele frequency (%)		Reference
			CC	CT	TT	C	T	
Malay	404 (328/76)	29.0±9.8	130 (32.2)	195 (48.3)	79 (19.5)	56.3	43.7	Present study
Caucasian (Israel&Spain)	340 (nd)	nd	132 (38.8)	169 (49.7)	39 (11.5)	63.7	36.3	(10)
Saudi	116 (nd)	nd	39 (33.6)	59 (50.9)	18 (15.5)	59.0	41.0	(11)
Korean	434 (167/267)	47.0±15.0	112 (25.8)	215 (49.5)	107 (24.7)	50.6	49.4	(12)
	148 (81/67)	10.8±3.9	35 (23.6)	79 (53.4)	34 (23.0)	50.3	49.7	(13)
Quilombo*	206 (98/108)	nd	22 (10.7)	111 (53.9)	73 (35.4)	37.6	62.4	(18)
African-American	95 (25/70)	20.5±2.7	9 (9.5)	36 (37.9)	50 (52.6)	28.4	71.6	(19)
Han Chinese	513 (187/326)	42.5±8.7	170 (33.1)	203 (39.6)	140 (27.3)	53.0	47.0	(20)
	130 (0/130)	nd	39 (30.0)	61 (46.9)	30 (23.1)	53.5	46.5	(21)
Japanese	649 (184/465)	nd	166 (25.6)	327 (50.4)	156 (24.0)	50.7	49.3	(22)
Greek	181 (68/113)	53.7±12.0	137 (75.7)	38 (21.0)	6 (3.3)	86.2	13.8	(23)
Taiwanese	505 (257/248)	39.7±12.3	89 (17.6)	263 (52.1)	153 (30.3)	43.7	56.3	(25)
West-Ukrainian	48 (18/30)	49.1±6.3	22 (45.8)	24 (50.0)	2 (4.2)	70.8	29.2	(33)
Caucasian (UK)$^{\alpha}$	427 (171/256)	50.0±16.0	208 (48.7)	171 (40.0)	48 (11.3)	68.7	31.3	(34)
Caucasian (Pisa)	225 (72/153)	42.2±11.0$^{\beta}$	110 (48.9)	99 (44.0)	16 (7.1)	70.9	29.1	(35)
Egyptian	222 (108/114)	38.5±12.4	70 (31.5)	118 (53.2)	34 (15.3)	58.1	41.9	(36)
Russian	200 (99/101)	36.0±8.2	95 (47.5)	90 (45.0)	15 (7.5)	70.0	30.0	(37)

SD: standard deviation, M/F: male/female; nd: not determined, *: isolated Brazilian populations of African ancestry, β: standard error of the mean; α: 96% of subjects were Caucasians

Table 4. Comparisons of genotype and allele frequencies of rs1805124 in Malays and other reported healthy ethnics worldwide

Ethnic	n (M/F)	Age (years), mean±SD	Observed genotype, n (%)			Allele frequency (%)		Reference
			TT	TC	CC	T	C	
Malay	404 (328/76)	29.0±9.8	269 (66.6)	120 (29.7)	15 (3.7)	81.4	18.6	Present study
Taiwanese	137 (46/91)	70.2±9.1	130 (94.9)	7 (5.1)	0 (0.0)	97.4	2.6	(24)
Chinese	296 (197/99)	58.4±10.8	281 (94.9)	11 (3.7)	4 (1.4)	96.8	3.2	(26)
	80 (18/62)	59.9±9.3	46 (57.5)	32 (40.0)	2 (2.5)	77.5	22.5	(31)
	81 (52/29)	62.5±7.6	60 (74.1)	20 (24.7)	1 (1.2)	86.4	13.6	(38)
Caucasian (Minnesota)	312 (236/76)	nd	197 (62.7)	103 (32.8)	12 (3.8)	79.7	20.3	(27)
Tunisian	106 (59/47)	64.0±13.0	69 (65.1)	32 (30.2)	5 (4.7)	80.2	19.8	(28)
Finnish	5043 (2352/2691)	51.4±14.1	3227 (64.0)	1613 (32.0)	201 (4.0)	80.0	20.0	(29)
Japanese	232 (135/97)	39.0±12.5	186 (80.2)	43 (18.5)	3 (1.3)	89.4	10.6	(30)
European-Caucasian	251 (185/66)	nd	170 (67.7)	70 (27.9)	11 (4.4)	81.7	18.3	(39)
Russian	411 (348/63)	37.2±17.1	253 (61.6)	143 (34.8)	15 (3.6)	78.9	21.1	(40)
Ihoba Chinese	100 (nd)	nd	77 (77.0)	23 (23.0)	0 (0.0)	88.5	11.5	(41)
Miao Chinese	98 (nd)	nd	71 (72.5)	25 (25.5)	2 (2.0)	85.2	14.8	(42)
Bai Chinese	200 (100/100)	nd	118 (59.0)	71 (35.5)	11 (5.5)	76.8	23.2	(43)
Jordanian	500 (320/180)	38.2±9.7	315 (63.0)	160 (32.0)	25 (5.0)	79.0	21.0	(44)

SD: standard deviation, M/F: male/female; nd: not determined

needed to be explored further. However, the investigation of the SNPs is worthy for the future benefits of society and should be proceeded through a genome-wide association approach and require multiple data of the ethnics to solidify the results.

CONCLUSION

IBS is a critical health concern as it can negatively affect the life value of oneself and increase the financial burden to the societies. This study has reported the genotype distributions and allele frequencies of rs5443 and rs1805124, the IBS-associated SNPs in the Malay population from Malaysia. Though pathophysiological, environmental, and psychological factors would also influence, the analysis of gene polymorphisms can always become the first hint to predict IBS susceptibility in individuals or in the ethnics. Based on the presented findings in the study and all cited studies above, it is understandable that the rs5443 and rs1805124 variants are diversified among populations and commonly present in healthy individuals and ethnicities. Nevertheless, the findings from this study have shed light on future research in order to explore more on the association of gene polymorphisms and the common health problem such as IBS and its causes. Future research should be conducted to determine the susceptibility of rs5443 and rs1805124 with the IBS-related causal and symptoms among local Malay patients to affirm the findings.

Ethics Committee Approval: This research was admitted by Universiti Sultan Zainal Abidin (UniSZA) Human Research Ethics Committee (UHREC), Terengganu, Malaysia (Reference number: UniSZA.C/2/UHREC/628-2/73; Date: 19/02/2019) and the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM), Kelantan, Malaysia (Reference number: USM/JEPeM/ 19020149; Date: 08/04/2019). Subjects were 404 healthy, unrelated Malays obtained and informed consent from Development of Ethno-pharmacogenetics Relatedness and Personalised (Grant no. 1001/PSK/8620013).

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Evaluation of Pediatric Immune Thrombocytopenia (ITP) Cases and Risk Factors for Chronic ITP - Single Center Experience

Pediatrik İmmün Trombositopeni (İTP) Vakalarının ve Kronik İTP için Risk Faktörlerinin Değerlendirilmesi - Tek Merkez Deneyimi

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ABSTRACT

Aim: Immune thrombocytopenia (ITP) is the most common acquired bleeding disorder in childhood. The study aimed to assess the demographic and clinical characteristics, and treatment responses and to evaluate their effects on chronicity in pediatric ITP cases.
Material and Methods: Primary ITP patients aged 1 month to 18 years, who were diagnosed and followed up in the Pediatrics Clinic of Tokat Gaziosmanpaşa University Hospital between January 2010 and December 2018, were retrospectively analyzed.
Results: Thirty-eight patients with a diagnosis of primary ITP were included in the study. The mean age of the patients was 94.3±53.4 (14-199) months. The female/male ratio was 1. Twenty (57.1%) patients had acute ITP, and 15 (42.9%) patients had chronic ITP. There was no significant difference between the acute ITP group and the chronic ITP group in demographic, clinical features, laboratory findings, and treatment responses. In the first 12 months, the number of admissions with a platelet count of <20 000 /mm^3, the number of admissions requiring treatment, and the rate of treatment given during follow-up were significantly higher in the chronic ITP group (p=0.001, p=0.001, and p<0.001, respectively).
Conclusion: To be aware of the risk factors for the development of chronic ITP will lead to the identification of high-risk patients, decisions about treatment and follow-up, and prevent unnecessary interventions and anxiety that may occur in the patient and his/her family. According to the results of this study, frequent relapses in the first year after the diagnosis of ITP may be considered a marker for chronic ITP.
Keywords: Child; acute immune thrombocytopenia; chronic immune thrombocytopenia; risk factors.

ÖZ

Amaç: İmmün trombositopeni (İTP) çocukluk çağının en sık görülen edinilmiş kanama bozukluğudur. Bu çalışmada, pediatrik İTP vakalarında demografik ve klinik özellikler ile tedavi yanıtlarının incelenmesi ve bunların kronikleşmeye olan etkilerinin değerlendirilmesi amaçlandı.
Gereç ve Yöntemler: Ocak 2010 ve Aralık 2018 tarihleri arasında Tokat Gaziosmanpaşa Üniversitesi Hastanesi Çocuk Sağlığı ve Hastalıkları Kliniği'nde tanı alan ve takip edilen, 1 ay ile 18 yaş arası primer İTP hastaları geriye dönük olarak incelendi.
Bulgular: Primer İTP tanısı olan 38 hasta bu çalışmaya dahil edildi. Hastaların yaş ortalaması 94,3±53,4 (14-199) ay idi. Kız/erkek oranı 1 idi. 20 (%57,1) hastada akut İTP, 15 (%42,9) hastada kronik İTP vardı. Akut İTP grubu ile kronik İTP grubu arasında demografik, klinik özellikler, laboratuvar bulguları ve tedavi yanıtları açısından anlamlı bir farklılık yoktu. İlk 12 ayda trombosit sayısı <20.000 /mm^3 olan başvuru sayısı, tedavi gerektiren başvuru sayısı ve takipte tedavi verilme oranı kronik İTP grubunda anlamlı olarak daha yüksekti (sırasıyla, p=0.001, p=0.001 ve p<0.001).
Sonuç: Çocuklarda primer İTP'de kronikleşme için risk faktörlerinin bilinmesi, yüksek riskli hastaların tanımlanarak takip ve tedavinin planlanmasına, gereksiz girişimlerin, hasta ve ailesinde meydana gelebilecek anksiyetenin önüne geçilmesine yardımcı olacaktır. Bu çalışmanın sonuçlarına göre, İTP hastalarında tanı sonrası ilk bir yıl içinde trombositopeni ataklarının sık görülmesi, kronik İTP için bir belirteç olarak kabul edilebilir.
Anahtar kelimeler: Çocuk; akut immün trombositopeni; kronik immün trombositopeni; risk faktörleri.

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INTRODUCTION

Immune thrombocytopenia (ITP) is an immune-mediated, acquired, common hematological disease characterized by decreased platelet count (<100 000 /mm3) and increased bleeding risk due to autoantibodies against platelets. ITP is classified as primary and secondary according to the presence of an underlying disease (1). Primary ITP is a diagnosis of exclusion and characterized by isolated thrombocytopenia in the absence of other causes which may be associated with thrombocytopenia such as systemic lupus erythematosus, Hepatitis C infection, or lymphoproliferative diseases (2). Another classification is based on the duration of the disease as newly diagnosed, persistent, or chronic ITP. Patients recovering from the disease within three months are defined as newly diagnosed/acute ITP whereas cases with persistent thrombocytopenia more than 12 months are defined as chronic ITP. Risk factors for chronic ITP were frequently studied in the literature and gender, age, degree of thrombocytopenia at admission, preceding viral infection or vaccination history, and sudden onset were found significant in some studies (3).

ITP is a benign disease and serious life-threatening bleeding such as intracranial hemorrhage in ITP patients is extremely rare, 0.6-1% (4-6). However, it is known that the disease is associated with some degree of anxiety and decreased quality of life especially in chronic ITP, both for the patient and his/her family (7-9). Therefore, identifying the risk factors and high-risk patients for chronic ITP and predicting the course of the disease is important for preventing unnecessary interventions and anxiety that may occur in the patient and his/her family.

In this study, we aimed to investigate the demographic, clinical, and laboratory characteristics, treatment responses, and risk factors for chronic ITP in children diagnosed and followed up in our center between 2010 and 2018.

MATERIAL AND METHODS

In this study, 38 patients aged between 1 month and 18 years who were diagnosed with primary ITP between January 2010 and December 2018 in Tokat Gaziosmanpaşa University Hospital, Department of Pediatrics were included. To create the list of patients, we performed a search via the International Classification of Diseases (ICD) codes. Codes covering 'purpura and other hemorrhagic conditions' (D69.0-D69.9) including primary ITP code (D69.3) were searched and the files of the patients with primary ITP were examined (Figure 1). Demographic information, clinical and laboratory findings, and treatments given to these patients were recorded. Among the platelet indices; mean platelet volume (MPV), platelet percentage in the blood (plateletcrit, PCT), platelet distribution width (PDW), the ratio of large platelets to normal ones (platelet large cell ratio, PLCR), and platelet mass index (PMI, platelet count multiplied by MPV) were evaluated. Exclusion criteria in the study were having the diagnosis in another center and secondary thrombocytopenia. The study was approved by the Ethics Committee of Tokat Gaziosmanpaşa University (04.12.2018, 276).

Statistical Analysis

IBM SPSS Statistics 19.0 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.) was used for statistical analysis. In addition to descriptive statistical methods (mean, standard deviation, frequency), the Chi-square test was used in the comparison of qualitative data between groups. The Shapiro-Wilk test was used to evaluate the normality of the data. Levene's test was used to determine the homogeneity. For comparing the means of quantitative variables between groups, independent samples t test for normally distributed variables and Mann-Whitney U test for non-normal distributed variables were used. Pearson correlation coefficient was used for the strength and direction of the linear relationship between the variables. A p value <0.05 was considered significant.

RESULTS

A total of 38 patients diagnosed with primary ITP were included in this study. The female/male ratio was 1. The mean age was 94.3±53.4 (14-199) months. Signs of bleeding were present in 34 (89.4%) of the patients at the time of admission, there was one patient with severe bleeding (menorrhagia). The most common physical finding was ecchymosis on the skin which was present in 18 (47.3%) patients. Preceding infection was detected in 21 (55.2%) patients and the most common was upper respiratory tract infection. The general characteristics of the patients were given in Table 1.

Records of three patients were not sufficient for determining the course and discriminating between acute and chronic ITP and these were excluded in the comparison of acute and chronic ITP groups due to the uncertainty of course. Among the remaining 35 patients, 20 (57.1%) had acute ITP, 15 (42.9%) had chronic ITP, and no patient had persistent ITP. Comparison between acute and chronic ITP groups revealed no significant difference in demographic, clinical, or laboratory parameters (Tables 2 and 3).

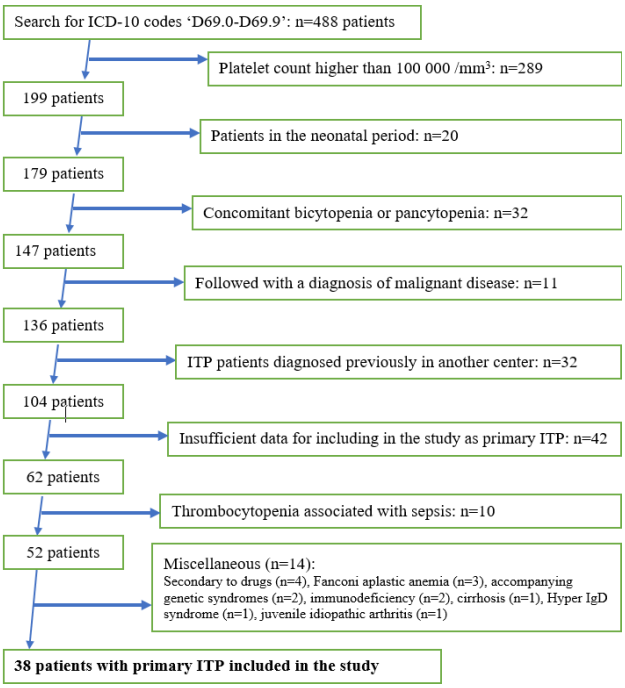

Figure 1. Identification of study patients
ITP: Immune thrombocytopenia, Ig: Immunoglobulin

Intravenous immunoglobulin (IVIG) was administered to 35 of 38 (92.1%) patients as initial therapy, and three patients were followed without pharmacological treatment. Among those 35 patients, three patients' records were not sufficient for discriminating between acute and chronic ITP. Of the remaining 32 patients, 19 (59.4%) were acute ITP, and 13 (40.6%) were chronic ITP. Two of the three patients who were followed up without treatment had chronic ITP, and one patient remained with acute ITP (Figure 2). In total, there were 20 (19 IVIG, 1 without treatment) patients in the acute ITP group, and 15 (13 IVIG, 2 without treatment) patients in the chronic ITP group. There was no significant difference between the acute and chronic ITP groups in IVIG doses (p=0.853), and platelet counts which were measured at 24, 48, and 72 hours after IVIG treatment (p values were 0.137, 0.610, and 0.498, respectively). In the chronic ITP group, during the first 12 months after diagnosis, the number of admissions with a platelet count under 20 000 /mm^3 and the number of admissions requiring treatment was significantly higher than in the acute ITP group (both p values were 0.001, Table 4).

In the chronic ITP group, during follow-up, 4 (26.7%) patients received IVIG treatment, 8 patients (53.3%) received IVIG + steroid treatment, and splenectomy was performed in 3 (20.0%) patients in whom remission was achieved. The rate of receiving medical treatment during follow-up in the chronic ITP group was significantly higher than in the acute ITP group (p<0.001).

In the study, in the acute ITP group, there was a strong negative correlation between the erythrocyte sedimentation rate (ESR) measured at the time of diagnosis and the platelet count at 72 hours after IVIG treatment (r=-0.980, p=0.012). In the same group, a strong

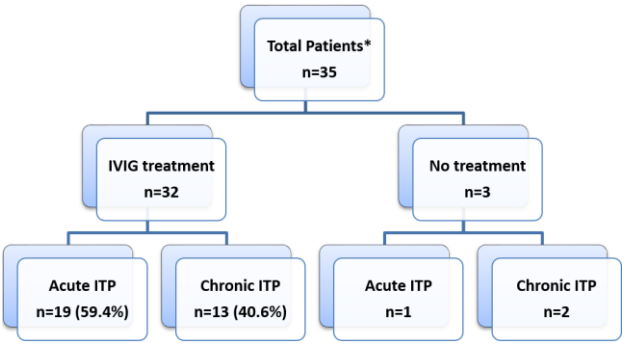

Figure 2. The course of the study patients according to initial treatment

*: The notes of three patients were not sufficient to decide about the course and they were not included in this figure. ITP: immune thrombocytopenia, IVIG: intravenous immunoglobulin

Table 1. Demographic and clinical features of the study patients (n=38)

Age groups, n (%)	
<24 months	5 (13.1)
24-72 months	9 (23.7)
>72 months	24 (63.2)
Gender (male), n (%)	19 (50.0)
Positive bleeding signs in PE, n (%)	34 (89.4)
Symptoms/signs at presentation, n (%)	
Petechia and purpura on skin	9 (23.7)
Ecchymosis on skin	18 (47.4)
Epistaxis	6 (15.8)
Menorrhagia	1 (2.6)
No bleeding sign	4 (10.5)
Thrombocytopenia detected incidentally	2 (5.3)
Fatigue	1 (2.6)
Abdominal pain	1 (2.6)
Previous infection history, n (%)	21 (55.2)
Upper respiratory tract infection	17 (44.8)
Acute gastroenteritis	3 (7.9)
Pneumonia	1 (2.6)
Season at presentation, n (%)	
Spring	8 (21.1)
Summer	11 (28.9)
Autumn	9 (23.7)
Winter	10 (26.3)

SD: standard deviation, min: minimum, max: maximum, PE: physical examination

Table 2. Comparison of demographic and clinical characteristics between acute and chronic ITP groups

	Acute ITP (n=20)	Chronic ITP (n=15)	p
Gender (male), n (%)	8 (40)	8 (53.3)	0.433
Age (months), mean±SD (min-max)	81.7±47.3 (17-158)	101.0±51.9 (14-175)	0.260
Age groups, n (%)			
<24 months	4 (20)	1 (6.7)	
24-72 months	4 (20)	4 (26.7)	0.696
>72 months	12 (60)	10 (66.7)	
Season at presentation, n (%)			
Spring	4 (20)	2 (13.3)	
Summer	6 (30)	5 (33.3)	0.967
Autumn	4 (20)	4 (26.7)	
Winter	6 (30)	4 (26.7)	
Positive bleeding signs in PE, n (%)	19 (95)	12 (80.0)	0.250
Symptoms/signs at presentation, n (%)			
Petechia and purpura on skin	6 (30)	1 (6.7)	
Ecchymosis on skin	10 (50)	8 (53.3)	0.346
Epistaxis	2 (10)	3 (20.0)	
Menorrhagia	1 (5)	0 (0.0)	
Previous infection history, n (%)	13 (65)	6 (40)	0.142

ITP: immune thrombocytopenia, SD: standard deviation, min: minimum, max: maximum, PE: physical examination

Table 3. Comparison of the laboratory parameters at the time of diagnosis between acute and chronic ITP groups

	Acute ITP (n=20)	Chronic ITP (n=15)	p
Hb (g/dl)	11.99±1.98	12.57±1.20	0.325
HTC (%)	35.12±5.70	37.41±3.06	0.169
PLT (/mm^3)	14139.00±12747.07	14182.67±11730.99	0.992
MPV (fL)	11.06±1.64	14.50	-
PMI	290.65±148.68	567.00	-
PCT (%)	0.03 (0.02-0.04) [0.01-0.05]	0.03 (0-0.06) [0-0.06]	0.999
PDW (fL)	19.58±4.14	20.60±2.39	0.759
CRP (mg/L)	3.19 (0.7-4.1) [0.1-22]	3.2 (0.6-5.6) [0.1-54]	0.900
ESR (mm/hour)	13.5 (3-23) [2-33]	7 (4-19) [2-45]	0.852
ALT (u/L)	19.73±14.67	15.19±4.10	0.325
AST (u/L)	31.46±21.98	28.00±6.07	0.600

ITP: immune thrombocytopenia, Hb: hemoglobin, HTC: hematocrit, PLT: platelet count, MPV: mean platelet volume, PMI: platelet mass index [PLT (/mm3) x MPV (fl)], PCT: plateletcrit, PDW: platelet distribution width, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, ALT: alanine aminotransferase, AST: aspartate aminotransferase, data were shown as mean±standard deviation or median (25th-75th percentile) [minimum-maximum]

Table 4. Comparison of IVIG doses and response to treatment between acute and chronic ITP groups

	Acute ITP (n=20)	Chronic ITP (n=15)	p
Dose of IVIG (g/kg)	0.88±0.15	0.87±0.20	0.853
PLT at 24th hour of IVIG treatment (/mm^3)	32.000±16.700	47.580±26.450	0.137
PLT at 48th hour of IVIG treatment (/mm^3)	75.830±45.340	91.600±81.450	0.610
PLT at 72nd hour of IVIG treatment (/mm^3)	145.690±82.300	119.650±75.890	0.498
Number of admissions with PLT <20 000 /mm^3 in the first 12 months	0 (0-0) [0-0]	2 (0-4) [0-14]	**0.001**
Number of admissions requiring treatment in the first 12 months	0 (0-0) [0-0]	1 (0-4) [0-10]	**0.001**

IVIG: intravenous immunoglobulin, ITP: immune thrombocytopenia, PLT: platelet, data were shown as mean±standard deviation or median (25th-75th percentile) [minimum-maximum]

positive correlation was found between the PCT value at the time of diagnosis and the platelet count at the 24th hour after IVIG treatment (r=0.925, p=0.008). In the chronic ITP group, a strong positive correlation was found between platelet counts at the time of diagnosis and at 24 and 48 hours after IVIG treatment (r=0.789, p=0.011, and r=0.743, p=0.022, respectively).

DISCUSSION

ITP is the most common cause of acquired thrombocytopenia in childhood and is characterized by shortened platelet lifespan due to immune-mediated platelet destruction in the reticuloendothelial system, isolated thrombocytopenia, and increased megakaryocytes in the bone marrow. Although primary ITP is a benign disease with a remission rate of 65-80% in children, it is known that it may become chronic at a rate of 25-30% (4). In the current study, demographic features, clinical characteristics, and laboratory findings at the time of diagnosis were not statistically different between acute and chronic ITP groups. In literature, possible factors related to the development of chronic ITP have been widely investigated. In a prospective study, Edslev et al. (10) found that symptoms lasting less than 2 weeks, age <10 years at diagnosis, preceding infection history, platelet count <5 000 /mm^3 at diagnosis, purpuric rash on mucous membranes and male gender were associated with improvement in the first 12 months in children with newly diagnosed ITP. In another study, abrupt onset and age under five years were found to be factors reducing the development of chronic ITP (11). In a systematic review and meta-analysis; female gender, age ≥11, no previous infection or vaccination history, insidious onset, platelet count ≥20 000 /mm^3 at diagnosis, and ANA positivity were associated with chronic ITP while mucosal bleeding was found to be related to decreased risk for chronic ITP (12). Similarly; female gender, age >10 years, no preceding infection, and platelet count ≥20 000 /mm³ at the time of diagnosis were found to be risk factors for chronic ITP in some recent studies (13-15).

The effect of initial treatment on the course of the disease has also been widely assessed in the literature. In general, it is considered that there is no relation between the treatment regimen and the natural course of ITP. However, in some recent studies, it is suggested that the agents used in treatment may have different effects. Some studies have shown that initial IVIG treatment reduces the development of chronic ITP (15-18) whereas others have found that it has an increasing effect (19) and some suggested it has no effect on chronicity (13,20,21). In a study from Thailand, it was found that pediatric ITP patients who were followed without treatment or who received steroids alone had less chronic ITP than those who received combined IVIG and methylprednisolone therapy (11). From Türkiye, Yıldız et al. (22) found that the relapse rate was lower in the untreated group than in the treated patients. In a

randomized controlled trial by Heitink-Polle et al. (18), initial treatment with IVIG was associated with decreased chronic ITP rate compared to follow-up without treatment. In our center, since IVIG was the first-line treatment and except for three cases followed without treatment vast majority of the patients were initially given IVIG, it was not possible to evaluate the effect of treatment on the development of chronic ITP.

In this study, among the patients who received IVIG initially, the rate of chronic ITP was found as 42.9% which is quite higher than the literature. This study was a retrospective study and the patients were identified by searching ICD codes. Probably the rate of correct recording of the ICD code and detection in the retrospective search was higher in chronic ITP patients who were admitted many times and received treatment with frequent relapses, compared to patients who were followed up without treatment and spontaneously improved. In addition, we think that some patients whose file notes were not sufficient and therefore not included in the study, may actually be acute ITP who were followed up without treatment and recovered spontaneously. All these factors may have contributed to the high rate of chronic ITP in this study.

In the current study, there was an important difference between acute and chronic ITP groups in the number of admissions. In the first 12 months after diagnosis, the number of admissions requiring treatment or admissions with a platelet count of <20 000 /mm3 was significantly higher in the chronic ITP group than the acute ITP group. Accordingly, frequent relapses after the diagnosis may be a predictor for chronic ITP.

It is known that some of the platelet indices are helpful in the diagnosis of ITP, and many studies have reported that they are useful in distinguishing between ITP and other causes of thrombocytopenia, especially hematological malignancies (23,24). However, there are few studies on the prognostic importance of these indices in ITP. In the study of Ahmed et al. (25), it was found that the rate of relapse and chronic ITP is lower in children if MPV is <8 fL at the time of diagnosis. Similarly, some adult studies have reported that MPV may be a marker for ITP relapse (26,27). In the study by Adly et al. (28), it was stated that the immature platelet fraction at admission was higher in chronic ITP patients than in acute ITP patients, and this parameter could be a marker for chronic ITP. In the current study, no significant difference was found between acute and chronic ITP groups for thrombocyte indices PDW, PCT, MPV, and PMI but this may be related to the small sample size of the study population. Since these indices are cheap and easy to work, we think that studies on the relation between these and chronic ITP with large patient groups will be valuable and promising.

In this study, we found a strong negative correlation between the ESR at admission and the platelet count at 72 hours after IVIG treatment in the acute ITP group. Although the acute-chronic course of the disease could not be known at presentation, these parameters may help predict the early IVIG response in patients.

This study has some limitations. The most important limitation is that it was a retrospective study which also led to the low number of study patients. We think some of the primary ITP patients missed out due to the shortcomings in the recording of ICD codes and inadequate file notes. Consequently, the rate of patients who received treatment and the rate of chronic ITP were higher than most of the studies in the literature.

CONCLUSION

In children with primary ITP being aware of the risk factors for the development of chronic ITP will lead to the identification of high-risk patients, decisions about treatment, prevent unnecessary interventions and anxiety that may occur in the patient and his/her family. According to the results of this study, frequent relapses in the first year after the diagnosis of ITP may be considered as a marker for chronic ITP. Prospective studies with large patient series are needed to determine clinical and laboratory risk factors more accurately.

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The Effect of Strabismus Surgery on Refractive Error and Anterior Segment Measurement

Şaşılık Cerrahisinin Refraksiyon Kusuru ve Ön Segment Ölçümleri Üzerine Etkisi

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ABSTRACT

Aim: This study aimed to investigate the effects of horizontal strabismus surgery on refractive error and anterior segment parameters.
Material and Methods: Fifty-four eyes of 27 patients were included in this study. Patients underwent repeated refraction measurements and anterior segment evaluation preoperatively, first week, first month, third month, and sixth month postoperatively. Patients were divided into three groups, those who underwent resection (group 1), recession (group 2), and healthy eyes without any surgical intervention (group 3).
Results: The mean age of the patients was 24.4±11.1 years and 14 (51.9%) were female. There was a statistically significant difference in central corneal thickness in group 2 before and after surgery (p=0.037). The mean central corneal thickness was highest in the first week with 548.14±40.42 µm and lowest in the first month with 541.50±41.75 µm after surgery. There was a statistically significant difference in cell density (p=0.004) and mean cell area (p=0.004) between groups 1 and 2 in the first week after surgery. The cell density level was statistically significantly higher in group 1 in all postoperative measurements. In addition, the mean cell area level was statistically significantly lower in group 1.
Conclusion: Strabismus surgery has an effect on anterior segment parameters in addition to correcting eye movements and visual axis. Depending on the type of intervention, the number of affected anterior segment parameters also varies. In the management of the patient in the postoperative period, the anterior segment structures should be carefully evaluated at each examination and the findings should be noted.
Keywords: Anterior segment; refraction changes; strabismus surgery.

ÖZ

Amaç: Bu çalışmada horizontal şaşılık cerrahisinin, refraksiyon kusuru ve ön segment parametrelerine olan etkilerinin araştırılması amaçlanmıştır.
Gereç ve Yöntemler: Bu çalışmaya 27 hastanın toplam 54 gözü dahil edildi. Hastalara cerrahi öncesi, cerrahi sonrası birinci hafta, birinci ay, üçüncü ay ve altıncı ayda tekrarlayan refraksiyon ölçümü ve ön segment değerlendirilmesi yapıldı. Hastalar, rezeksiyon (kısaltma) yapılanlar (grup 1), resesyon (geriletme) yapılanlar (grup 2) ve herhangi bir cerrahi müdahale geçirmemiş sağlıklı gözler (grup 3) olmak üzere üç gruba ayrıldı.
Bulgular: Hastaların yaş ortalaması 24,4±11,1 yıl ve 14'ü (%51,9) kadındı. Grup 2'de cerrahi öncesi ve sonrası dönemde merkezi kornea kalınlığında istatistiksel olarak anlamlı bir farklılık saptandı (p=0,037). Ortalama merkezi kornea kalınlığı ameliyat sonrası 548,14±40,42 µm ile birinci haftada en yüksek, 541,50±41,75 µm ile birinci ayda en düşüktü. Cerrahi sonrası birinci haftada, grup 1 ve 2 arasında, hücre yoğunluğu (p=0,004) ve ortalama hücre alanı (p=0,004) değerlerinde istatistiksel olarak anlamlı bir farklılık vardı. Cerrahi sonrası tüm ölçümlerde hücre yoğunluğu düzeyi grup 1'de istatistiksel olarak anlamlı derecede yüksekti. Ayrıca, ortalama hücre alanı düzeyi grup 1'de istatistiksel olarak anlamlı derecede düşüktü.
Sonuç: Şaşılık cerrahisi, göz hareketlerini ve görme eksenini düzeltmenin yanı sıra ön segment parametreleri üzerine de etkilidir. Müdahalenin tipine bağlı olmak üzere, etkilenen ön segment parametrelerin sayısı da değişmektedir. Cerrahi sonrası dönemdeki hastanın yönetiminde her muayenede ön segment yapıları dikkatli değerlendirilmeli ve bulgular not edilmelidir.
Anahtar kelimeler: Ön segment; refraksiyon değişiklikleri; şaşılık cerrahisi.

INTRODUCTION

The foundation of modern strabismus surgery dates back to 1839 when Johann Friedrich Dieffenbach performed the first successful strabismus surgery. Interestingly, this first successful strabismus surgery was also the surgery in which the first surgical complication was reported (1). The traditional view of strabismus surgery is that it is more difficult to plan than to perform. Nevertheless, some undesirable conditions, called complications, sometimes have little clinical significance and sometimes may affect the visual acuity, physical appearance, and future life of the patients (2).

Refractive changes are among the complications of strabismus surgery. This is mostly attributed to the fact that surgical interventions to the extraocular muscles change the vectorial forces transmitted to the cornea via the scleral pathway. Other theories on this subject are postoperative scleral wound healing, edema in the orbit and eyelids, change in ciliary body blood flow, and change in crystalline lens shape and curvature (3). In addition, if the anterior ciliary arteries are damaged during strabismus surgery, the supply of the anterior segment may be disrupted and changes in parameters may occur accordingly (4). Also, surface tension changes in adjacent tissues due to changes in muscle tension after surgery may result in changes in corneal and anterior segment measurements (5).

This study aimed to investigate postoperative changes in refractive error and anterior segment parameters after strabismus surgery.

MATERIAL AND METHODS

The study was conducted prospectively in the Department of Ophthalmology, Karadeniz Technical University, between October 01, 2017, and July 01, 2019. Patients who underwent surgery did not have other ocular surface disorders such as keratoglobus, keratoconus, and keratitis, were compliant, attended all follow-up visits regularly, and healthy volunteer participants without any history of ocular surgery or trauma were included. Participants with previous ocular surgery or monocular eye were excluded.

The study was conducted in accordance with the Declaration of Helsinki and the necessary approval was obtained from the Ethics Committee of Karadeniz Technical University (date: 17.07.2017, number: 124). In addition, written informed consent was obtained from the relatives of the patients in the pediatric period and all participants in the adult period.

Patients were divided into three groups, who underwent resection (group 1), recession (group 2), and healthy eyes without any surgical intervention (group 3).

All of the participants in three groups underwent binocular autorefractometry (Plusoptix A09, Nidek, Japan), best corrected visual acuity (BCVA) level (Snellen), anterior and posterior segment slit-lamp examination, prism covering test, and deviation and direction of strabismus, Non-contact tonometry (NT-530P, Nidek, Japan), optical biometry (AL-SCAN Optical Biometer, Nidek, Japan), specular microscopy (CEM-530, Nidek, Japan) and pupillometry (MonPack 2, Metrovision, France) measurements were evaluated.

Biometric measurements with a signal-to-noise ratio (SNR) >2 were considered reliable and recorded. During the biometric measurement, flat and steep keratometry values (K1 and K2, respectively), horizontal corneal diameter (limbus-to-limbus distance, LLM), anterior chamber depth (ACD), and axial length (AL) values calculated from the 2.4- and 3.3-mm optical zone to be obtained from the device screen were determined. The cell number, cell density (CD, cell number/mm^2), mean cell area (MCA, μm^2), standard deviation (SD, μm^2), coefficient of variation (CV, %), area of the largest cell (max, μm^2), area of the smallest cell (min, μm^2), hexagonality (Hex, %) and central corneal thickness (CCT, μm) of the corneal endothelium were recorded. Refractive error was determined in spherical equivalent (diopter, D). All measurements were performed by the same investigator using the same room conditions and the same instruments. During routine follow-up examinations at one week, one month, three months, and six months postoperatively, measurements were compared with the preoperative period.

Statistical Analysis

All data obtained from the participants were evaluated with IBM SPSS v.22.0 (IBM Corp., Armonk, NY, USA). The conformity of all data obtained in the study to normal distribution was evaluated by the Kolmogorov-Smirnov test. Continuous data conforming to normal distribution were presented as mean±standard deviation. The means of two dependent groups were compared by paired t-test, and the means of more than two dependent groups were compared by repeated measures ANOVA. In independent groups, the means of two groups were compared by Student's t-test, and the means of more than two groups were compared by one-way ANOVA. The statistical significance level was set at 0.05.

RESULTS

A total of 54 eyes of 27 patients were included in the study. There were 15 patients (7 males, 8 females) in Group 1 and 12 patients (6 males, 6 females) in Group 2. The mean age of the patients was 24.4±11.1 years. According to the type of strabismus, 18 patients had alternating exotropia (XT), 2 patients had monocular exotropia, 6 patients had alternating esotropia (ET) and 1 patient had monocular esotropia. Twenty-three patients had no systemic comorbidity, one patient had factor VII deficiency, one patient had epilepsy, one patient had goiter, and one patient had attention deficit hyperactivity disorder. The mean age of the participants in the control group (n=20) was 24.6±5.6 years and 10 (50%) were female (Table 1). The patient groups and the control group were similar in terms of age (p=0.562) and gender (p=0.978). Specular microscopic data showed no statistically significant difference between the groups in the preoperative period.

In intragroup analysis, no statistically significant difference was found between preoperative and postoperative measurements in group 1 (p=0.949). However, in group 2, there was a significant difference in CCT values between preoperative and postoperative measurements (p=0.037). The mean CCT was highest in the first week after surgery with 548.14±40.42 μm and lowest after surgery with 541.50±41.75 μm.

In inter-group analysis, the CD level was statistically significantly higher in group 1 in all postoperative

measurements. In addition, the MCA level was statistically significantly lower in group 1 (Table 2).

In optical biometry measurements, there was no statistically significant difference between the groups in the parameters measured in the preoperative and/or postoperative periods (Table 3).

In intra-group analysis, AL in group 1 was lowest in the third postoperative month with 23.07±0.68 mm and highest in the sixth postoperative month with 23.11±0.74 mm (p=0.022). In group 2, AL was lowest at one week postoperatively with 23.07±0.68 mm and highest at one month postoperatively with 23.71±1.38 mm (p=0.014). K1 value was highest at one month postoperatively with 42.44±1.58 D and lowest at six months postoperatively with 42.20±1.88 D (p=0.047). K2 value was highest at the first postoperative week with 44.31±1.57 D and lowest at the sixth postoperative month with 43.85±1.78 D (p=0.003). ACD was lowest at the first postoperative week with 3.52±0.34 mm and highest at the preoperative period with 3.58±0.34 mm (p=0.002). LLM was highest in the preoperative period with 11.98±0.49 mm and lowest in the sixth postoperative month with 11.10±0.48 mm (p<0.001). In pupillometry measurements, pupil diameter (PD) was lowest in group 1 with 6.02±0.77 mm in the first postoperative month and highest with 6.71±0.71 mm in the third postoperative month (p=0.005). In all other intragroup and intergroup analyses, there was no statistically significant difference between the groups in the parameters measured in the preoperative and/or postoperative periods (Table 4).

Table 1. Demographic data of the participants

	Group 1 (n=15)	Group 2 (n=12)	Group 3 (n=20)	p
Age (years)	24.3±9.2	24.7±7.1	24.6±5.6	0.562
Gender, n (%)				
Male	7 (46.7)	6 (50)	10 (50)	0.978
Female	8 (53.3)	6 (50)	10 (50)	

DISCUSSION

Although the interventions performed in strabismus surgery are on extraocular muscles, some changes in the corneal endothelium, biometric measurements of the anterior segment, iris, and pupillary reaction have been reported in the postoperative period. There are several causes of corneal stress during and after extraocular muscle surgery. The first of these is anterior segment ischemia (6). The presence of ischemia risk has caused the number of muscles to be intervened during strabismus surgery to be limited (7). In the case of postoperative ischemia, the corneal endothelial cell layer does not have the ability to repair the damage. Therefore, the repair is achieved by expansion of the remaining cells, amitotic nucleus division, migration, and formation of the rosette phenomenon (8,9). The second cause of corneal stress after surgery of extraocular muscles is the development of inflammation and its spread to the anterior chamber. As a result, corneal endothelium may be affected, with inflammation treatment being among the causes (10).

Table 2. Specular microscopy data of the participants

	Preoperative	1st week	1st month	3rd month	6th month	p
Cell Number						
Group 1	152.09±36.09	153.18±49.16	137.33±47.25	148.78±32.10	154.88±31.68	0.720
Group 2	122.73±41.21	123.82±39.09	128.95±42.91	119.62±36.18	122.10±37.79	0.475
Group 3			116.76±28.60			
p	0.116	0.154	0.843	0.127	0.120	
Cell Density (/mm²)						
Group 1	2908.82±284.09	2941.27±309.41	2911.67±301.48	2981.78±262.69	2924.25±240.50	0.717
Group 2	2723.59±309.17	2688.18±269.39	2698.64±236.61	2727.29±260.35	2760.55±290.15	0.164
Group 3			2689.62±270.44			
p	0.132	**0.010**	**0.024**	**0.007**	**0.008**	
Mean Cell Area (µm²)						
Group 1	351.64±29.84	341.73±32.35	345.00±32.98	346.33±26.79	341.63±23.45	0.754
Group 2	371.73±42.48	375.86±39.91	373.32±33.38	369.86±35.48	365.45±37.44	0.107
Group 3			375.38±38.20			
p	0.149	**0.013**	**0.027**	**0.009**	**0.008**	
Hexagonality (%)						
Group 1	69.18±4.05	69.64±5.73	65.56±10.32	67.78±4.89	68.38±4.72	0.820
Group 2	66.64±4.77	68.27±5.57	68.14±5.60	68.10±6.47	65.60±6.75	0.105
Group 3			67.86±4.13			
p	0.412	0.252	0.963	0.829	0.460	
Central Corneal Thickness (µm)						
Group 1	530.82±29.11	529.27±39.52	539.00±37.96	536.11±35.08	534.25±38.92	0.949
Group 2	546.41±42.14	548.14±40.42	541.50±41.75	543.62±42.65	544.60±40.22	**0.037**
Group 3	544.19±38.71	544.14±38.66	544.00±39.2	543.65±40.58	545.30±40.27	0.921
p	0.593	0.390	0.924	0.876	0.742	

Table 3. Optical biometry data of the participants

	Preoperative	1st week	1st month	3rd month	6th month	p
Axial Length (mm)						
Group 1	23.08±0.66	23.13±0.62	23.11±0.62	23.07±0.68	23.11±0.74	**0.022**
Group 2	23.67±1.39	23.07±0.68	23.71±1.38	23.63±1.40	23.71±1.39	**0.014**
Group 3			23.58±0.95			
p	0.375	0.496	0.433	0.472	0.490	
Anterior Chamber Depth (mm)						
Group 1	3.76±0.16	3.72±0.23	3.71±0.20	3.67±0.23	3.70±0.22	0.927
Group 2	3.58±0.34	3.52±0.34	3.55±0.34	3.53±0.33	3.53±0.35	**0.002**
Group 3			3.54±0.36			
p	0.248	0.180	0.529	0.436	0.479	
Flat Keratometry (D)						
Group 1	42.71±0.73	42.77±0.70	42.86±0.77	42.70±0.89	42.68±0.68	0.170
Group 2	42.37±1.79	42.33±1.63	42.44±1.58	42.26±1.77	42.20±1.88	**0.047**
Group 3			42.41±1.95			
p	0.642	0.468	0.525	0.553	0.414	
Steep Keratometry (D)						
Group 1	43.53±1.05	43.69±1.09	43.93±1.17	43.86±1.28	43.71±1.09	0.660
Group 2	43.84±1.70	44.31±1.57	44.14±1.60	43.90±1.70	43.85±1.78	**0.003**
Group 3			43.41±1.76			
p	0.601	0.196	0.251	0.342	0.617	
Limbus-to-Limbus Distance (mm)						
Group 1	12.27±0.32	12.15±0.52	12.13±0.33	12.17±0.26	12.16±0.32	0.756
Group 2	11.98±0.49	11.65±0.56	11.87±0.47	11.96±0.49	11.10±0.48	**<0.001**
Group 3			11.96±0.44			
p	0.081	0.070	0.251	0.288	0.410	

Table 4. Pupillometry data of the participants

	Preoperative	1st week	1st month	3rd month	6th month	p
0 Candela/ m^2 (Luminance)						
Group 1	6.34±0.41	6.30±0.43	6.02±0.77	6.71±0.71	6.63±0.51	**0.005**
Group 2	6.40±0.65	6.29±0.74	6.29±0.65	6.08±0.79	6.22±0.64	0.538
Group 3			6.25±0.61			
p	0.704	0.751	0.343	0.062	0.164	
1 Candela/ m^2 (Luminance)						
Group 1	5.30±0.63	5.48±0.75	5.20±0.87	5.64±1.21	5.58±0.99	0.274
Group 2	5.50±0.76	5.34±0.85	5.25±1.01	4.84±0.88	5.20±0.90	0.095
Group 3			5.26±0.74			
p	0.498	0.429	0.353	0.144	0.803	
10 Candela/ m^2 (Luminance)						
Group 1	4.06±0.79	4.42±0.77	3.78±0.58	4.22±0.91	4.18±1.06	0.104
Group 2	4.25±0.83	4.08±0.84	3.97±0.75	3.78±0.87	3.98±0.79	0.236
Group 3			4.10±0.75			
p	0.779	0.167	0.364	0.357	0.831	
100 Candela/ m^2 (Luminance)						
Group 1	2.85±0.37	2.81±0.23	2.64±0.19	3.02±0.51	2.73±0.21	0.166
Group 2	2.87±0.43	2.77±0.34	2.70±0.29	2.71±0.41	2.80±0.59	0.638
Group 3			2.77±0.40			
p	0.634	0.323	0.503	0.259	0.882	
200 Candela/ m^2 (Luminance)						
Group 1	2.53±0.22	2.51±0.19	2.40±0.12	2.57±0.27	2.40±0.13	0.599
Group 2	2.55±0.35	2.48±0.24	2.43±0.19	2.40±0.19	2.48±0.40	0.170
Group 3			2.47±0.23			
p	0.664	0.401	0.846	0.236	0.913	

In a study by Denis and Toesca (11) evaluating endothelial cell density, pleomorphism, and polymegatism by non-contact specular microscopy in children undergoing strabismus surgery, it was revealed that strabismus surgery did not result in a significant decrease in endothelial cell number, but pleomorphism changes were seen in aggressive interventions.

Gusek-Schneider et al. (12) evaluated endothelial cell density in eyes in which strabismus surgery was performed and found no significant change in endothelial cell density when the preoperative and postoperative periods were compared.

In our study, no significant corneal endothelial cell change was detected in specular microscopy measurements before and after surgery in the patient group. However, in group 2, central corneal thickness increased in the early postoperative period. This increase disappeared in the sixth month after surgery and there was no statistically significant difference with the preoperative period. Therefore, although the effects of strabismus surgery on anatomical structures differ according to the periods, it is necessary to follow up to determine whether the changes detected are permanent or temporary and to avoid making decisions in the early period.

Emre et al. (13) evaluated the change in anterior segment parameters after horizontal strabismus surgery performed in 18 eyes of 12 patients. However, they did not find a statistically significant change between the preoperative and postoperative periods.

Noh et al. (5) investigated anterior segment parameters and refractive changes in eyes in which external rectus surgery was performed and reported statistically significant changes in spherical equivalent, mean keratometry values, corneal astigmatism, anterior chamber volume, central and peripheral anterior chamber depth values in the first week after surgery. In the first month after surgery, observed that the changes in other parameters except the spherical equivalent gradually decreased.

Hutcheson KA (14) mentioned the importance of factors related to suturing and muscle placement techniques during surgery in the case of postoperative astigmatism. He theorized that if a muscle is sutured too close to the limbus or tied by resection causing excessive tension, the corneal or scleral curvature may change.

The diopter and axis of corneal astigmatism appear to change in eyes undergoing strabismus surgery. In a study by Karakosta et al. (15), a mean astigmatism difference of 0.43 D was observed between the patient and control groups after surgery. Furthermore, a 0.50 D astigmatism change was found in both the lateral and medial rectus muscle groups. Therefore, these changes should be considered when planning surgery to prevent clinically insignificant astigmatism from becoming clinically significant.

In a study by Mezad-Kours et al. (16) in 31 eyes of 22 patients who underwent strabismus surgery in adulthood, a postoperative spherical equivalent myopic shift and a change in the direction of rule-compliant astigmatism were observed. The induced surgical refractive change was clinically significant (≥0.5 D) in 11 eyes of the 9 patients (40.9% of patients).

In our study, axial length measurements in group 1 and most of the parameters (AU, K1, K2, ACD, WWD) in group 2 were found to be significantly different in optical biometric measurements in the preoperative and postoperative period in the patient group. In accordance with the literature, anterior segment changes are observed in the postoperative period in patients undergoing both resection and recession surgery. One of the main anatomical goals of strabismus surgery is to achieve parallelism of the visual axes. Our surgeries should provide functional achievements as well as anatomical success. While evaluating the functional achievements, the effects of surgery should be considered and caution and caution should be taken about additional interventions and/or treatment recommendations to be made in the early period.

The small number of patients in our study is one of the main shortcomings. In addition, the inability to perform anterior segment angiography to objectively assess the effect on anterior segment circulation due to muscle intervention is another shortcoming. Since our institution did not have a corneal topography device during the study period, this aspect of the effects could not be evaluated. However, the prospective nature of our study is one of its strengths.

It is not known whether the results in future studies will be similar to the six-month measurement results in our study. Therefore, prospective studies with a larger number of cases, longer duration, and wider participation may contribute to obtaining more detailed results on this subject.

CONCLUSION

Strabismus surgery is effective on anterior segment parameters as well as correcting eye movements and visual axis. In the management of the patient in the postoperative period, the anterior segment should be carefully evaluated at each examination, the findings should be noted and the patients should be followed closely.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Karadeniz Technical University (17.07.2017, 124).

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The Relationship of Chronic Diseases with Anxiety and Depression in Patients Over 65 Years of Age

65 Yaş Üstü Hastalarda Kronik Hastalıkların Anksiyete ve Depresyon ile İlişkisi

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ABSTRACT

Aim: With the increasing life expectancy, the elderly population is gradually increasing. Considering the difficulty in recognizing and treating mental disorders in the elderly, clinicians should be careful about risk factors. This study aimed to investigate the relationship of chronic diseases with depression and anxiety levels and to raise awareness for mental illnesses in this age group.

Material and Methods: This study was conducted with 100 patients over 65 years of age. Patients with a diagnosis of psychiatric illness and using psychotropic drugs for the last 6 months were not included in the study. After the sample of the study was formed, the sociodemographic data form prepared by the researchers and the Hospital Anxiety and Depression Scale were applied to the patients.

Results: Anxiety levels were found to be significantly higher in female patients (p=0.032). A positive correlation was found between age and depression levels (r=0.225, p=0.025). No statistically significant difference was found in anxiety and depression levels between the patient groups with and without chronic disease (p=0.122, and p=0.668, respectively).

Conclusion: Chronic diseases, duration of the disease, and use of medication were not found to be associated with anxiety and depression levels, while anxiety levels were found to be higher in female patients, and also a significant positive correlation was found between age and depression levels. Mental disorders are a subject that needs to be examined in detail in elderly patients. The mental illnesses of elderly patients should not be ignored and should always be considered by clinicians.

Keywords: Elderly; depression; anxiety; chronic disease.

ÖZ

Amaç: Beklenen yaşam sürelerinin artması ile birlikte yaşlı nüfusu giderek artmaktadır. Yaşlılarda ruhsal hastalıkların tanınması ve tedavi edilmesinin zorluğu göz önünde bulundurulduğunda risk faktörleri açısından klinisyenlerin dikkatli olması gerekmektedir. Bu çalışma kronik hastalıkların depresyon ve anksiyete düzeyleri ile ilişkisini araştırmak ve bu yaş grubunda ruhsal hastalıklara yönelik farkındalık yaratmayı amaçlamaktadır.

Gereç ve Yöntemler: Bu çalışma 65 yaş üstü olan 100 hasta ile yapılmıştır. Son 6 aydır psikiyatrik hastalık tanısı olan ve psikotrop ilaç kullanımı olan hastalar çalışmaya dahil edilmemiştir. Çalışmanın örneklemi oluşturulduktan sonra, hastalara araştırmacılar tarafından hazırlanmış olan sosyodemografik veri formu ve Hastane Anksiyete ve Depresyon Ölçeği uygulanmıştır.

Bulgular: Kadın hastalarda anksiyete düzeylerinin anlamlı olarak daha yüksek olduğu bulunmuştur (p=0,032). Yaş ve depresyon düzeyleri arasında pozitif yönde anlamlı bir korelasyon olduğu saptanmıştır (r=0,225, p=0,025). Kronik hastalığı olan ve olmayan hasta grupları arasında anksiyete ve depresyon düzeyleri bakımından istatiksel olarak anlamlı bir farklılık saptanmamıştır (sırasıyla, p=0,122 ve p=0,668).

Sonuç: Kronik hastalıklar, hastalığın süresi ve ilaç kullanımı anksiyete ve depresyon düzeyleri ile ilişkili bulunmazken kadın hastalarda anksiyete düzeyi daha yüksek bulunmuş ve ayrıca yaşla depresyon düzeyleri arasında da pozitif yönde anlamlı bir korelasyon saptanmıştır. Ruhsal bozukluklar yaşlı hastalarda detaylı bir şekilde incelenmesi gereken bir konudur. Yaşlı hastaların ruhsal hastalıkları göz ardı edilmemeli ve klinisyenler tarafından her zaman dikkate alınmalıdır.

Anahtar kelimeler: Yaşlı; depresyon; anksiyete; kronik hastalık.

INTRODUCTION

The elderly population is increasing all over the world. Along with the increasing elderly population, chronic physical and mental diseases are also increasing. The elderly may have more than one chronic physical illness. Physical and social problems that occur with aging can lead to psychosocial crises (1). 15% of the world's population is elderly (2). With aging, the person's health problems increase, social relations decrease, and therefore the person may have mental illnesses. It is difficult for the elderly to both cope with the diseases and maintain their lives (3). Chronic diseases also increase the frequency of psychiatric diseases such as depression and anxiety in these patients. Depression and anxiety in the elderly can often be overlooked. With the decrease in social life and increase in life problems, a tendency to depression and anxiety can be seen (4). However, in elderly patients, not being able to recognize depression and anxiety or noticing it late affects the treatment negatively (5). Clinicians need to be more careful in the treatment of chronic diseases of elderly individuals. Patients should be evaluated psychiatrically, and if necessary, they should be referred to a psychiatrist (6). Medical and family support are important to maintain the required life quality. Interdepartmental cooperation is required in terms of both providing psychoeducation to this patient group and treating psychiatric diseases (7). Anxiety and depressive symptoms may occur during the course of chronic physical illnesses. These symptoms, which are sometimes not obvious, can often be overlooked and not recognized. However, these symptoms may adversely affect the treatment of chronic physical disease. Therefore, early diagnosis and treatment of such symptoms will positively affect the patient's life quality and reduce hospitalizations (8). Many studies have investigated the psychiatric symptoms of the elderly with chronic diseases. However, the etiology, clinical appearance, and treatment approaches of mental symptoms in elderly patients with chronic physical disease have not been fully revealed. Therefore, in this study, we aimed to contribute to the existing literature by investigating the relationship between the diagnosis of the disease, sociodemographic characteristics, and anxiety and depressive symptom levels in elderly patients with chronic physical diseases.

MATERIAL AND METHODS

The study was conducted with patients over the age of 65 who applied to the Internal Medicine Clinic between March 2022 and June 2022. The sample size of the research was calculated with G*Power v.3.1. It was concluded that the total sample size should be 88 with 5% type I error, 90% test power, and 0.7 effect size (9,10). 113 patients were included in the study, considering that there may be missing data. However, 13 patients could not complete the scales, so a total of 100 patients were included in the study sample. First of all, the patients were informed about the study, their consent was obtained, and a voluntary consent form was signed. The patient group was literate and had the academic capacity to fill in the scales. Patients with a diagnosis of psychiatric illness and using psychotropic drugs for the last 6 months, who did not have enough education to understand the tests, who had mental or social retardation, and who did not approve to participate in the study were not included in the study. A sociodemographic data form prepared by the researchers and the Hospital Anxiety and Depression Scale were applied to the patients. Ethics committee approval of the study was obtained (24.03.2022, 75). All practices in this study were made in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Sociodemographic Data Form

Basic sociodemographic and clinical data such as age, gender, education, marital status, disease duration, presence of chronic illness, and diagnosis were questioned. It was prepared by the researchers for this study.

Hospital Anxiety and Depression Scale

This scale consists of 14 items, and is a reliable scale measuring depression and anxiety levels, especially in hospitalized patients. The scale was developed by Zigmond in 1983 (11). Turkish validity and reliability of the scale was conducted by Aydemir et al. (12). In the validity and reliability analysis of the scale, the Cronbach alpha value was found as 0.85 for the anxiety subscale and 0.77 for the depression subscale. The cut-off score for the anxiety subscale was 10 and the cut-off score for the depression subscale was 7. Even-numbered questions of the scale show depression level, while odd-numbered questions show anxiety level. High scores on the scale are associated with increased levels of depression and anxiety (13). It was found that the scale had acceptable internal consistency and did not vary between genders in a study conducted between the ages of 65 and 80 (14).

Statistical Analysis

IBM SPSS v.25 program was used in the statistical analysis of the research data. Descriptive statistics were presented as mean±standard deviation, median, 25^{th}-75^{th} percentile, minimum, maximum, and percentage. The conformity of the variables to the normal distribution was examined using Kolmogorov-Smirnov test. Because of the non-normal distribution of the data, The Mann-Whitney U test was used between two independent groups, and Spearman correlation was used to analyze the correlation. The statistical significance level was accepted as 0.05.

RESULT

A hundred patients were included in the study. Of the patients, 67 were female, 33 were male, the youngest was 65 years old, and the oldest was 89 years old. The mean age was 72.3±6.3 years. While 82 of the patients were primary school graduates, 11 were high school graduates, and 7 were university graduates. In addition, 74 of them were married, 3 of them were single, 20 of them were widowed, and 3 of them were divorced. While 79 of the patients had at least one chronic disease, 21 of them did not have any chronic disease. 64 patients had hypertension, 23 patients had coronary artery disease, 6 patients had chronic obstructive pulmonary disease, 28 patients had diabetes mellitus, 12 patients had hypothyroidism, 19 patients had hypercholesterolemia, 2 patients had breast cancer, 3 patients had osteoporosis, 1 patient had benign prostate hypertrophy, 1 patient had chronic urticarial, and 1 patient had allergic rhinitis. The median duration of chronic disease was 10 (range, 1-50) years. Of the patients,

79 were using drugs. The total number of drugs taken in a day was at least 1 and a maximum of 10, and the median number of drugs used by the patients was 2.

When the relationship between the clinical characteristics of the patients and their anxiety and depression level was examined, a significant positive correlation was found between age and depression level (r=0.225, p=0.025). No significant correlation was found between the duration of the chronic disease and the number of drugs used, and the levels of anxiety and depression (Table 1).

While the depression level of the patients did not differ significantly according to gender (p=0.065), anxiety level were found to be significantly higher in female than in male patients (p=0.032). When the patients were grouped as having or not having a chronic disease, no statistically significant difference was found between the groups with or without the disease both in anxiety (p=0.122) and depression (p=0.668) levels (Table 2).

Similarly, when chronic diseases were considered one by one, no difference was found in anxiety and depression levels according to the presence of each disease (Table 3).

DISCUSSION

In the present study, chronic diseases and clinical features of patients over the age of 65, who constitute an important patient group in terms of health services, were examined. In the study, 79% of patients over 65 years of age who applied to the outpatient clinic had at least one chronic disease. The median number of drugs used by the patients per day was 2, and the most common chronic disease was hypertension with a rate of 64%. In the literature, the rates of chronic diseases in patients over 65 years of age are similar. In a study conducted by Dişçigil et al. (15) in primary care, 82.2% of patients over the age of 65 were found to have at least one chronic disease, and in accordance with our study, the most common chronic disease in patients was found to be hypertension with a rate of 55.6%. In the study of Taşkın Şayir et al. (16), the most common chronic disease was hypertension with a rate of 80%, and 91% of the patients in the study had been using drugs for at least 3 months. In another study, the rate of chronic disease in people over the age of 65 was 66.1% (17). In a large sample study, the rate of polypharmacy in people older than 60 years was 14.9% (18). In another study, 83% of patients had one or more chronic diseases. 86% of the patients used at least one drug related to their chronic diseases (5). Chronic disease and excessive use of drugs, which are frequently seen in the elderly population, can cause mental illnesses. The most common psychiatric disorders in elderly patients are depressive disorder and dementia (19). However, anxiety disorders are also common in elderly patients and cause health problems in the elderly population (20). Health problems of elderly patients increase with the presence of mental illness in old age. These patients need more medical support (21). In a meta-analysis, the most common mental disorder in elderly patients was found to be a major depressive disorder (22). However, in another study, it was found that

Table 1. The correlation between clinical characteristics of the patients and anxiety and depression levels

	HADS-A		HADS-D	
	r_s	p	r_s	p
Age	0.098	0.334	0.225	**0.025**
Duration of disease	0.066	0.512	0.028	0.780
Number of used drugs	0.036	0.719	-0.051	0.611
Number of chronic diseases	0.050	0.620	0.036	0.720

HADS-A: hospital anxiety and depression scale-anxiety, HADS-D: hospital anxiety and depression scale-depression, r_s: Spearman's rho

Table 2. Comparison of anxiety and depression levels in terms of gender and presence of chronic disease

	Gender		p
	Female (n=67)	Male (n=33)	
HADS-A	7 (5) [0-18]	4 (6) [1-18]	**0.032**
HADS-D	5 (6) [0-16]	7 (6) [0-14]	0.065

	Chronic Disease		p
	Yes (n=79)	No (n=21)	
HADS-A	7 (6) [0-18]	5 (6) [0-15]	0.122
HADS-D	7 (6) [0-16]	6 (7) [0-13]	0.668

HADS-A: hospital anxiety and depression scale-anxiety, HADS-D: hospital anxiety and depression scale-depression, *: median (interquartile range) [minimum-maximum]

Table 3. Comparison of anxiety and depression levels of the patients according to the chronic diseases

	HT + (n=64)	HT - (n=36)	
HADS-A	7 (5) [1-18]	6 (6) [0-18]	0.097
HADS-D	7 (6) [0-16]	6 (7) [0-14]	0.347

	CAD + (n=23)	CAD - (n=77)	
HADS-A	7 (7) [0-18]	7 (6) [0-15]	0.696
HADS-D	6 (6) [0-13]	7 (6) [0-16]	0.506

	COPD + (n=6)	COPD - (n=94)	
HADS-A	5 (7) [2-12]	7 (6) [0-18]	0.590
HADS-D	3.5 (7.75) [0-10]	6.5 (6) [0-16]	0.210

	DM + (n=28)	DM - (n=72)	
HADS-A	7 (5.75) [1-18]	7 (6) [0-18]	0.700
HADS-D	6.5 (5) [0-14]	6 (6) [0-16]	0.823

	HTr + (n=12)	HTr - (n=88)	
HADS-A	7 (9.25) [1-17]	7 (6) [0-18]	0.655
HADS-D	8 (8.5) [1-14]	6 (6) [0-16]	0.987

	HC + (n=19)	HC - (n=81)	
HADS-A	3 (7) [1-14]	6 (7) [0-12]	0.259
HADS-D	7 (5) [0-18]	7 (6) [0-16]	0.508

HADS-A: hospital anxiety and depression scale-anxiety, HADS-D: hospital anxiety and depression scale-depression, HT: hypertension, CAD: coronary artery disease, COPD: chronic obstructive pulmonary disease, DM: diabetes mellitus, HTr: Hypothyroidism, HC: hypercholesterolemia, *: median (interquartile range) [minimum-maximum]

anxiety disorder was most common in the elderly and its incidence was 38.2%. In the same study, the rate of depression was 21.7%, and the frequency of co-occurrence of depression and anxiety was 21.1% (23). In another meta-analysis conducted on the elderly population, the incidence of anxiety disorder was 11% (24). In the present study, the relationship between the anxiety and depression levels of the elderly population and clinical features was examined. Accordingly, depression levels increased with age in the patient group, but no relationship was found between age and anxiety levels. In addition, a positive and significant relationship was found between age and depression levels in the patient group, but no relationship was found between age and anxiety levels. In a study by Hacıhasanoğlu et al., in which they investigated the relationship of chronic diseases with clinical features in primary care, a significant positive correlation was found between age and depression and anxiety levels in patients with chronic diseases. However, the sample of this study consisted of not only patients over 65 years of age (25). There are also results in the literature that are inconsistent with our study. In a study by Giordana et al., it was found that the frequency of anxiety disorder decreased with age (26). Similarly, the 12-month prevalence rates of anxiety disorders were examined in a multicenter and large-scale study. The prevalence of anxiety disorders in the elderly population was found to be 17.2%. In our study, women's anxiety levels were found to be higher than men, but no relationship was found between age and anxiety levels. One study found that the prevalence of any anxiety disorder was 40% to 47% more common in adults aged 75-84 compared to those aged 65-74. In another study, the prevalence of any anxiety disorder was 40% to 47% in adults aged 75-84 years. This rate was higher than the elderly in the 65-74 age range. That is, the frequency of anxiety increases with age (27). In the literature, there are data about the relationship between anxiety and depression levels and age. In our study, a positive and significant relationship was found between age and depression levels. Another finding in our study is that anxiety disorder levels in women were significantly higher than in men. There was no difference between genders in depression levels. However, in a study in the literature, depression levels were found to be higher in women over 65 years of age than in men (5). Likewise, in another study by Hacıhasanoğlu et al. (28), depression levels were found to be higher in women over 65 years of age. In a study by Silva et al. (29), depression in the elderly was found to be higher in women. In our study, no relationship was found between the presence of chronic disease and depression and anxiety levels in patients. Likewise, no relationship was found between the type of chronic disease, the duration of the disease, or the use of medication with anxiety and depression levels. In the literature, there are data on the relationship between chronic diseases and depression and anxiety levels in elderly patients, indicating that chronic diseases increase the level of anxiety and depression in general. However, in some studies, similar to the present study, no relationship was found between chronic diseases and anxiety and depression levels. Studies conducted on this subject also show that depression levels increase in the presence of chronic diseases. In a study by Bingöl et al. (5), patients with chronic diseases and regular drug use were found to have higher depression levels. In a study conducted by Dişçigil et al. (15) in primary care, depression rates increased as the number of chronic diseases increased, and if the patient had 2 or more chronic diseases, then depression rates increased 6.2 times. In a study by Şanal Karahan et al. (30), the anxiety levels of patients over the age of 65 with chronic disease were found to be higher than those without chronic disease. A positive correlation was found between the number of drugs used and the number of chronic diseases and anxiety levels in the patient group in the study. In a similar study, the rates of depression were found to be higher in the elderly with chronic diseases (31,32). In a study by Chen et al. (17), it was found that the presence of 2 or more chronic diseases was positively associated with anxiety and depression levels. Consistent with our study, no relationship was found between depression and chronic illness in a study by Saltan A. (33). Likewise, in the study of Altay et al. (34), the presence of chronic disease and drug use were not found to be associated with the level of depression. There are different results on this subject in the literature. Studies examining the relationship between chronic disease and depression and anxiety levels in elderly patients are needed. Elderly patients may not be able to describe their complaints well. Although there are studies in the literature that have reached similar results to the present study, it is thought that the presence of a chronic illness for a long time may increase the level of mental disorders such as anxiety disorder and depressive disorder. However, more research is needed on this subject, especially on anxiety disorders.

Depression is one of the important problems of old age. The presence of chronic diseases and the effect of daily living activities related to these diseases can increase the depression levels of patients. In elderly patients, depression can also be seen in somatic symptoms as well as core symptoms of depression such as not being able to enjoy life. In this case, depression can sometimes be overlooked (35). Likewise, anxiety disorders are also observed in old age. The incidence of anxiety disorder due to medical diseases is also increasing in elderly patients. Sometimes it can be difficult to diagnose anxiety disorder due to the complexity of symptoms in patients (36). In addition, anxiety can be overlooked in elderly patients because it is often observed together with depression. In addition, the symptoms of mental disorders can be considered as normal signs of old age depending on chronic diseases. Therefore, it is difficult to diagnose anxiety disorder in elderly patients (37). Chronic diseases, socioeconomic factors, and stressful life events may increase the frequency of anxiety disorders in elderly patients (38).

The limitations of this study are that it is a single-center study, and the findings cannot be generalized to all individuals over the age of 65. Although the patients are the ones who come to the regular outpatient clinic controls, the fact that the compliance of the patients to the treatment has not been evaluated is another limitation of the study.

CONCLUSION

As a result, life expectancy is increasing all over the world. In this case, the health problems of old age are increasing. Mental disorders are a subject that needs to be examined

in detail in elderly patients. Difficulties in diagnosing patients, treatment processes, and social and family support are very important. The mental illnesses of elderly patients should not be ignored and should always be considered by clinicians. The importance of health data of the elderly patient population is increasing day by day. More research is needed on this subject.

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Effect of Hydroxytyrosol on Prdx6 Expression in Diabetic Rat Liver

Hidroksitirozolün Diyabetik Sıçan Karaciğerinde Prdx6 Ekspresyonu Üzerindeki Etkisi

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ABSTRACT

Aim: Oxidative stress caused by hyperglycemia, which is the most important complication of diabetes mellitus, causes liver damage. Hydroxytyrosol is a polyphenolic compound abundant in olive oil that protects the liver against oxidative damage. Peroxiredoxin 6 (Prdx6) is an anti-oxidative enzyme known to exist in the liver. The aim of this study was to investigate the effect of hydroxytyrosol on Prdx6 expression in diabetes-induced liver injury.
Material and Methods: Male Wistar rats were grouped into four as the control group (n=10), hydroxytyrosol group (n=10), streptozotocin group (n=10), and hydroxytyrosol+streptozotocin group (n=10). Blood glucose levels of the animals were measured after streptozotocin injection and at the end of the experiment. The general structure of the liver was examined with a hematoxylin-eosin stain. Prdx6 protein expression was determined with an immunohistochemical method.
Results: In the streptozotocin+hydroxytyrosol group, blood glucose level was found to be lower when compared with the streptozotocin group ($p<0.001$), and histopathological findings in hepatocytes were found to decrease. Prdx6 expression was found to be similar in the control and hydroxytyrosol groups ($p=0.590$). However, it was found to be higher in streptozotocin and streptozotocin+hydroxytyrosol groups ($p<0.001$). Prdx6 expression of the streptozotocin+hydroxytyrosol group was found lower than the streptozotocin group ($p<0.001$).
Conclusion: Hydroxytyrosol, the anti-oxidative activity of which has been proven in many studies, was found to relieve blood glucose levels in diabetic rats, cause histopathological changes in hepatocytes, and decrease anti-oxidative Prdx6 expression. This decrease suggested that instead of inhibiting Prdx6, hydroxytyrosol reduced oxidative stress irrespective of Prdx6.
Keywords: Diabetes mellitus; liver; hydroxytyrosol; Prdx6; streptozotocin.

ÖZ

Amaç: Diabetes mellitusun en önemli komplikasyonu olan hipergliseminin sebep olduğu oksidatif stres, karaciğer hasarına neden olmaktadır. Hidroksitirosol, zeytinyağında bol miktarda bulunan ve karaciğeri oksidatif hasara karşı da koruyan polifenolik bir bileşiktir. Peroksiredoksin 6 (Prdx6), karaciğerde varlığı bilinen bir anti-oksidatif enzimdir. Bu çalışmanın amacı, hidroksitirozolün diyabete bağlı karaciğer hasarındaki koruyucu rolünde Prdx6 ekspresyonunun etkisini araştırmaktı.
Gereç ve Yöntemler: Erkek Wistar ratlar kontrol grubu (n=10), hidroksitirozol grubu (n=10), streptozotosin grubu (n=10) ve hidroksitirozol+streptozotosin grubu (n=10) olmak üzere dört gruba bölündü. Hayvanların kan glukoz seviyesi streptozotosin enjeksiyonu sonrasında ve deney sonunda da ölçüldü. Karaciğerin genel yapısı ise hematoksilen-eozin boyasıyla incelendi. Prdx6 proteinin ekspresyonu immunohistokimya yöntemi ile belirlendi.
Bulgular: Streptozotosin+hidroksitirozol grubunda kan glukoz seviyesi streptozotosin grubu ile kıyaslandığında daha düşük olarak bulundu ($p<0,001$) ve hepatositlerdeki histopatolojik bulgularda ise azalma olduğu saptandı. Prdx6 ekspresyonu kontrol ve hidroksitirozol gruplarında benzer olarak bulundu ($p=0,590$). Ancak streptozotosin ve streptozotosin+hidroksitirozol gruplarında onlardan daha yüksek olduğu saptandı ($p<0,001$). Streptozotosin+hidroksitirozol grubu Prdx6 ekspresyonu streptozotosin grubuna göre daha düşük olarak bulundu ($p<0,001$).
Sonuç: Birçok çalışmada antioksidatif etkinliği kanıtlanmış olan hidroksitirozol diyabetik ratlarda kan glukoz seviyesini düşürdüğü, hepatositlerdeki histopatolojik değişikliklere neden olduğu ve antioksidatif Prdx6 ekspresyonunu azalttığı bulunmuştur. Bu azalma bize, hidroksitirozolün doğrudan Prdx6'yı inhibe etmesinden ziyade, Prdx6'dan bağımsız bir şekilde oksidatif stresi azaltmasına bağlı olarak gerçekleşiyor olabileceğini düşündürdü.
Anahtar kelimeler: Diyabetes mellitus; karaciğer; hidroksitirozol; Prdx6; streptozotosin.

INTRODUCTION

Diabetes mellitus (DM) is widely observed in the world. In addition, DM is a complex metabolic disease (1-3). DM is characterized by hyperglycemia. This is due to a lack of insulin secretion or insulin resistance (3). It is thought that approximately 220 million people in the world have DM. World Health Organization (WHO) predicts that if no solution is found for the treatment of this disease, the number of patients will double by 2030 (4). Both environmental and genetic factors support the occurrence of DM. The most striking main symptom of DM is hyperglycemia (5). Uncontrolled hyperglycemia in DM causes excessive free radical formation, which in turn causes oxidative stress by disrupting the oxidant-antioxidant balance (6). Deterioration in carbohydrate, protein, lipid, and nucleic acid metabolism caused by increased free radicals causes oxidative stress and inflammation. Oxidative stress and inflammation cause hepatopathy in the liver and damage to different organs (7). The liver is an organ with intense free radical reactions. Therefore, oxidative stress markers are quite high in the liver in the early stages of diabetes (3). The oxidative stress-induced liver injury affects insulin binding to the insulin receptor on the liver cell surface and insulin signaling (8). While this situation affects glucose and lipid balance negatively, it may also cause a large number of metabolic disorders (4). Insulin resistance in DM also causes the accumulation of free fatty acids, which is associated with steatosis, inflammatory steatohepatitis, cirrhosis, and liver failure (9). DM has also been associated with many diseases such as liver enzyme disorders, cirrhosis, non-alcoholic liver disease, and acute liver failure. Moreover, death due to diabetic liver disease is also remarkable (10).

Peroxiredoxins (Prdxs) are multifunctional enzymes. This enzyme family is found in many organisms. They have important roles in protecting cells against oxidative damage (11). The Prdxs family consists of six antioxidant enzymes. Prdx6 is the last member of this family (12). Prdx6 differs from other family members in that it has one conserved cysteine residue and has the ability to bind and reduce phospholipid hydroperoxides (13). While Prdx6 is widely expressed in all tissues, it is expressed in higher levels in the liver, pancreas, and kidney (14). Since the liver is a detoxifying organ and neutralizes circulating oxidants, higher expression of Prdx6 is observed in hepatocytes (15). Prdx6 has antioxidant protective properties against reactive oxygen species (ROS) in liver tissue (14). Prdx6 inhibits the oxidative damage caused by ischemia-reperfusion, hypoxia, and a high-fat diet in the liver (16-18).

Hydroxytyrosol (HT) is the most active biological extract found in olives and olive oil. HT has both in vivo and in vitro antioxidant, anti-inflammatory, and neuroprotective effects (19,20). HT shows its anti-inflammatory effects by inhibiting the expression of inflammatory cytokines (21) and MMP9 and COX2 in active monocytes (22). HT, which is a powerful scavenger against free radical species, has a protective effect from oxidative stress (20). HT has protective effects also on the liver (23). HT shows anti-fibrotic and anti-inflammatory effects by regulating oxidative stress in the liver (24). HT has also been shown to have a protective effect against liver damage (25). Another study has shown a combined HT and Vitamin E application to reduce fibrosis associated with non-alcoholic fatty liver disease (26).

When the aforementioned studies are examined, it can be seen that HT and Prdx6 are important in protecting the liver from oxidative damage. However, HT's effects on Prdx6 expression in liver damage due to oxidative stress caused by diabetes are not known. In this study, we investigated the effects of HT, which is known to have antioxidant and anti-inflammatory effects in olive and olive oil, on the expression of Prdx6, which is an antioxidant enzyme in diabetes-induced liver damage, in the diabetic rat model.

MATERIAL AND METHODS

Animals and Chemicals

Forty male Wistar rats with a weight of 250-300 gr were used in the study. The animals were housed at a room temperature of 24±2 ºC and a humidity of 50±5 % in 12-hour light:12-hour dark periods. The animals were provided with food and water ad libitum. Four groups were formed. These were control (only sterile distilled water injection), HT, streptozotocin (STZ), and STZ+HT. Before starting the study, the animals were included by looking at their blood glucose levels. 55 mg/kg STZ (Sigma, S0130) was dissolved in sterile distilled water and administered intraperitoneally as a single dose to STZ and STZ+HT group animals. The blood glucose levels of animals were measured 48 hours after the injection. Those with a blood glucose level of ≥250 mg/dL were considered diabetic. Next, 30 days 10 mg/kg HT (Sigma/Cayman-70604) was administered intraperitoneally to HT and STZ+HT groups. Blood glucose levels were measured from tail blood on days 3 and 30 after the STZ injection. At the end of the experiment, the animals were sacrificed and their liver tissues were removed.

Tissue Preparation

In all groups, after liver tissues were removed, they were fixated in 4% formaldehyde for 24 hours for immunohistochemistry. They were then dehydrated with ethyl alcohol series of 70%, 80%, and 90%, respectively. Next, they were cleared with xylene and blocked by embedding in paraffin.

Morphological Evaluation

4 µm thick sections were taken on a normal slide from the paraffin blocks of each group. After they were deparaffinized and rehydrated, the slides were stained with hematoxylin and eosin. The slides were subsequently followed back and covered with entellan. The hematoxylin and eosin-stained slides were evaluated histopathologically.

Immunohistochemistry

Sections of 4 µm thickness from paraffin blocks were taken on positively charged slides. The sections were deparaffinized in xylene and rehydrated by keeping them in decreasing-grade alcohols. Sections were boiled in citrate buffer (pH 6.0) in a 750-watt microwave oven for 7 minutes. It was then cooled to room temperature. Sections were washed 3 times for 5 minutes (3x5') with phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and washed 3x5' with PBS. Sections were blocked with UltraV block (Thermo Scientific™ TL-125-UB) for 7 min at room

temperature in a humidified chamber. They were then incubated with the Prdx6 primary antibody (1:300 dilution, Abcam; ab73350, Rabbit) at +4 ºC overnight. The next day, the sections were washed 3x5' with PBS. It was incubated with biotinylated secondary antibody (Thermo Scientific™ TL-125-PB) for 60 min at room temperature and washed 3x5' with PBS. It was then incubated with streptavidin (Thermo Scientific™ TL-125-PH) for 15 minutes at room temperature. Sections were washed 3x5' with PBS. Immunoreactivity was developed with diaminobenzidine (DAB). Slides were counterstained with hematoxylin, traced back, and sealed with entellan.

Immunohistochemistry and hematoxylin and eosin-stained sections were photographed under an Olympus Cx41 microscope with an AxioCam Zeiss digital camera. After 10 photos at 400X magnification were taken from each of the immunostaining slides, the intensity of the immunostaining was quantitatively analyzed using ImageJ software (http://imagej.nih.gov/ij/).

Statistical Analysis

All data were analyzed with GraphPad Prism 9 (GraphPad Software) in terms of statistical significance. After the normality of data obtained with ImageJ was evaluated with the Shapiro-Wilk test, descriptive statistics were expressed as mean±standard deviation. Data were analyzed with One-way ANOVA followed by the Holm-Sidak method for multiple comparisons. Blood glucose levels were analyzed by two-way repeated measures ANOVA with applied Tukey's multiple comparisons tests. A p value of <0.05 was considered statistically significant.

RESULTS

Blood Glucose Results

In the study, blood glucose levels of day 3 and 30 measurements were similar in the control and HT groups which were not given STZ injection (p=0.757, p=0.902, respectively, Table 1, Figure 1). In STZ and STZ+HT groups, blood glucose levels were significantly increased when compared with control and HT groups in day 3 and 30 measurements (p<0.001, Table 1, Figure 1). No statistically significant difference was found between groups on day 3 blood glucose measurements in STZ and STZ+HT groups (p=0.999, Table 1, Figure 1). However, in day 30 measurements, the STZ+HT group's blood glucose level was significantly lower when compared with the STZ group (p=0.015, Figure 1). On day 30 STZ group's blood glucose level increased compared to the day 3 STZ group's blood glucose level (p<0.001, Figure 1). However, the blood glucose levels of the day 3 and day 30 STZ+HT groups were similar (p>0.999, Figure 1).

Morphological Evaluation Results

The histological structure of the cells in the control and HT groups was normal (Figure 2a-d). In the STZ group, it was found that the intensity of eosinophilic staining decreased in the cytoplasm of hepatocytes and the microvesicular white areas were found to increase (Figure 2e,f). There were no histopathologic findings in hepatocytes and other cells in the STZ+HT group (Figure 2g,h).

Immunochemistry Results

Prdx6 immunohistochemical staining was both cytoplasmic and nuclear. Expression of Prdx6 in the liver was remarkable mostly in the cytoplasm and nuclei of hepatocyte cells. Prdx6 expression of control and HT groups was similar (p=0.590, Table 1, Figure 3A-3B). In control and HT groups, nuclear expression was higher than cytoplasmic expression (Figure 3A-a,c). However, cytoplasmic expression was higher in the hepatocytes lining the central vein. Cellular distribution of control and

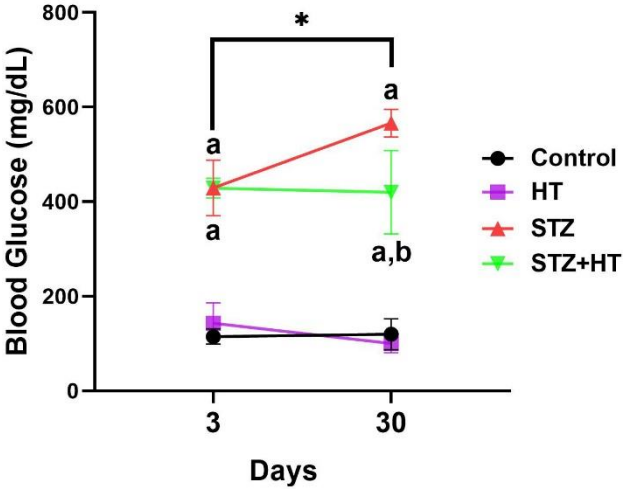

Figure 1. Blood glucose levels on days 3 and 30

HT: hydroxytyrosol, STZ: streptozotocin, statistical significance (p<0.05) compared with [a]: control, [b]: HT, and [c]: STZ, and [*]: between the day 3 and 30 STZ groups

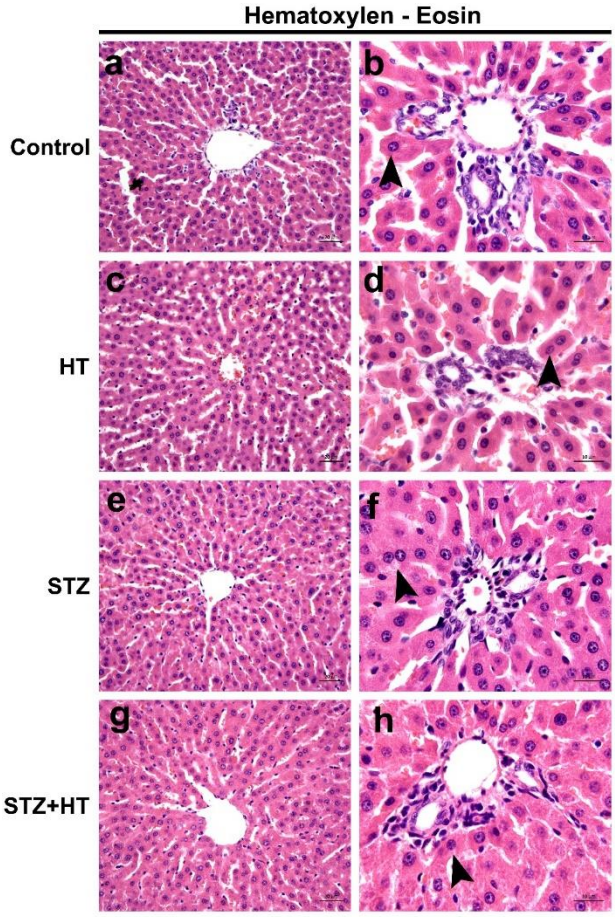

Figure 2. Representative micrographs of morphological evaluation of the liver by hematoxylin & eosin staining

HT: hydroxytyrosol, STZ: streptozotocin, black arrowhead: hepatocyte cells. a, c, e, and g photos were magnified at 400X; while b, d, f, and h were magnified at 1500X

Table 1. Comparison of the blood glucose and Prdx6 measurements between groups

	Control	HT	STZ	STZ+HT	p
Prdx6	4.858±0.414	4.753±0.507	7.125±0.518*	6.222±0.444*#	**<0.001**
Blood glucose					
Day 3	114.8±16.18	142.8±43.36	429.0±58.75*	428.8±20.97*	**<0.001**
Day 30	119.6±37.72	100.2±19.31	566.0±29.36*	419.8±88.11*#	**<0.001**
General	117.2±24.47	121.5±38.80	497.5±84.44*	424.3±60.57*	**<0.001**

Prdx6: peroxiredoxin 6, HT: hydroxytyrosol, STZ: streptozotocin, statistical significance ($p<0.05$) compared with *control and HT, and #STZ.

HT groups Prdx6 expression was positive in hepatocyte cells and negative in Kupffer cells. While the expression of endothelial and bile duct cells was very intensely positive in the control group, it was intensely positive in the HT group (Table 2, Figure 3A-a,c). Prdx6 expression of STZ and STZ+HT groups was higher than the control and HT groups ($p<0.001$, Table 1, Figure 3A-3B). The expression pattern of STZ and STZ+HT groups was both cytoplasmic and nuclear (Figure 3A-e,g). In the STZ group, nuclear and cytoplasmic staining was more intense than in the other groups. In addition, the cytoplasmic Prdx6 expression around the central vein was more intense when compared with the control and HT group (Figure 3A-e). In the STZ group, the cellular distribution of Prdx6 was very intensely positive in hepatocyte cells, and weakly positive in endothelial and bile duct cells, while it was negative in Kupffer cells (Table 2, Figure 3A-e). It was noteworthy that Prdx6 expression was decreased in the STZ+HT group when compared with the STZ group ($p<0.001$, Table 1, Figure 3A-3B). The cytoplasmic staining pattern of this group was decreased (Figure 3A-g). In addition, cytoplasmic staining of Prdx6 around the central vein was the most intense in this group (Figure 3A-g). In the STZ+HT group, the cellular distribution of Prdx6 was intensely positive in hepatocyte and endothelial cells, and positive in bile duct cells, while it was negative in Kupffer cells (Table 2, Figure 3A-g).

DISCUSSION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia that occurs when the body cannot produce or use enough insulin (27). Hyperglycemia, which occurs as a result of diabetes, causes damage due to oxidative stress in many organs including the liver (7). HT is a polyphenolic compound that is abundant in olive and olive oil and which plays a protective role in the liver (23). In diabetic animals which were administered HT, a decrease was observed in blood glucose levels and histopathological findings in the cytoplasm of hepatocytes were found to be relieved. This shows that HT can have a protective role against liver damage due to diabetes.

When previously conducted studies are examined, it can be seen that HT and Prdx6 have been shown to have a role in protecting the liver against oxidative stress (14,24). Reactive oxygen species have an important role in the pathogenesis of liver diseases (28). After all, the liver is one of the important organs affected by reactive oxygen species. Therefore, it is very sensitive against the effects of oxidative damage caused by hyperglycemia (29). Prdx6 expression is high in hepatocytes which have a role in

Table 2. Semi-quantitative distribution of Prdx6 labeling

Cells	Control	HT	STZ	STZ+HT
Hepatocyte cells	+	+	+++	++
Endothelial cells	+	+++	(+)	++
Bile duct cells	++	++	(+)	+
Kupffer cells	-	-	-	-

Prdx6: peroxiredoxin 6, HT: hydroxytyrosol, STZ: streptozotocin, label 0: negative; (+): weak positive; +: positive; ++: dense positive; +++: very dense positive

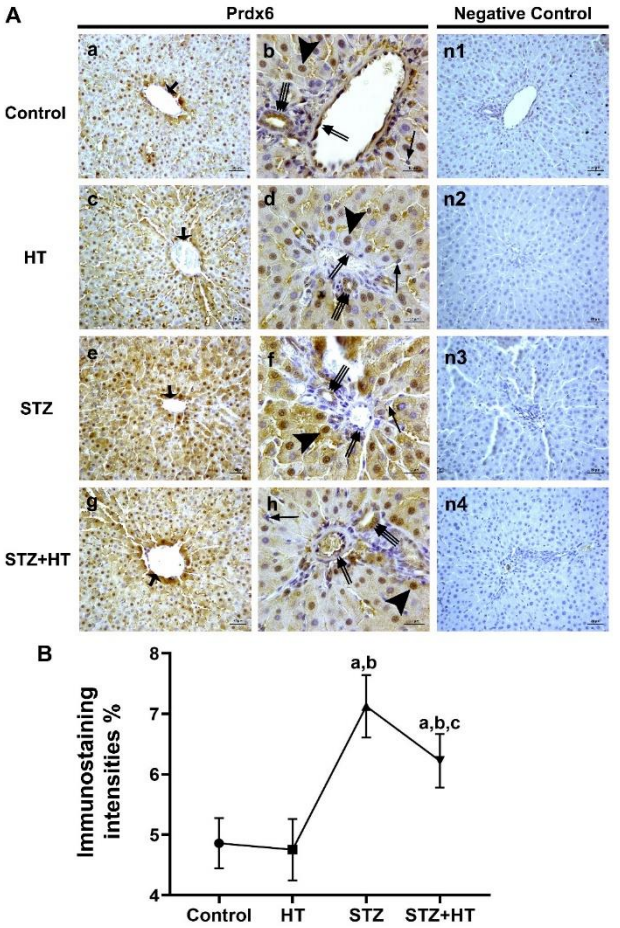

Figure 3. A. Representative micrographs of immunohistochemical analysis of Prdx6, **B.** Quantitative analysis graph of immunohistochemical staining intensity

Prdx6: peroxiredoxin 6, HT: hydroxytyrosol, STZ: streptozotocin, thick short arrow: central vein, arrowhead: hepatocyte cells, arrow: Kupffer cells, double arrow: endothelial cells, triple arrow: bile duct cells, statistical significance ($p<0.05$) compared with [a]: control, [b]: HT, and [c]: STZ. a, c, e, g, n1, n2, n3, and n4 photos were magnified at 400X, while b, d, f, and h were magnified at 1500X

detoxification and inhibiting oxidants in the blood (15). Prdx6 also protects the liver from reactive oxygen species (14). Similarly, HT shows anti-fibrotic and anti-inflammatory effects by balancing oxidative balance in the liver (24). Prdx6 is protective against ischemia-reperfusion, hypoxia, and damage due to oxidative stress triggered by a high-fat diet (16-18). Prdx6 also supports fatty acid oxidation in rats fed with a high-fat diet (30). These studies show that HT and Prdx6 protect the liver against oxidative damage caused by oxidative stress. The reason why there were no differences in Prdx6 expression between control and HT groups in the present study may be the fact that since the absence of diabetes-induced hyperglycemia is not considered a deterioration in oxidant-antioxidant balance, this may not affect Prdx6 expression. In addition, the fact that HT did not change Prdx6 expression in the HT group when compared with the control suggested HT may not have any inhibiting or stimulating effect on Prdx6 expression in the liver. However, in the STZ group, as a result of increased oxidative stress due to hyperglycemia, the expression of Prdx6, which is an important antioxidant enzyme, may have increased significantly. The result that Prdx6 expression decreased when HT was administered in the STZ+HT group was also interesting. The reason for this decrease may be due to the fact that HT reduces oxidative stress caused by diabetes-induced hyperglycemia irrespective of Prdx6. We thought that Prdx6 expression, which increased as a result of oxidative stress due to STZ-induced diabetes, may have decreased in parallel with the decrease in oxidative stress with the administration of HT. As a result, HT, which is an important protector of the liver against oxidants, may not be able to reduce or eliminate the oxidative stress in the liver caused by diabetes-related hyperglycemia through the Prdx6 enzyme. While HT regulates the oxidative balance in the liver, Prdx6 expression in liver cells may be decreased due to the decrease in oxidative stress.

CONCLUSION

HT can reduce blood glucose and improve histopathological findings by reducing oxidative stress due to hyperglycemia through an anti-oxidative pathway or pathways in diabetic rats. However, the decrease of Prdx6 after HT administration to diabetic animals suggested that HT might suppress oxidative stress by using other anti-oxidative pathways in liver cells rather than this pathway.

Ethics Committee Approval: The study was approved by the Experimental Animals Local Ethics Committee of Düzce University (21.12.2022, 12/04).

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The Effect of Coenzyme Q10 as a Prophylactic Treatment in Episodic Migraine

Epizodik Migrende Koenzim Q10'un Profilaktik Tedavi Olarak Etkisi

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ABSTRACT

Aim: Migraine is a neurological disorder characterized by recurring and often severe headaches. The aim of this study was to evaluate the effectiveness of coenzyme Q10 (CoQ10) in episodic migraine prophylaxis.

Material and Methods: In a prospective follow-up study, 80 patients with episodic migraine were enrolled according to the International Classification of Headache Disorders 3rd edition and divided into two groups. One group consisted of 40 patients who received CoQ10 oral 200 mg twice daily as monotherapy for three months, while the other group consisted of 40 patients who received CoQ10 as an adjunct therapy in combination with other prophylactic antimigraine drugs. The Migraine Disability Assessment (MIDAS) questionnaire was administered to both groups before and after three months of therapy. Throughout the three-month therapy period, patients recorded daily symptoms, allowing for observation of changes in symptom severity, number, and duration of attacks from baseline.

Results: Both groups that received CoQ10 supplementation exhibited positive responses. The group receiving adjunct therapy showed a statistically more significant reduction in the MIDAS ($p<0.001$), duration ($p<0.001$), and frequency ($p<0.001$) of attacks compared to the monotherapy group. Vomiting ($p<0.001$) in the adjunct therapy group completely disappeared and sound sensitivity ($p=0.002$) showed a dramatic response to treatment.

Conclusion: CoQ10 appears to have more beneficial effects as an adjunct than monotherapy in reducing the duration, frequency, and presenting symptoms such as nausea, vomiting, and light sensitivity, in addition to sound sensitivity that responded only to adjunct therapy than monotherapy among Iraqi patients with episodic migraine.

Keywords: Migraine disorders; coenzyme Q10; prophylaxis.

ÖZ

Amaç: Migren, tekrarlayan ve sıklıkla şiddetli baş ağrılarıyla karakterize bir nörolojik hastalıktır. Bu çalışmanın amacı, epizodik migren profilaksisinde koenzim Q10'un (CoQ10) etkinliğini değerlendirmektir.

Gereç ve Yöntemler: Prospektif bir takip çalışmasında, epizodik migreni olan 80 hasta Başağrısı Bozukluklarının Uluslararası Sınıflaması 3. basımına göre çalışmaya dahil edildi ve iki gruba ayrıldı. Bir grup, üç ay boyunca monoterapi olarak günde iki kez oral 200 mg CoQ10 alan 40 hastadan oluşurken, diğer grup ise diğer profilaktik antimigren ilaçlarla birlikte yardımcı tedavi olarak CoQ10 alan 40 hastadan oluşuyordu. Her iki gruba da tedaviden önce ve üç aylık tedaviden sonra Migren Dizabilite Değerlendirme (Migraine Disability Assessment, MIDAS) anketi uygulandı. Üç aylık tedavi periyodu boyunca, hastaların günlük semptomları kaydetmesiyle, başlangıçtan itibaren semptom şiddeti, atak sayısı ve süresindeki değişikliklerin gözlemlenmesine olanak sağlandı.

Bulgular: CoQ10 takviyesi alan her iki grup da olumlu yanıtlar gösterdi. Ek tedavi alan grup, monoterapi grubuna göre MIDAS'ta ($p<0,001$), atak süresi ($p<0,001$) ve sıklığında ($p<0,001$) istatistiksel olarak daha belirgin bir azalma gösterdi. Ek tedavi grubunda kusma ($p<0,001$) tamamen kayboldu ve ses duyarlılığı ($p=0,002$) tedaviye dramatik bir yanıt gösterdi.

Sonuç: CoQ10 Iraklı epizodik migren hastalarında monoterapiye göre sadece yardımcı tedaviye yanıt veren ses duyarlılığına ek olarak süreyi, sıklığı ve bulantı, kusma ve ışığa duyarlılık gibi semptomların ortaya çıkışını azaltmada yardımcı olarak monoterapiden daha yararlı etkilere sahip gibi görünmektedir.

Anahtar kelimeler: Migren bozuklukları; koenzim Q10; profilaksi.

INTRODUCTION

Migraine is a chronic disorder characterized by recurrent and often severe headaches, commonly accompanied by symptoms such as nausea, vomiting, and sensitivity to light and sound (1). Migraine stands as a prevalent neurological condition on a global scale, impacting a staggering number of individuals surpassing 1 billion. Its prevalence is estimated at around 10%, with 6% affecting males and 14% impacting females. However, the occurrence of migraines varies across different regions, with higher rates observed in Europe at 15% and North America at 13%, while relatively lower rates are found in Asia at 9% and Africa at 5% (2). The primary goal of migraine treatment is to reduce the frequency and intensity of migraine attacks, and while there are several medications available, only a few of them act as preventive measures. There is substantial evidence that supports the positive impacts of Coenzyme Q10 (CoQ10) and L-carnitine supplements in reducing lactate levels in the bloodstream and alleviating symptoms associated with migraines (3).

Moreover, it has been hypothesized that inhibiting cortical spreading and the subsequent accumulation of MMP-9, a protein involved in the breakdown of the blood-brain barrier, could contribute to nerve inflammation and the worsening of migraines (4). The disruptions in the brain's energy demand and/or production observed in migraines are likely significant enough to induce oxidative stress (5). While limited clinical trials have been conducted, some have shown the potential of CoQ10 as a prophylactic treatment for migraines (6,7). Micronutrients like magnesium, vitamin B2, and CoQ10 have received significant attention for their potential in managing migraines (8). However, studies specifically investigating the effectiveness of CoQ10 as monotherapy versus adjunct therapy with other drugs are lacking.

Therefore, the aim of this study was to investigate the effectiveness of CoQ10 in migraine prophylaxis and its impact on reducing the frequency and severity of migraine attacks.

MATERIAL AND METHODS

A prospective follow-up study, from January 2021 to January 2022, was conducted in the outpatient neurology clinic of the Azadi Teaching Hospital in Kirkuk. Informed oral consent was obtained from all patients. The study focused on 80 patients diagnosed with episodic migraine, who were divided into two groups. One group comprised 40 patients who received CoQ10 oral 200 mg twice daily as monotherapy for three months. The other group consisted of 40 patients who received the same dose as adjunct therapy alongside other prophylactic antimigraine drugs.

The dose adjustment plan for CoQ10 supplementation was based on a review article (9) that synthesized findings from six relevant studies investigating the use of CoQ10 in various doses ranging from 30 mg to 800 mg daily. The review article provided a comprehensive analysis of the dose-response relationship and efficacy of CoQ10 supplementation in similar populations.

Considering the range of doses examined in the reviewed studies, we selected a daily dose of 400 mg of CoQ10 as the appropriate dosage for our study. This dose was determined based on the reported efficacy and safety profile in previous literature, aiming to provide a sufficient therapeutic effect while minimizing the potential for adverse effects.

The diagnosis and classification of migraine patients in the study were based on the International Classification of Headache Disorders 3rd edition (ICHD-3). Prior to and following the three-month therapy period, the Migraine Disability Assessment (MIDAS) questionnaire was conducted in both groups. Over the course of the three months, daily monitoring and recording of changes in mean duration, number of migraine attacks per day, severity, and accompanying symptoms such as nausea, vomiting, vertigo, and light and sound sensitivity took place. The study excluded participants who met any of the following criteria: pregnancy, breastfeeding, other types of headaches apart from episodic migraine, smoking, history of brain injury in the last two years, history of allergies, stroke, inflammation, autoimmune diseases, other chronic diseases such as cardiovascular diseases, diabetes, rheumatoid arthritis, digestive disorders, or neurological diseases, history of antioxidant supplements in the last three months, history of contraceptives, non-steroidal inflammatory drugs, corticosteroids, history of alcohol consumption, change in the dose or type of regular drugs, or evidence of incompatibility with supplement intake.

This study was approved by the clinical research ethics committee of the University of Kirkuk - College of Medicine (date: 15.11.2022, number: 19).

Statistical Analysis

Data entry and analysis were performed using IBM SPSS Statistics v.26 (Statistical Package for Social Sciences, IBM Inc., Chicago, USA) software. Descriptive statistics included frequency and percentage for categorical data, while mean and standard deviation were calculated for continuous data. Normality assumptions were assessed using the Kolmogorov-Smirnov test, and skewness values. Categorical variables were compared using the Chi-square test, or Fisher's exact test for small frequency cells. For quantitative parameters, independent t-tests were employed to compare the means, and Mann-Whitney U tests were used to compare the number and duration of migraine attacks between both studied groups. Statistical significance was set at a p-value of less than 0.05.

RESULTS

The analysis involved 80 patients, with an average age of 30.49±8.06 (range, 15-45) years. The majority of the patients were female (n=57, 71.3%). There were no statistically significant differences in sex (p=0.083) and age (p=0.134) between the two groups.

There was a statistically significant difference (p<0.001) in the MIDAS questionnaire score prior to treatment between the adjunct therapy and monotherapy groups, with the adjunct therapy group showing a higher severity. However, the adjunct therapy group exhibited a greater decrease, from 3.63±0.49 to 1.05±0.22, in the MIDAS score compared to the monotherapy group which dropped from 3.00±0.78 to 2.75±0.84. Moreover, there was a statistically significant difference (p=0.003) in the number of migraine attacks before treatment between the adjunct therapy and monotherapy groups, with the adjunct therapy

group experiencing more frequent attacks. This difference became even more significant (p<0.001) after treatment initiation in the adjunct therapy group (median number of attacks dropped from 9 to 0) compared to the monotherapy group (dropped from 7 to 3). Additionally, there was a statistically significant difference (p<0.001) in the reduction of migraine attack duration per hour between the adjunct therapy and monotherapy groups when comparing before treatment initiation (Table 1).

There were no significant changes in presenting symptoms related to the aura, vertigo, nausea, and light sensitivity before and after therapy in both groups, except for vomiting (p<0.001), which was significantly more prevalent in the adjunct therapy group before starting therapy but completely disappeared after therapy. Moreover, sound sensitivity (p=0.002) showed a dramatic response to treatment in the adjunct therapy group compared to the monotherapy group (Table 2).

Table 1. Comparison of demographics, MIDAS, number of attacks, and duration of attacks between groups

	Adjunct Therapy (n=40)	Monotherapy (n=40)	p
Sex, n (%)			
Female	32 (80)	25 (62.5)	0.083
Male	8 (20)	15 (37.5)	
Age (years), mean±SD	29.12±8.18	31.85±7.81	0.134
MIDAS, mean±SD			
Before Treatment	3.63±0.49	3.00±0.78	**<0.001**
After Treatment	1.05±0.22	2.75±0.84	**<0.001**
Number of attacks, median (min-max)			
Before Treatment	9 (5-24)	7 (3-10)	**0.003**
After Treatment	0 (0-1)	3 (1-5)	**<0.001**
Attack duration (hour), median (min-max)			
Before Treatment	21 (10-72)	24 (8-72)	0.400
After Treatment	1 (0-6)	5 (1-24)	**<0.001**

MIDAS: migraine disability assessment, SD: standard deviation, min: minimum, max: maximum

Table 2. Comparison of migraine symptoms before and after CoQ10 treatment between groups

	Adjunct Therapy (n=40)	Monotherapy (n=40)	p
Aura, n (%)			
Before Treatment	4 (10.0)	5 (12.5)	>0.999
After Treatment	3 (7.5)	5 (12.5)	0.712
Nausea, n (%)			
Before Treatment	40 (100)	38 (95.0)	0.493
After Treatment	2 (5.0)	0 (0.0)	0.493
Vomiting, n (%)			
Before Treatment	40 (100)	14 (35.0)	**<0.001**
After Treatment	0 (0.0)	0 (0.0)	N/A
Light sensitivity, n (%)			
Before Treatment	33 (82.5)	30 (75)	0.412
After Treatment	0 (0.0)	1 (2.5)	>0.999
Sound sensitivity, n (%)			
Before Treatment	24 (60.0)	23 (57.5)	0.820
After Treatment	0 (0.0)	9 (22.5)	**0.002**
Vertigo, n (%)			
Before Treatment	24 (60.0)	22 (55.0)	0.651
After Treatment	0 (0.0)	3 (7.5)	0.240

CoQ10: coenzyme Q10, N/A: not applicable

DISCUSSION

This study aimed to assess the effectiveness of CoQ10 as a prophylactic treatment for episodic migraines among Iraqi patients and compare its use as monotherapy versus adjunct therapy with other prophylactic antimigraine drugs. Migraine imposes a significant burden on patients' lives, particularly during moderate to severe attacks (10). Numerous medications have been approved for both the acute and preventive treatment of migraines (11).

To provide a brief summary of the study and discuss the relevant literature, it is important to note that the mean age of the patients included in this study was 30 years. This finding aligns with a previous study conducted in Iraq which also reported a mean age of 35 years for migraine patients (12). However, a recent study from Italy showed a higher mean age of 49 years among migraine patients (13). It should be noted that the Italian study focused on patients with established chronic migraine, which may account for the higher mean age in their sample. Additionally, the higher prevalence of aggravating factors, such as stress, in Iraq could contribute to the younger age of migraine patients in our study.

Regarding gender distribution, the majority (71.3%) of patients in our study were female, consistent with other Iraqi studies reporting that 60-70% of migraine sufferers are female (14,15). This observation aligns with the general understanding that females tend to express migraine symptoms more frequently than males (16,17).

Migraine without aura was the most common type of migraine in our study, with over 88% of participants not experiencing aura. This finding is in line with previous studies that have shown migraine without aura to be the most prevalent type (15,18).

In terms of the treatment approach, all patients in our study were prescribed CoQ10 as a prophylactic treatment for their migraines. We observed notable reductions in the MIDAS score, number of migraine attacks, and duration of headache attacks after treatment. The MIDAS score demonstrated a significant decrease after treatment, indicating an improvement in migraine-related disability. Similarly, the number of migraine attacks experienced by patients showed a significant reduction after treatment.

Additionally, the duration of migraine attacks was notably decreased. These findings align with previous studies, particularly clinical trials, which have reported significant reductions in MIDAS, number, and duration of migraine attacks following CoQ10 treatment (3,19,20).

Some studies have also shown a decrease in serum lactate levels among patients receiving CoQ10 as a prophylactic treatment for migraines (3,19). However, a meta-analysis study highlighted that while CoQ10 may reduce the frequency of migraine attacks, it does not appear to reduce their severity or duration (21). It is important to note that meta-analyses may have high heterogeneity, and their results should be interpreted with caution.

One of the diagnostic criteria for migraine listed by the ICHD-3 is the presence of phonophobia and photophobia during attacks. Phonophobia, or sensitivity to sound, is commonly seen during acute attacks (22,23). In our study, 59% of the patients were associated with phonophobia. However, it is important to note that we considered these symptoms during both attack and inter-attack periods, whereas previous studies focused only on symptoms during attacks. Interestingly, we found that sound sensitivity did not respond well to CoQ10 as monotherapy compared to adjunct therapy with other prophylactic antimigraine drugs.

Coenzyme Q10 supplementation has shown promising effects when used in combination with other supplements, as demonstrated in two separate studies (8,24). In their research, a fixed combination of Andrographis paniculata, feverfew, vitamin B2, magnesium, and CoQ10 supplementation was utilized for episodic migraine prophylaxis. The results indicated a significant improvement in migraine attacks, duration, and symptoms in both study groups. Notably, the adjunct treatment group exhibited a significantly lower MIDAS score, number of migraine attacks, and duration of migraine attacks compared to the monotherapy group. This suggests that adjunct therapy may yield better outcomes than monotherapy.

These findings align with the growing understanding of the metabolic mechanisms involved in migraine pathogenesis. It has been proposed that migraines may be triggered by a deficiency of cerebral energy or the accumulation of oxidative stress beyond the control of antioxidants (25). CoQ10, as an antioxidant, may help mitigate these mechanisms and provide therapeutic benefits in migraine management.

Furthermore, a recent study conducted in Iran focused on the efficacy of CoQ10 as a prophylactic treatment for migraine headaches in children (26). The study concluded that CoQ10 holds great value as a treatment option for children with migraines, particularly due to its relatively fewer side effects, especially during long-term use. This suggests that CoQ10 supplementation may be a viable and well-tolerated treatment approach for pediatric migraine patients.

Overall, these findings highlight the potential benefits of CoQ10 supplementation, as a prophylactic treatment in improving migraine outcomes and reducing the burden of migraines in both adults and children. Further research and clinical trials are warranted to better understand the precise mechanisms of action and optimize the use of CoQ10 in migraine management.

One limitation of our study is that the number of patients included was not sufficiently high, which can be attributed to the fact that it was self-funded. As a result, we had to carefully consider practical factors when determining the sample size. Although a larger sample size would have been preferable to improve statistical power and precision, limitations such as limited resources forced us to adopt a balanced approach that prioritized feasibility while still allowing us to draw meaningful conclusions.

CONCLUSION

Coenzyme Q10 has shown greater efficacy as an adjunct therapy compared to monotherapy in reducing the duration, frequency, and presenting symptoms of episodic migraines among Iraqi patients. The addition of CoQ10 to the treatment regimen resulted in significant improvements in symptoms such as nausea, vomiting, light sensitivity, and sound sensitivity, with the latter specifically responding only to adjunct therapy rather than monotherapy.

Ethics Committee Approval: The study was approved by the Ethics Committee of the University of Kirkuk - College of Medicine (15.11.2022, 19).

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Investigation of the Relationship Between Health and Food Literacy and Healthy Eating Obsession in Call Center Employees

Çağrı Merkezi Çalışanlarında Sağlık ve Gıda Okuryazarlığı ile Sağlıklı Yeme Takıntısı Arasındaki İlişkinin İncelenmesi

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ABSTRACT

Aim: The aim of this study was to examine the relationship between food literacy, health literacy, and healthy eating obsession in call center employees.
Material and Methods: The cross-sectional descriptive study was conducted with 545 participants working in a call center, of whom 68.1% (n=371) were female and 31.9% (n=174) were male. A questionnaire consisting of sociodemographic data form, and perceived food literacy, health literacy, and ORTO-R scales were applied to the participants.
Results: While 51.7% (n=282) of the participants had a normal body mass index, 41.7% (n=227) were overweight. Perceived food literacy (p=0.008) and ORTO-R (p=0.004) scores of female participants were higher than male participants, and those who were married had higher perceived food literacy scores than singles (p=0.003). Underweight individuals scored higher perceived food literacy than normal-weight individuals, and normal-weight individuals scored higher than overweight individuals (p=0.004). ORTO-R scores of the overweight group were higher than the other two groups and the difference was statistically significant (p<0.001). According to the regression analysis, health literacy decreased by 5.026 units for those who do not think they are eating healthy, and by 9.943 units for those who do not know how a healthy diet should be. Overweight participants exhibit more orthorexic eating behavior (p<0.001).
Conclusion: Effective and continuous training programs will be beneficial for call center employees who have inactive and sedentary working conditions due to their work, in order to determine their deficiencies or inadequacies in terms of gaining food literacy, health literacy, and healthy eating behavior.
Keywords: Call center employees; health literacy; food literacy; eating disorder.

ÖZ

Amaç: Bu çalışmanın amacı, çağrı merkezi çalışanlarında gıda okuryazarlığı, sağlık okuryazarlığı ve sağlıklı beslenme takıntısı arasındaki ilişkinin incelenmesidir.
Gereç ve Yöntemler: Kesitsel tipte olan çalışma, bir çağrı merkezinde çalışan %68,1 (n=371) kadın ve %31,9 (n=174) erkek olmak üzere 545 katılımcı ile yapıldı. Katılımcılara sosyodemografik veri formu ile birlikte algılanan gıda okuryazarlığı, sağlık okuryazarlığı ve ORTO-R ölçeklerinden oluşan bir anket uygulandı.
Bulgular: Katılımcıların %51,7'si (n=282) normal vücut kitle indeksine sahipken %41,7'si (n=227) ise fazla kiloluydu. Kadın katılımcıların algılanan gıda okuryazarlığı (p=0,008) puanları ve ORTO-R (p=0,004) puanları erkek katılımcılara göre daha yüksek olup evli olanların ise bekarlara göre algılanan gıda okuryazarlığı puanları daha yüksekti (p=0,003). Düşük kilolu bireyler normal kilolu bireylere göre daha yüksek gıda okuryazarlığı algısı puanı alırken normal kilolu bireyler de fazla kilolu bireylere göre daha yüksek puan aldı (p=0,004). Fazla kilolu grubun ORTO-R puanları diğer iki gruba göre de daha yüksekti ve aradaki fark istatistiksel olarak anlamlıydı (p<0,001). Regresyon analizine göre sağlık okuryazarlığı sağlıklı beslenmediğini düşünenlerde 5,026 birim, sağlıklı beslenmenin nasıl olması gerektiğini bilmeyenlerde ise 9,943 birim azalmaktadır. Fazla kilolu katılımcılar daha fazla ortoreksik yeme davranışı sergilemektedir (p<0,001).
Sonuç: İşleri gereği hareketsiz ve hareketsiz çalışma koşullarına sahip olan çağrı merkezi çalışanlarının gıda okuryazarlığı, sağlık okuryazarlığı ve sağlıklı beslenme davranışı kazandırma açısından eksikliklerini veya yetersizliklerini belirlemeye yönelik etkili ve sürekli eğitim programları faydalı olacaktır.
Anahtar kelimeler: Çağrı merkezi çalışanları; sağlık okuryazarlığı; gıda okuryazarlığı; yeme bozukluğu.

INTRODUCTION

Nowadays, developing and changing living conditions cause big cities to live and stay awake 24 hours a day. Therefore, call centers which are the working areas of living cities, have to provide 24-hours service (1,2). Work-specific factors such as variable working hours, limited workspace, the system that sometimes does not allow short breaks, and inactivity can cause many health problems, especially nutritional disorders in employees (3). It is reported that unhealthy eating patterns cause an increase in the burden of obesity, nutrition-related non-communicable diseases, and related deaths, as well as environmental problems (4,5). Eating attitude is one of the biggest behavioral health problems today.

Social values, which are in constant change, also affect human feeding behavior. In recent years, there has been a clear awareness that healthy nutrition has positive effects on health in societies. However, obsessing over this type of behavior toward healthy foods causes a negative result in health and quality of life (6). Based on the definition by the Centers for Disease Control and Prevention (CDC), food literacy is the degree to which individuals have the capacity to obtain, process, and understand basic food and food preparation information for appropriate food selection (7), while nutritional literacy is the individual's capacity to access, understand, interpret and apply basic nutritional information and services in a way that improves their health (8). Health literacy (HL) is extremely important, as achieving higher levels of literacy in societies is associated with a range of health outcomes. Persons with poor health literacy are less sensitive to health education, less affected by the implementation of disease prevention programs, and less successful in the management of chronic diseases (9).

Food literacy, on the other hand, is based on the premise of applying basic literacy skills to concepts related to nutrition. This includes but is not limited to, the skills and behaviors required to choose healthy foods, and plan and prepare healthy meals that meet individual nutritional needs. A healthy eating plan plays an important role in preventing overweight/obesity and subsequent health conditions. However, understanding what a healthy diet includes is complex and may require high cognitive skills (10). Health and food literacy are crucial for both successful weight management and making healthy lifelong choices (11). Health and nutrition literacy plays an important role in the development of healthy eating behaviors (12).

The aim of this study was to examine the relationship between food literacy, health literacy, and healthy eating obsession in call center employees.

MATERIAL AND METHODS

In this cross-sectional descriptive study, the data collection process was carried out via Google Forms. Participants were first asked about their willingness to participate in the study. The research was conducted with a total of 545 participants, 371 female and 174 male, working in the call center. Ethics committee approval was obtained from the Ankara Medipol University (20.12.2022, 194).

Data Collection Tools

Sociodemographic Data Form: An introductory information form, which included questions about the participants' sociodemographic characteristics such as age, gender, and education level, was applied. Body weight and height were recorded according to the statements of the participants. Body mass index (BMI) values were calculated by the researchers by dividing the body weight by the square of the height. BMI values were evaluated according to the classification of the World Health Organization (WHO), <18.5 underweight, 18.5-24.9 normal body weight, 25.0-29.9 slightly overweight, and ≥30.0 obese (1).

Perceived Food Literacy Scale (PFLS): The scale was developed by Poelman et al. (13) in 2018, and a validity and reliability study for the Turkish version was conducted by Tarı Selçuk et al. (14) in 2020. It consists of 29 items in total, and 8 sub-dimensions as food preparation skills, resilience and resistance, healthy snack styles, social and conscious eating, examining food labels, daily food planning, healthy budgeting, and healthy food stockpiling. Individuals' agreement with each statement was determined by a five-point Likert scale, 5: always done to 1: never done. As the scores of individuals on the scale increase, their awareness of eating also increases. In the scale, items 2, 10, 12, 19, 26, 27, 28, and 29 are scored by reversing. The sum of the scores obtained from all items in the scale indicates the perceived food literacy level and high scores indicate the high level of food literacy. The score range for the whole scale is 48-141. In this study, the internal reliability coefficient (Cronbach alpha) of the scale was found to be 0.86.

Health Literacy Scale (HLS): The 47-item Health Literacy Survey in Europe (HLS-EU) form developed by Sorensen was later simplified as the HLS with the collaboration of Toçi, Bruzari, and Sorenson. Sezer and Kadıoğlu (12) conducted the validity and reliability study. The scale consists of 25 items and four subscales. Access to information includes five items (items 1-5), the score range of this subscale is 5-25. Understanding information includes seven items (items 6-12), the score range in this subscale is 7-35. The appraisal/evaluation subscale includes eight items (items 13-20), the score range on this scale is 8-40. The application/use subscale includes five items (items 21-25), the score range on this scale is 5-25. The score range for the whole scale is 25-125. The items of the scale are in a Likert structure as 5: I have no difficulty, 4: I have little difficulty, 3: I have some difficulty, 2: I have a lot of difficulty, 1: I am unable to do / I have no ability / impossible. All items of the scale have a positive structure, there is no reverse item. Low scores indicate insufficient, problematic, and poor health literacy, while high scores indicate adequate and very good. The higher the score, the higher the individual's health literacy level. The score range for the whole scale is 6-30 (15). In this study, the internal reliability coefficient (Cronbach alpha) of the scale was found to be 0.91.

ORTO-R: This scale was used to measure the intensity of participants' orthorexic behaviors. It consists of 6 items, developed by Rogoza et al. (16). Arusoglu et al. (5) conducted the validity and reliability study. The level of agreement of individuals with each statement is determined using a 5-point Likert scale. The person is asked to score 5 points if he/she always does the specified situations to 1 point if he/she never does. The scores of individuals from the scale were used for intergroup

comparisons (16). In this study, the internal validity coefficient (Cronbach alpha value) of the scale was found to be 0.83.

Statistical Analysis

The data obtained in the study were analyzed using the IBM SPSS v.22 program. Number, percentage, mean, and standard deviation were used for descriptive statistical methods in the evaluation of the data. The t-test was used to compare continuous data between two independent groups, and the One-way ANOVA test was used to compare more than two independent groups. Scheffe test was used as a complementary posthoc analysis to determine the differences after the ANOVA test. Pearson correlation and regression analysis were applied between the continuous variables. Linear regression (enter) was used to determine the effects of correlated independent variables on health literacy and perceived food literacy. The logistic regression model was also used to determine the relationship between perceived food literacy and health literacy. The findings were evaluated at the 95% confidence interval and at the 0.05 significance level.

RESULTS

A total of 545 individuals, 68.1% (n=371) female, and 31.9% (n=174) male, participated in the study. The majority of the participants (74.9%, n=408) graduated from university, and 48.4% (n=264) were married. Of the participants, 91.9% (n=501) knew how to have a healthy diet, 68.3% (n=372) were not doing physical exercise, and 84.0% (n=458) did not define obesity in their first-degree relatives. In addition, 51.7% (n=282) of the participants had a healthy normal BMI, while 41.7% (n=227) were overweight.

Cronbach alpha values of PFLS, HLS, and ORTO-R were found to be 0.84, 0.78, and 0.81, respectively, and the total scores were 100.18±14.77 for PFLS, 111.40±13.59 for HLS, and 17.55±4.38 for ORTO-R.

PFLS (p=0.008) and ORTO-R (p=0.004) scores of female participants were higher than male participants, and those who were married had higher PFLS total scores than singles (p=0.003). Individuals who think they eat healthy have higher HLS (114.53±12.04) and PFLS (105.24±14.71) scores, but lower ORTO-R scores (17.05±3.96) than those who don't think they eat healthy (p<0.001). It was observed that those who knew how to have a healthy diet and those who did physical exercise scored higher on HLS and PFLS (p<0.001). PFLS and ORTO-R differed significantly between the groups according to BMI. In the PFLS scores, underweight individuals scored higher than normal-weight individuals, and normal-weight individuals scored higher than overweight individuals (p=0.004). ORTO-R scores of the overweight group were higher than the other two groups and the difference was statistically significant (p<0.001).

There were weak and moderate positive correlations between both total and sub-dimension scores of PFLS and HLS (Table 2). While food preparation skills, one of the sub-dimensions of the PFLS showed a positive weak correlation with ORTO-R (r=0.157, p<0.001), ORTO-R is also correlated with healthy snack styles (r=0.109, p=0.011), examination of food labels (r=0.256, p<0.001), and daily food planning (r=0.261, p<0.001), and also the total score of the PFLS (r=0.128, p=0.003).

Multiple regression analysis was performed to reveal how health literacy is affected by thinking about living healthy and knowing how to eat healthy. A one-unit increase in those who think they live healthily affects health literacy by 5.026 units, while the same increase in knowing how to live healthily affects health literacy by 9.943 units. A total of 12.5% change in the level of health literacy is associated

Table 1. Comparison of the scales scores according to the demographic characteristics

Gender	Male (n=174)	Female (n=371)	p
HLS	110.51±15.62	111.82±12.52	0.295
PFLS	97.75±14.94	101.33±14.57	**0.008**
ORTO-R	16.78±4.13	17.91±4.54	**0.004**

Marital Status	Married (n=264)	Single (n=281)	p
HLS	111.67±13.96	111.15±13.25	0.654
PFLS	101.58±14.99	98.87±14.54	**0.003**
ORTO-R	17.31±4.21	17.77±4.52	0.216

Age Group	19-29 years (n=228)	30-39 years (n=253)	≥40 years (n=64)	p
HLS	110.27±13.90	111.78±13.53	113.58±12.40	0.143
PFLS	98.67±14.30	101.80±15.43	99.20±12.65	0.064
ORTO-R	17.78±4.39	17.53±4.46	16.79±3.96	0.277

BMI Group	Underweight (n=36)	Normal (n=282)	Overweight (n=227)	p
HLS	115.36±10.44	111.40±13.84	110.78±13.65	0.172
PFLS	105.19±15.86[a]	101.33±15.23[b]	97.97±13.81[c]	**0.004**
ORTO-R	17.41±4.80[ab]	16.92±4.25[a]	18.35±4.36[b]	**0.001**

PFLS: perceived food literacy scale, HLS: health literacy scale, BMI: body mass index

Table 2. Correlation between perceived food literacy and health literacy

	Accessing Information		Understanding Information		Appraising Information		Applying Information		HLS Total		ORTO-R	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Food preparation skills	0.273	**<0.001**	0.352	**<0.001**	0.334	**<0.001**	0.273	**<0.001**	0.363	**<0.001**	0.157	**<0.001**
Resilience and resistance	0.231	**<0.001**	0.242	**<0.001**	0.327	**<0.001**	0.431	**<0.001**	0.361	**<0.001**	-0.057	0.185
Healthy snack styles	0.242	**<0.001**	0.264	**<0.001**	0.308	**<0.001**	0.346	**<0.001**	0.340	**<0.001**	0.109	**0.011**
Social and conscious eating	0.104	**0.015**	0.147	**0.001**	0.148	**0.001**	0.092	**0.032**	0.146	**0.001**	0.032	0.455
Examination of food labels	0.067	0.117	0.104	**0.015**	0.158	**<0.001**	0.213	**<0.001**	0.161	**<0.001**	0.256	**<0.001**
Daily food planning	0.070	0.102	0.089	**0.037**	0.117	**0.006**	0.168	**<0.001**	0.131	**0.002**	0.261	**<0.001**
Healthy budgeting	0.299	**<0.001**	0.311	**<0.001**	0.366	**<0.001**	0.421	**<0.001**	0.409	**<0.001**	0.128	**0.003**
Healthy food stockpiling	0.038	0.377	0.022	0.612	0.028	0.510	0.025	0.555	0.033	0.447	0.074	0.085
PFLS Total	0.291	**<0.001**	0.335	**<0.001**	0.386	**<0.001**	0.420	**<0.001**	0.421	**<0.001**	0.128	**0.003**

PFLS: perceived food literacy scale, HLS: health literacy scale

with thinking about eating healthy and knowing how to eat healthy (R^2=0.125). PFLS is negatively affected by 6.314 units from the male gender, 2.812 units from being single, 9.269 units from not considering a healthy diet, and 5.866 units from not doing physical exercise. Gender, marital status, thinking that eating healthy, and exercising affect the perceived food literacy at a rate of 31.8% (R^2=0.318). The effect of health literacy on perceived food literacy was tested with simple linear regression analysis and the results were found to be statistically significant (p<0.001). A significant relationship was found between perceived food literacy and health literacy. The adjusted R^2 value shows the generalizability of the model and the created model explains 17.7% of the total variance.

DISCUSSION

Despite the rapid developments in the food industry in recent years, exposure to important hazards arising from unhealthy foods and bad eating habits continues, as is the case with call center workers who live sedentarily and have to work constantly at a desk (17). From this point of view, this study aimed to investigate the relationships between food literacy and health promotion literacy of call center workers. In addition, no studies have been found in the literature on the food and health literacy of call center employees who have very sedentary living conditions. While 51.7% (n=282) of the participants in our study had a healthy BMI, 41.7% (n=227) were overweight. Factors affecting nutritional literacy include sociodemographic factors such as age, gender, education level, marital status, and income level. In order for individuals to lead a healthy life and gain healthy eating habits, it is necessary to increase food and nutrition literacy. The results of this study showed that both food and health literacy are closely related to sociodemographic and individual factors. In line with the findings of previous studies (18,19), statistically significant relationships were found between food and nutritional knowledge, food skills, endurance, and eating behaviors. The most striking finding was that females' PFLS scores were higher than males, while married females had a higher PFLS total score than singles. It has been found that gender and marital status play an important moderator role between food literacy and healthy eating habits in call center employees. Especially married and female participants had higher awareness of food literacy than males in terms of their resilience levels. It is assumed that since females are generally more sensitive about their appearance than males, they attach more importance to maintaining their healthy weight and therefore they constantly try to eat a balanced diet. It is thought that those who are married have higher health and food literacy because they are more organized and have more responsibilities. According to the results of the regression analysis, being male, being single, not thinking about a healthy diet, and not doing physical exercise are the factors that reduce PFLS. These factors explain 18% of the variance for PFLS. Participants who did not know how a healthy diet should be, smoking status, and BMI did not have a significant effect on PFLS.

It has been determined that individuals who think they eat healthy have higher HLS scores but lower ORTO-R scores than those who do not. In addition, it was determined that among the call center employees, those who do physical exercise and knew how to eat healthily had higher food and health literacy levels, and those who were overweight had a much higher orthorexic behavior. These results show that call center workers should be encouraged to develop not only declarative but also psychological and self-regulating aspects of food literacy in a balanced way through many different educational and cultural ways in order to lead a healthier life (13). In addition, health literacy was also significantly associated with food literacy levels, consistent with previous findings. This confirms that food and health are inextricably linked, meaning that all relevant variables need to be carefully considered in relation to each other. Knutsson et al. (20) reported that those who work constantly at a desk and/or in shifts exhibit more orthorexic eating behavior than those who work in daytime and active working conditions. In our study, it was determined that about half of the call center employees were overweight and these people developed more orthorexic behaviors. While the obsession with healthy eating may directly affect the lives of individuals and cause nutritional deficiencies and moreover eating behavior disorders, in some cases, the dietary rules applied by the individual in order to improve health may have harmful consequences on health (21). In the literature, it is stated that age, interest in healthy nutrition, and being compatible

with innovations in healthy nutrition are affected by health literacy, there is an increase in health literacy as age increases, and also knowing how to eat healthy is directly related to health literacy (22,23). In a study, it was determined that those with a BMI in the normal range and those who work in the health field, either themselves or one of their family members, had a higher level of nutritional literacy (24,25). While it has been reported that the level of nutritional literacy decreases with increasing age (26), there are studies reporting that there is no relationship between nutritional literacy and gender (27), or that female participants have higher nutritional literacy than male participants (28,29). This may be due to the fact that females are more interested in nutrition and health than males, have more knowledge and skills in food preparation, and are responsible for family nutrition.

Low health and food literacy is known to pose a barrier to healthy eating. Because healthy eating is an important part of a healthy life. In this direction, the primary purpose of the workplace physician is to maximize the health, physical, mental, and social well-being of the employee and maintain this level, and provide the necessary training. Therefore, it is among the primary duties of the workplace physician to raise awareness of healthy nutrition by increasing the health and food literacy levels of the call center employees who are constantly working at the desk. The more the occupational physician focuses on health and food literacy training, the better the physiological and psychological health of the employees will be.

In our study, it was determined that an increase in those who did not think they had a healthy diet decreased HLS by 5.026 units and those who did not know how to eat healthy decreased it by 9.943 units. It can be said that these factors explain 12.5% of the variance for HLS. In order to develop a healthy lifestyle, perceived food literacy and health literacy should be evaluated together.

According to our research results, perceived food literacy explains 17.7% of health literacy. While Apaydın Demirci and Çelik (30) reported that perceived food literacy explains 9.9% of healthy lifestyle behavior, İncedal Sonkaya et al. (31) stated that eating behavior explains 20.2% of the change in the dependent variable of health literacy, Çakıcı and Yıldız (32) reported that health awareness explains 34.4% of healthy eating behavior. Although more studies are needed, there are important research results that perceived food literacy may have a protective effect on health (33).

CONCLUSION

It is thought that effective and continuous training programs will be beneficial for call center employees who have inactive and sedentary working conditions due to their work, in order to determine their deficiencies or inadequacies in terms of gaining food literacy, health literacy, and healthy eating behavior. The working conditions of call center employees significantly affect the physical and psychological health of individuals. In order to eliminate the said negativities, employees should be informed about the health risks created by the work environment and their demands for training should be taken into consideration. In order to achieve this, it is important to plan regular trainings, to make occupational medicine active, preventive medicine, qualified, and accessible. It is necessary for the occupational physician to continue his/her profession without being under the pressure of any structure such as the employer, or state through the law, and the right to make free decisions based on scientific knowledge should be protected.

Ethics Committee Approval: The study was approved by the Non-interventional Clinical Research Ethics Committee of Ankara Medipol University (20.12.2022, 194).

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The Expression of Caspase-3 and GRIM-19 in Non-mucinous Lung Adenocarcinoma and Their Clinicopathologic Significance

Müsinöz Olmayan Akciğer Adenokarsinomlarında Caspase-3 ve GRIM-19 Ekspresyonu ile Bu Proteinlerin Klinikopatolojik Önemi

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ABSTRACT

Aim: The current study aimed to investigate apoptotic proteins such as caspase-3 and GRIM-19 protein expression in non-mucinous lung adenocarcinomas and their clinicopathologic significance.
Material and Methods: This study was performed on 81 patients diagnosed with non-mucinous lung adenocarcinoma between January 1, 2010, and June 1, 2020. Immunohistochemical analysis was performed to examine the expressions of caspase-3 and GRIM-19, and the association between these proteins and clinicopathological parameters was investigated.
Results: Caspase-3 nuclear positivity was more common in high-grade non-mucinous lung adenocarcinomas (p<0.001). Caspase-3 cytoplasmic expression was stronger in tumors with advanced-stage (p=0.021) and lymph node metastases (p=0.020). GRIM-19 expression was low in tumors with high-grade non-mucinous lung adenocarcinomas (p=0.002), and tumors with lymphovascular invasion (p=0.021). The median follow-up time was 31.7 (range, 1-145 months. The overall 5-year survival rate of patients with low and high GRIM-19 expression tumors was 48% and 92%, respectively. GRIM-19 expression significantly affected the 5-year overall survival rate (p=0.008), but not the 5-year disease-free survival rate (p=0.368).
Conclusion: We revealed a significant association between caspase-3 and GRIM-19 expressions and poor clinicopathologic features and prognosis. For the first time in the literature, we revealed an association between low GRIM-19 expression and worse clinical outcomes in patients with non-mucinous lung adenocarcinoma. Caspase-3 and GRIM-19 may become potential therapeutic targets and novel potential predictive biomarkers for non-mucinous lung adenocarcinoma patients.
Keywords: Apoptosis; Caspase-3; GRIM-19; lung adenocarcinoma.

ÖZ

Amaç: Bu çalışmada, müsinöz olmayan akciğer adenokarsinomlarında, apoptotik protein olarak bilinen caspase-3 ve GRIM-19 protein ekspresyonu ve bu proteinlerin klinikopatolojik öneminin araştırılması amaçlandı.
Gereç ve Yöntemler: Bu çalışma, 1 Ocak 2010 ile 1 Haziran 2020 tarihleri arasında müsinöz olmayan akciğer adenokarsinomu tanısı alan 81 hasta üzerinde gerçekleştirildi. Caspase-3 ve GRIM-19 ekspresyonlarını incelemek için immünohistokimyasal analiz yapıldı ve bu proteinler ile klinikopatolojik parametreler arasındaki ilişki araştırıldı.
Bulgular: Caspase-3 nükleer pozitifliği yüksek dereceli müsinöz olmayan akciğer adenokarsinomlarında daha yaygın bulundu (p<0,001). Caspase-3 sitoplazmik ekspresyonu ileri evre (p=0,021) ve lenf nodu metastazı (p=0,020) olan tümörlerde daha güçlü saptandı. GRIM-19 ekspresyonu, yüksek dereceli müsinöz olmayan akciğer adenokarsinomlu tümörlerde (p=0,002) ve lenfovasküler invazyonlu tümörlerde (p=0,021) düşük idi. Ortanca takip süresi 31,7 (aralık, 1-145) ay idi. Düşük ve yüksek GRIM-19 ekspresyonlu tümörlere sahip hastaların 5 yıllık genel sağkalım oranı sırasıyla %48 ve %92 idi. GRIM-19 ekspresyonu genel 5 yıllık sağkalım oranı üzerinde anlamlı bir etkisi olduğu (p=0,008), ancak 5 yıllık hastalıksız sağkalım oranı üzerinde anlamlı bir etkisi olmadığı (p=0,368) saptandı.
Sonuç: Caspase-3 ve GRIM-19 ekspresyonu ile kötü klinikoptolojik özellikler ve prognoz arasında anlamlı bir ilişki olduğunu gösterdik. Literatürde ilk kez, düşük GRIM-19 ekspresyonunun müsinöz olmayan akciğer adenokarsinomunda daha kötü bir klinik gidişle ilişkili olduğunu ortaya koyduk. Caspase-3 ve GRIM-19 müsinöz olmayan akciğer adenokarsinomu hastaları için yeni potansiyel prognostik biyobelirteçlerin yanı sıra potansiyel terapötik hedefler haline gelebilir.
Anahtar kelimeler: Apoptoz; Caspase-3; GRIM-19; akciğer adenokarsinomu.

INTRODUCTION

Invasive non-mucinous adenocarcinoma is the most common subtype of lung carcinoma and is responsible for the highest number of cancer-related fatalities globally (1,2). The aggressive nature of lung adenocarcinomas persists despite significant progress in treatment alternatives, attributed to mutations in multiple oncogenes, tumor suppressor genes, and apoptotic proteins (3).

Initiation of apoptosis involves a series of sequential steps triggered by the activation of a group of cysteine proteases known as "caspases" (4). Caspase-3, a well-known member of the caspase enzyme family, assumes a crucial role in both the intrinsic and extrinsic pathways of apoptosis (4). The extrinsic pathway primarily involves death receptor proteins located on the cytoplasmic membrane, whereas the intrinsic pathway is initiated by a disruption of mitochondrial membrane permeability, leading to the release of cytochrome c from the intermembranous space of mitochondria into the cytoplasm. Subsequently, cytochrome c activates caspase-3, initiating a cascade in the intrinsic apoptotic pathway (4,5). In addition to its apoptotic influence, caspase-3 released from apoptotic cells exerts an impact on the proliferation, differentiation, and survival of neighboring malignant or normal cells (4).

On the other hand, Liu et al. (6) demonstrated that mitochondrial permeability changes lead to spontaneous cytochrome c leakage and sublethal caspase-3 activation specifically in cancer cells, rather than in normal cells. Sublethal caspase-3 activation does not cause apoptosis but leads to spontaneous DNA double-strand breaks, chromosomal instability, and gene mutations, and promotes malignant transformation (6). Thus, caspase-3 is crucial for cell maintenance. Previous studies have demonstrated caspase-3 expression in some malignant tumors, such as breast, colorectal, cervical, squamous cell carcinoma, and glioma (7). However, the literature currently lacks sufficient data on caspase-3 expression in lung adenocarcinoma and its impact on prognosis.

The interferon (IFN) family of cytokines is known for its effectiveness in antiviral and immune responses, as well as its antitumor effects. The primary effect of IFNs is the elimination of infected or neoplastic cells by inducing cell cycle inhibition or apoptosis. Retinoic acid (RA), a natural metabolite of vitamin A, is involved in cell growth, differentiation, and embryogenesis (8). The combination therapy of IFN and RA exhibits a synergistic effect, leading to more effective inhibition of tumor growth. Previous studies have revealed enhanced tumor growth inhibition with IFN/RA combination therapy (8-10). GRIM-19 is involved in the IFN-β/RA-induced cell death pathway and has been shown to synergistically suppress cell cycle progression (8-10) and exhibit tumor suppressor effects (11). Loss or significant reduction of GRIM-19 expression has been reported in various malignancies, such as cervical, ovarian, kidney, colorectal, and hepatocellular carcinoma (10,12). However, it has been reported that GRIM-19 overexpression induces apoptosis in the human breast carcinoma cell line MCF-7, and gastric cancer cells, and suppresses hepatocellular carcinoma growth (13,14). A molecular study conducted by Wang et al. (15) revealed a decrease in GRIM-19 RNA and protein levels, and tumor cell growth was suppressed by GRIM-19 overexpression, promoting tumor cell apoptosis in vivo and in vitro in lung adenocarcinoma. Despite this, the precise relationship between GRIM-19 expression, clinicopathological features, and its influence on tumor progression remains incompletely understood.

This study aimed twofold: firstly, to evaluate the expression of caspase-3 and GRIM-19 in invasive non-mucinous lung adenocarcinomas, and secondly, to explore the association between their expression and clinicopathological parameters, potentially offering valuable prognostic insights.

MATERIAL AND METHODS

The Clinical Research Ethics Committee of Baskent University (Project no: KA23/162; Date: 27 April 2023) approved the current study which was compatible with the ethical guidelines of the Declaration of Helsinki. This study included 81 patients diagnosed with non-mucinous lung adenocarcinoma after lobectomy or pneumonectomy between January 1, 2010, and June 1, 2020. Patients receiving preoperative chemotherapy and/or radiotherapy treatment and patients with mucinous adenocarcinoma were excluded. Two pathologists (MT, EYA) re-evaluated all histopathological slides. Clinical follow-up findings of all patients were obtained from hospital records. The classification of tumors was carried out according to the diagnostic criteria established by the World Health Organization (WHO) in 2021, and the staging was performed using the American Joint Committee on Cancer (AJCC) 8th edition (1).

Tissue microarray (TMA) blocks were prepared by removing tissue from two representative foci involving different areas with a diameter of 2 mm. Immunohistochemically, rabbit polyclonal Caspase-3 antibody (IgG isotype, 1:100; Genetex) and rabbit monoclonal GRIM-19 antibody (clone EPR4471 (2), 1:200; Epitomics) were applied on the 4μm-thick slices of TMA blocks with Dako Omnis system and are stained with EnVision FLEX staining kits. Appropriate positive controls were used.

Two pathologists (AOA, MT) independently evaluated the immunohistochemistry-stained slides. The nuclear caspase-3 immunoreactivity was assessed semiquantitatively by determining the percentage of cells exhibiting positive staining: <10% staining was classified as negative, while >10% staining was classified as positive (16). For the cytoplasmic staining intensity of caspase-3, the tumor was graded as follows: 1 for mild expression, 2 for moderate expression, and 3 for strong expression (17). GRIM-19 expression in the cytoplasm, with or without a nucleus, was also evaluated semiquantitatively. The intensity was scored as 0 for no staining, 1 for mild staining, 2 for moderate staining, and 3 for strong staining. The percentage of positive tumor cells was scored from 0 to 100%. An h-score was calculated by multiplying the intensity score and the percentage of staining. An h-score ≤120 indicated low expression, while an h-score >120 indicated high expression (18).

Caspase-3 and GRIM-19 expression in the non-mucinous lung adenocarcinoma tissues is illustrated in Figure 1 and Figure 2, respectively.

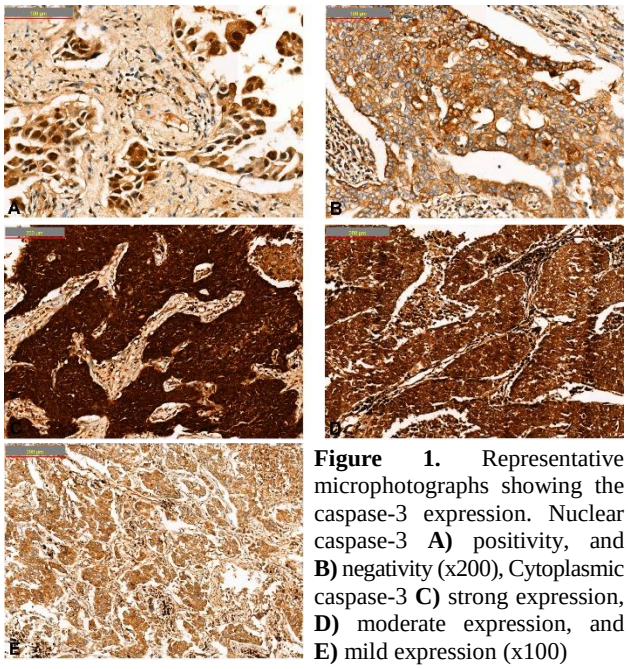

Figure 1. Representative microphotographs showing the caspase-3 expression. Nuclear caspase-3 **A)** positivity, and **B)** negativity (x200), Cytoplasmic caspase-3 **C)** strong expression, **D)** moderate expression, and **E)** mild expression (x100)

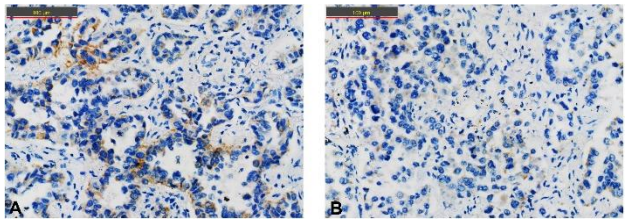

Figure 2. Representative microphotographs showing the GRIM-19 expression. **A)** high, and **B)** low GRIM-19 expression (x200)

Table 1. Clinicopathologic characteristics of patients with non-mucinous lung adenocarcinoma

Age (years), median (range)	65 (36-85)
Age, n (%)	
<65 years	39 (48.1)
≥65 years	42 (51.9)
Gender, n (%)	
Female	20 (24.7)
Male	61 (75.3)
Smoking status, n (%)	
Current smoker	38 (46.9)
Ex-Smoker	25 (30.9)
Never Smoker	18 (22.2)
Surgery, n (%)	
Lobectomy	71 (87.7)
Pneumonectomy	10 (12.3)
Tumor size (cm), median (range)	2.4 (0.8-11.5)
Tumor size, n (%)	
<2.5 cm	40 (49.4)
≥2.5 cm	41 (50.6)
Histology, n (%)	
Lepidic	4 (4.9)
Aciner	46 (56.8)
Papillary	8 (9.9)
Micropapillary	3 (3.7)
Solid	20 (24.7)
Stage, n (%)	
Stage I	44 (54.3)
Stage II	10 (12.3)
Stage III	24 (29.6)
Stage IV	3 (3.7)
Lymphovascular invasion, n (%)	
Absent	36 (44.4)
Present	45 (55.6)
Lymph node metastases, n (%)	
Absent	50 (61.7)
Present	31 (38.3)
Perineural invasion, n (%)	
Absent	72 (88.9)
Present	9 (11.1)
Necrosis, n (%)	
Absent	39 (48.1)
Present	42 (51.9)
Visseral pleura involvement, n (%)	
Absent	34 (42.0)
Present	47 (58.0)
Recurrence, n (%)	
No recurrence	70 (86.4)
Recurrence	11 (13.6)
Follow-up status, n (%)	
Dead of disease	30 (37.0)
Alive with recurrent disease	3 (3.7)
Alive with no evidence of disease	46 (56.8)
Dead of nondisease	2 (2.5)

Statistical Analysis

Statistical analyses were made with the IBM SPSS v.25 program. Descriptive analyzes were presented using median and minimum-maximum values. Analytical methods (Kolmogorov-Smirnov test) were used to determine whether the variables were normally distributed. Since normal distribution could not obtain, non-parametric tests were performed. The Pearson chi-square or Fisher's exact test was used to compare the qualitative variables and represented by numbers and percentages. The Univariate Kaplan-Meier method was used to estimate overall survival (OS) and disease-free survival (DFS) rates and results were compared using the long-rank test. Statistical significance was considered only for p-values below 0.05.

RESULTS

Among the 81 patients enrolled in the study, 61 (75.3%) were male, while 20 (24.7%) were female. The median age was 65 (range, 36-85) years. Tumor diameters ranged from 0.8 cm to 11.5 cm, with a median of 2.4 cm. Among the tumors, 4 (4.9%) were grade 1 (all showed a lepidic pattern), 54 (66.7%) were grade 2 (46 showed an acinar, 3 showed a papillary), and 23 (28.4%) were grade 3 (3 showing a micropapillary, 20 showed a solid pattern). 44 (54.3%) tumors were classified as stage I, 10 (12.3%) as stage II, 24 (29.6%) as stage III, and 3 (3.7%) as stage IV disease. Early-stage tumors included stages I and II, while advanced-stage tumors included stages III and IV. The summary of the clinicopathological characteristics of the study cases was presented in Table 1.

Tumors showed staining in the tumor cells from 0% to 100% and varying intensities of cytoplasmic caspase-3 expression with or without nuclear expression. As shown in Table 2, 65 (80.2%) tumors exhibited caspase-3 nuclear positivity. Among the different histologic patterns, nuclear caspase-3 positivity was observed in none of the lepidic pattern adenocarcinomas, 41 (89.1%) of the acinar pattern, 4 (50%) of the papillary pattern, all (100%) of the micropapillary pattern, and 17 (85%) of the solid pattern tumors. Additionally, caspase-3 nuclear positivity was

found in none of 4 histologic grade 1 tumors, 45 (83.3%) of 54 histologic grade 2, and 20 (87%) of 23 histologic grade 3. Caspase-3 nuclear positivity demonstrated a significant association with tumor histology (p<0.001) and histologic grading (p<0.001). There were no significant associations observed between nuclear positivity of caspase-3 and age (p=0.469), gender (p=0.060), smoking status (p=0.208), tumor size (p=0.956), tumor stage (p=0.844), pT stage (p=0.979), lymphovascular invasion (p=0.289), lymph node metastases (p=0.519), perineural invasion (p=0.490), and necrosis (p=0.694).

Regarding caspase-3 cytoplasmic expression, 25 (30.9%) of 81 non-mucinous lung adenocarcinomas had mild expression, 40 (49.4%) had moderate, and 16 (19.8%) had strong expression. Among the early-stage tumors, 19 (35.2%) showed mild caspase-3 cytoplasmic expression, 29 (53.7%) showed moderate expression, and 6 (11.1%) showed strong expression. Among the advanced-stage tumors, 6 (22.2%) showed mild caspase-3 cytoplasmic expression, 11 (40.7%) showed moderate expression, and 10 (37.0%) showed strong expression. Caspase-3 cytoplasmic expression was stronger in advanced-stage compared to early-stage tumors, and a significant association was observed between caspase-3 cytoplasmic expression and tumor stage (p=0.021). Tumors with lymph node metastases exhibited stronger caspase-3 cytoplasmic expression compared to those without metastases (p=0.020). There were no significant associations observed between cytoplasmic expression of caspase-3 and age (p=0.583), gender (p=0.551), smoking status (p=0.598), tumor size (p=0.607), histology (p=0.731), histologic grade (p=0.345), pT stage (p=0.635), lymphovascular invasion (p=0.215), perineural invasion (p=0.595), and presence of necrosis (p=0.741).

For GRIM-19 expression, tumors displayed varying intensities of cytoplasmic expression without nuclear expression. 12 (14.8%) of 81 non-mucinous lung adenocarcinomas exhibited high GRIM-19 expression, and 69 (85.2%) exhibited low GRIM-19 expression. Low GRIM-19 expression was observed in 1 (25%) of the lepidic pattern adenocarcinomas, 42 (91.3%) of the acinar pattern, 6 (75%) of the papillary pattern, 2 (66.7%) of the micropapillary pattern, and 18 (90%) of the solid pattern tumors (p=0.005). Low GRIM-19 expression was observed in 1 (25%) of the histologic grade 1 tumors, 48 (88.9%) of the histologic grade 2 tumors, and 20 (87%) of the histologic grade 3 tumors. Tumors with higher histologic grades exhibited lower GRIM-19 expression compared to those with lower grades, indicating a significant association between GRIM-19 expression and histologic grading (p=0.002). Among the advanced-stage tumors, 26 (96.3%) showed low GRIM-19 expression, while among the early-stage tumors, 43 (79.6%) showed low expression, and a significant association was observed between GRIM-19 expression and tumor stage (p=0.047). Among tumors with lymphovascular invasion, 42 (93.3%) exhibited low GRIM-19 expression, while among tumors without lymphovascular invasion, 27 (75%) exhibited low expression, indicating a significant association between GRIM-19 expression and lymphovascular invasion (p=0.021). There were no significant associations observed between GRIM-19 expression and age (p=0.889), gender (p=0.452), smoking status (p=0.252), tumor size (p=0.502), pT stage (p=0.074), lymph node metastases (p=0.095), perineural invasion (p=0.740), and necrosis (p=0.444).

The median follow-up duration was 31.7 (range, 1-145) months. Out of the 81 patients, 30 (37.0%) died from carcinoma, while 2 (2.5%) patients died from other causes without evidence of recurrence and were considered censored. Three (3.7%) patients were still alive with the disease, and 46 (56.8%) were alive with no evidence of disease.

The 5-year OS rate showed a significant difference between patients with and without tumors exhibiting lymphovascular invasion (26% vs. 62%, p=0.009, Figure 3). The median overall survival time for patients with low GRIM-19 expression was 29.42 (range, 1-109) months, while for patients with high GRIM-19 expression, it was 44.35 (range, 14-145) months. The 5-year OS rate for patients with low GRIM-19 expression tumors was 48%, whereas, for patients with high GRIM-19 expression tumors, it was 92%. Univariate Kaplan-Meier/log-rank analyses showed that patients with tumors exhibiting low GRIM-19 expression had significantly lower 5-year OS rates compared to those with high GRIM-19 expression (p=0.008, Figure 4A). Nevertheless, there was no significant association between the 5-year OS rate and caspase-3 cytoplasmic expression (p=0.330, Figure 4B), caspase-3 nuclear expression (p=0.412, Figure 4C), and other clinicopathologic factors such as age (p=0.827), gender (p=0.429), smoking (p=0.738), tumor size (p=0.616), tumor histology (p=0.476), histologic grading (p=0.601), tumor stage (p=0.119), pT stage (p=0.076), lymph node metastases (p=0.353), perineural invasion (p=0.565), and presence of necrosis (p=0.169).

Only 11 (13.6%) experienced recurrence. The median time to recurrence was 21.74 (range, 4.9-41.7) months. The 5-year DFS rate showed no significant association with the GRIM-19 expression (p=0.368, Figure 5A), caspase-3 cytoplasmic expression (p=0.581, Figure 5B), caspase-3 nuclear expression (p=0.447, Figure 5C), and clinicopathologic factors such as age (p=0.785), gender (p=0.187), smoking (p=0.094), tumor size (p=0.090),

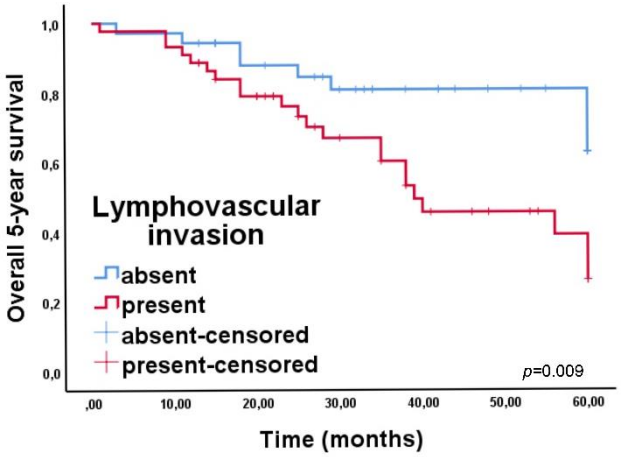

Figure 3. The Kaplan-Meier curves of lymphovascular invasion for 5-year overall survival rate. The 5-year OS rate of patients with tumors with lymphovascular invasion and no lymphovascular invasion was 26% and 62% (p=0.009)

Table 2. The association between caspase-3 and GRIM-19 expression and clinicopathologic features

	Age		**p**
	<65 years (n=39)	**≥65 years (n=42)**	
Caspase-3 nuclear expression, n (%)			
Negative	9 (23.1)	7 (16.7)	0.469
Positive	30 (76.9)	35 (83.3)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	13 (33.3)	12 (28.6)	
Moderate	17 (43.6)	23 (54.8)	0.583
Strong	9 (23.1)	7 (16.7)	
GRIM-19 expression, n (%)			
Low	33 (84.6)	36 (85.7)	0.889
High	6 (15.4)	6 (14.3)	
	Gender		**p**
	Female (n=20)	**Male (n=61)**	
Caspase-3 nuclear expression, n (%)			
Negative	7 (35)	9 (14.8)	0.060
Positive	13 (65)	52 (85.2)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	8 (40)	17 (27.9)	
Moderate	8 (40)	32 (52.5)	0.551
Strong	4 (20)	12 (19.7)	
GRIM-19 expression, n (%)			
Low	16 (80)	53 (86.9)	0.452
High	4 (20)	8 (13.1)	
	Smoking Status		**p**
	Current smoker (n=38)	**Ex-Smoker (n=25)**	**Never Smoker (n=18)**	
Caspase-3 nuclear expression, n (%)				
Negative	5 (13.2)	5 (20)	6 (33.3)	0.208
Positive	33 (86.8)	20 (80)	12 (66.7)	
Caspase-3 cytoplasmic expression, n (%)				
Mild	12 (31.6)	7 (28)	6 (33.3)	
Moderate	16 (42.1)	15 (60)	9 (50.0)	0.598
Strong	10 (26.3)	3 (12)	3 (16.7)	
GRIM-19 expression, n (%)				
Low	35 (92.1)	20 (80)	14 (77.8)	0.252
High	3 (7.9)	5 (20)	4 (22.2)	
	Tumor size		**p**
	<2.4 cm (n=40)	**≥2.4 cm (n=41)**	
Caspase-3 nuclear expression, n (%)			
Negative	8 (20)	8 (19.5)	0.956
Positive	32 (80)	33 (80.5)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	11 (27.5)	14 (34.1)	
Moderate	22 (55.0)	18 (43.9)	0.607
Strong	7 (17.5)	9 (22.0)	
GRIM-19 expression, n (%)			
Low	33 (82.5)	36 (87.8)	0.502
High	7 (17.5)	5 (12.2)	
	Histology					**p**
	Lepidic (n=4)	**Aciner (n=46)**	**Papillary (n=8)**	**Micropapillary (n=3)**	**Solid (n=20)**	
Caspase-3 nuclear expression, n (%)						
Negative	4 (100)	5 (10.9)	4 (50)	0 (0)	3 (15)	**<0.001**
Positive	0 (0)	41 (89.1)	4 (50)	3 (100)	17 (85)	
Caspase-3 cytoplasmic expression, n (%)						
Mild	3 (75)	14 (30.4)	2 (25)	1 (33.3)	5 (25)	
Moderate	1 (25)	22 (47.8)	4 (50)	2 (66.7)	11 (55)	0.731
Strong	0 (0)	10 (21.7)	2 (25)	0 (0)	4 (20)	
GRIM-19 expression, n (%)						
Low	1 (25)	42 (91.3)	6 (75)	2 (66.7)	18 (90)	**0.005**
High	3 (75)	4 (8.7)	2 (25)	1 (33.3)	2 (10)	
	Histologic Grade			**p**
	Grade 1 (n=4)	**Grade 2 (n=54)**	**Grade 3 (n=23)**	
Caspase-3 nuclear expression, n (%)				
Negative	4 (100)	9 (16.7)	3 (13)	**<0.001**
Positive	0 (0)	45 (83.3)	20 (87)	
Caspase-3 cytoplasmic expression, n (%)				
Mild	3 (75)	16 (29.6)	6 (26.1)	
Moderate	1 (25)	26 (48.1)	13 (56.5)	0.345
Strong	0 (0)	12 (22.2)	4 (17.4)	
GRIM-19 expression, n (%)				
Low	1 (25)	48 (88.9)	20 (87)	**0.002**
High	3 (75)	6 (11.1)	3 (13)	

Table 2. (continued) The association between caspase-3 and GRIM-19 expression and clinicopathologic features

	Tumor Stage		**p**
	Early Stage (I-II) (n=54)	**Advanced Stage (III-IV) (n=27)**	
Caspase-3 nuclear expression, n (%)			
Negative	11 (20.4)	5 (18.5)	0.844
Positive	43 (79.6)	22 (81.5)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	19 (35.2)	6 (22.2)	
Moderate	29 (53.7)	11 (40.7)	**0.021**
Strong	6 (11.1)	10 (37.0)	
GRIM-19 expression, n (%)			
Low	43 (79.6)	26 (96.3)	**0.047**
High	11 (20.4)	1 (3.7)	
	pT Stage		**p**
	pT1-2 (n=66)	**pT3-4 (n=15)**	
Caspase-3 nuclear expression, n (%)			
Negative	13 (19.7)	3 (20)	0.979
Positive	53 (80.3)	12 (80)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	19 (28.8)	6 (40.0)	
Moderate	33 (50.0)	7 (46.7)	0.635
Strong	14 (21.2)	2 (13.3)	
GRIM-19 expression, n (%)			
Low	54 (81.8)	15 (100)	0.074
High	12 (18.2)	0 (0)	
	Lymphovascular Invasion		**p**
	Absent (n=36)	**Present (n=45)**	
Caspase-3 nuclear expression, n (%)			
Negative	9 (25)	7 (15.6)	0.289
Positive	27 (75)	38 (84.4)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	12 (33.3)	13 (28.9)	
Moderate	20 (55.6)	20 (44.4)	0.215
Strong	4 (11.1)	12 (26.7)	
GRIM-19 expression, n (%)			
Low	27 (75)	42 (93.3)	**0.021**
High	9 (25)	3 (6.7)	
	Lymph Node Metastases		**p**
	Absent (n=50)	**Present (n=31)**	
Caspase-3 nuclear expression, n (%)			
Negative	11 (22)	5 (16.1)	0.519
Positive	39 (78)	26 (83.9)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	17 (34)	8 (25.8)	
Moderate	28 (56)	12 (38.7)	**0.020**
Strong	5 (10)	11 (35.5)	
GRIM-19 expression, n (%)			
Low	40 (80)	29 (93.5)	0.095
High	10 (20)	2 (6.5)	
	Perineural Invasion		**p**
	Absent (n=72)	**Present (n=9)**	
Caspase-3 nuclear expression, n (%)			
Negative	15 (20.8)	1 (11.1)	0.490
Positive	57 (79.2)	8 (88.9)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	21 (29.2)	4 (44.4)	
Moderate	36 (50.0)	4 (44.4)	0.595
Strong	15 (20.8)	1 (11.1)	
GRIM-19 expression, n (%)			
Low	61 (84.7)	8 (88.9)	0.740
High	11 (15.3)	1 (11.1)	
	Necrosis		**p**
	Absent (n=39)	**Present (n=42)**	
Caspase-3 nuclear expression, n (%)			
Negative	7 (17.9)	9 (21.4)	0.694
Positive	32 (82.1)	33 (78.6)	
Caspase-3 cytoplasmic expression, n (%)			
Mild	11 (28.2)	14 (33.3)	
Moderate	21 (53.8)	19 (45.2)	0.741
Strong	7 (17.9)	9 (21.4)	
GRIM-19 expression, n (%)			
Low	32 (82.1)	37 (88.1)	0.444
High	7 (17.9)	5 (11.9)	

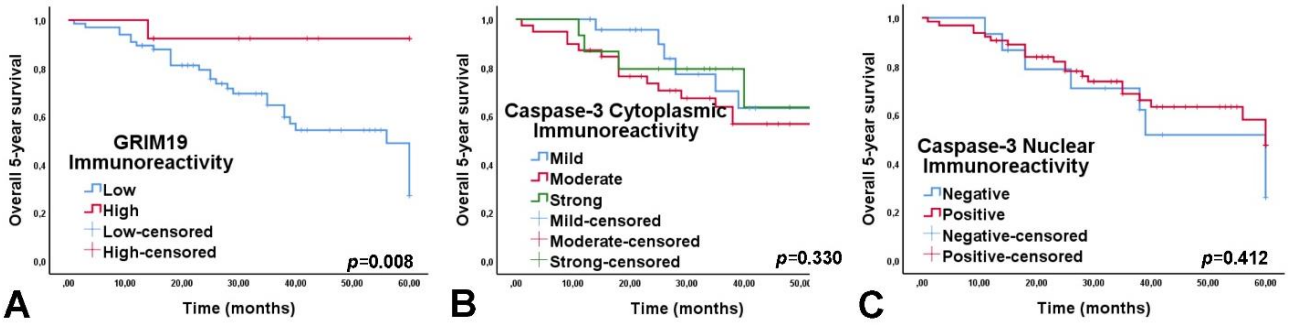

Figure 4. The Kaplan-Meier curves of GRIM-19 expression, caspase-3 cytoplasmic expression, and caspase-3 nuclear expression for 5-year overall survival. **A)** the 5-year OS rate of patients with a low GRIM-19 expression was significantly decreased compared with those with a high GRIM-19 expression (p=0.008), however, the 5-year OS rate showed no association with **B)** caspase-3 cytoplasmic expression (p=0.330), and **C)** caspase-3 nuclear expression (p=0.412)

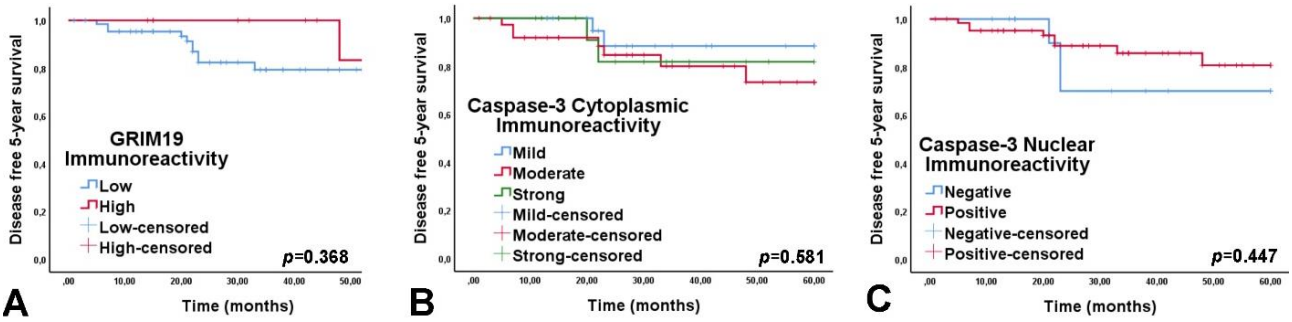

Figure 5. The Kaplan-Meier curves of GRIM-19 expression, caspase-3 cytoplasmic expression, and caspase-3 nuclear expression for 5-year disease-free survival. **A)** the 5-year DFS rate showed no association with GRIM-19 expression (p=0.368), **B)** caspase-3 cytoplasmic expression (p=0.581), and **C)** caspase-3 nuclear expression (p=0.447)

tumor histology (p=0.256), histologic grading (p=0.399), tumor stage (p=0.916), pT stage (p=0.264), lymphovascular invasion (p=0.139), lymph node metastases (p=0.534), perineural invasion (p=0.779), and presence of necrosis (p=0.384).

DISCUSSION

Invasive non-mucinous lung adenocarcinoma represents the most prevalent pathologic subtype among lung adenocarcinomas (2). The tumor growth rate depends on the balance between cellular proliferation and apoptosis, which determines tumor progression and prognosis (3). Caspase enzymes play an essential role in the apoptosis cascade. Besides their well-known apoptotic effects, caspase-3 may also cause spontaneous DNA double-strand breaks, chromosomal instability, and gene mutations, and promote malignant transformation through sublethal caspase-3 activation in cancer cells (6). Furthermore, caspase-3 has proliferative and angiogenic effects in tumor cells (4).

Caspase-3 overexpression has been demonstrated in many tumors (7). There is ongoing research on the significance of caspase-3 expression in the prognosis of malignant tumors, and the results are controversial. Several studies have shown that patients with breast, stomach, ovarian, cervical, and colorectal cancer, which exhibit high caspase-3 expression, have a worse prognosis (16,19). However, while some studies have indicated that a high level of caspase-3 expression is associated with better survival in patients with gastric and colorectal cancer (20,21), others have reported no significant relationship between caspase-3 expression and survival (22,23). Limited studies have been conducted on lung adenocarcinomas, and some of them have revealed that high caspase-3 expression is associated with longer survival rates in lung adenocarcinoma patients (24-26). However, Takata et al. (17) reported that patients with caspase-3 negative stage I lung adenocarcinoma had a higher 5-year OS rate compared to patients with caspase-3 positive tumors. In contrast to these results, our study demonstrated that caspase-3 positivity had no significant effect on 5-year OS or DFS rates.

Most of the available studies on lung carcinomas have not revealed any relationship between caspase-3 expression and prognostic factors such as histologic type, histologic grading, tumor stage, lymphovascular invasion, and lymph node involvement (17,24,25). However, Koomagi et al. (26) reported a significant association between caspase-3 expression and a lower incidence of lymph node involvement. In contrast, our study indicated that tumors with lymph node metastases and advanced tumors exhibited strong cytoplasmic expression of caspase-3, and there was a significant association between cytoplasmic caspase-3 expression and lymph node metastasis and tumor stage. Furthermore, we found that caspase-3 nuclear positivity was significantly associated with tumor histology and histologic grading in our current study. We observed that high-grade non-mucinous lung adenocarcinomas had a higher frequency of caspase-3 nuclear positivity. These findings suggest that nuclear

positivity and high cytoplasmic caspase-3 expression may be indicative of more aggressive behavior.

In addition to the well-known apoptosis pathways, rigorous research is ongoing to explore different mechanisms of cell death. GRIM-19, an apoptotic protein induced by the combination of IFN-β and RA, has emerged as a novel tumor suppressor (8,13). Overexpression of GRIM-19 has been shown to induce cell death through apoptosis (11,13,14). Notably, GRIM-19 expression is generally higher in normal tissues compared to malignant forms in various organs (27,28). Loss or reduced expression of GRIM-19 has also been observed in several tumors, including colorectal, breast, ovarian, cervical, and kidney tumors (7,28-31). Consistent with previous research, our study revealed that a majority of non-mucinous lung adenocarcinomas in our series (85.2%) exhibited low GRIM-19 expression. Furthermore, existing literature demonstrates a significant association between the loss or reduced expression of GRIM-19 and poor tumor differentiation, tumor stage, lymph node metastasis, and lymphovascular invasion in colorectal, breast, ovarian, and kidney tumors (28-31).

In the current literature, only a limited number of studies have investigated the impact of GRIM-19 on lung adenocarcinomas (15,27,32,33). Similar to our findings, Fan et al. (27) and Zhou et al. (32) reported a significant decrease in GRIM-19 expression in advanced lung adenocarcinomas compared to early-stage tumors. However, Wu et al. (33) did not observe a significant association between GRIM-19 expression and any histopathological findings, including tumor stage. In contrast to these studies, our research demonstrated that GRIM-19 expression was lower in tumors with higher histologic grades than in those with lower histologic grades. The loss of GRIM-19 expression exhibited a significant association with histologic grading. We also observed that tumors with lymphovascular invasion had lower levels of GRIM-19 expression compared to those without invasion.

Furthermore, our study revealed an interesting finding that patients with high GRIM-19 expression tumors had a higher 5-year OS rate compared to those with low GRIM-19 expression tumors, which has not been previously reported in the literature (92% and 48%, respectively). However, we did not observe a significant impact of GRIM-19 expression on the 5-year OS rate. It is worth noting that only a few studies in the available literature have investigated the significance of GRIM-19 expression on survival in malignant tumors. Hao et al. (29) reported that colon carcinoma patients with GRIM-19 negative tumors exhibited higher recurrence and metastatic rates, and negative tumors were associated with worse OS. Similarly, Ilelis et al. (18) demonstrated that patients with low GRIM-19 expression had worse OS and DFS rates compared to those with high GRIM-19 expression in high-grade serous ovarian carcinoma.

This study had several limitations. Firstly, the assessment of caspase-3 and GRIM-19 protein expressions were performed using TMA cores, which may have inherent limitations in terms of assessment accuracy. To mitigate this potential error, TMA blocks were obtained by sampling two cylindrical samples, each with a diameter of 2 mm, from different regions of each tumor. Secondly, protein expression was detected using immunohistochemistry, which may be subject to variability and subjective interpretation. Thirdly, the study was conducted at a single center, resulting in a limited number of patients included in our study. Therefore, it is crucial to validate these findings through additional multicenter studies involving a larger and more diverse patient cohort.

CONCLUSION

The expressions of caspase-3 and GRIM-19 may potentially contribute to tumorigenesis and tumor progression. Our study revealed significant associations between caspase-3 nuclear positivity, high cytoplasmic caspase-3 expression, and low GRIM-19 expression with unfavorable clinicopathological features, including tumor histology, tumor grade, tumor stage, lymphovascular invasion, and lymph node metastases. Moreover, our study demonstrated, for the first time in the literature, the significance of low GRIM-19 expression on the 5-year OS rate in patients with non-mucinous lung adenocarcinomas. These findings suggest that caspase-3 and GRIM-19 expression could serve as predictive markers for clinical behavior and potential targets for novel treatment options. However, further multicentric studies involving a larger number of patients are needed to determine the precise impact of these proteins in patients with non-mucinous lung adenocarcinoma.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Başkent University (27.04.2023, 162).

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Effects of Ultrasonography-Guided Transversus Abdominis Plane Block on Postoperative Analgesia, Gastrointestinal Motility, and Mobilization in Patients Delivering Cesarean Delivery Under Spinal Anesthesia: A Retrospective Study

Spinal Anestezi Altında Sezaryen Doğum Gerçekleştiren Hastalarda Ultrasonografi Kılavuzluğunda Uygulanan Transversus Abdominis Plan Bloğun Postoperatif Analjezi, Gastrointestinal Motilite ve Mobilizasyon Zamanına Etkisi: Retrospektif Çalışma

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ABSTRACT

Aim: The aim of this study was to investigate the effect of ultrasonography (USG)-guided transversus abdominis plane (TAP) block on postoperative analgesia, gastrointestinal motility, and mobilization time in patients who had a cesarean section under spinal anesthesia.
Material and Methods: The follow-up forms of the total 81 patients who had elective cesarean delivery under spinal anesthesia between March 2022 and June 2022 were reviewed retrospectively. The patients were divided into two groups, 41 patients as the TAP block applied group (group T) and 40 patients as the control group (group C). Demographic data of patients, visual analog scale (VAS) values at postoperative 2nd-, 4th-, 6th-, 12th-, and 24th-hour, tramadol requirements, non-steroidal anti-inflammatory drug (NSAID) and tramadol consumption, postoperative nausea-vomiting (PONV) status, initial gas release times and mobilization times were analyzed.
Results: The VAS scores of the patients in group T at the postoperative period 2nd-, 4th-, and 6th-hour were significantly lower than those of group C ($p<0.001$). However, the rate of PONV ($p=0.006$), tramadol requirement ($p=0.002$), amount of tramadol ($p=0.003$) and NSAID consumed ($p<0.001$), and mobilization time ($p=0.005$) were significantly lower in patients in group T. The time until the passage of flatus was short in Group T, although it was not significant ($p=0.072$).
Conclusion: The USG-guided TAP block significantly contributes to multimodal analgesia after cesarean delivery and may contribute to the early mobilization of patients and the return of gastrointestinal functions.
Keywords: Obstetrical analgesia; postoperative pain; TAP block; cesarean section; early ambulation; gastrointestinal motility.

ÖZ

Amaç: Bu çalışmanın amacı, spinal anestezi altında sezaryen doğum yapan hastalarda ultrasonografi (USG) kılavuzluğunda uygulanan transversus abdominis plan (TAP) bloğun postoperatif analjezi, gastrointestinal motilite ve mobilizasyon zamanına etkisini araştırmaktır.
Gereç ve Yöntemler: Mart 2022 ile Haziran 2022 tarihleri arasında spinal anestezi altında elektif sezaryen doğum gerçekleştirmiş olan toplam 81 hastanın takip dosyaları geriye dönük olarak incelendi. Hastalar, 41 hasta TAP blok uygulanan grup (grup T) ve 40 hasta kontrol grubu (grup K) olmak üzere iki gruba ayrıldı. Hastaların demografik verileri, postoperatif dönem 2., 4., 6., 12. ve 24. saatlerdeki vizüel analog skala (VAS) değerleri, tramadol gereksinimleri, non-steroid anti inflamatuar ilaç (NSAİİ) ve tramadol tüketimleri, postoperatif bulantı-kusma (postoperative nausea-vomiting, PONV) durumları, ilk gaz çıkış zamanları ile mobilizasyon zamanları analiz edildi.
Bulgular: Grup T'deki hastaların postoperatif 2., 4. ve 6. saatlerdeki VAS skorları grup K'ye göre anlamlı olarak daha düşüktü ($p<0,001$). Bununla birlikte grup T'deki hastalarda PONV görülme oranı ($p=0,006$), tramadol gereksinimi ($p=0,002$), tüketilen tramadol ($p=0,003$) ve NSAİİ ($p<0,001$) miktarı ile mobilizasyon süresi ($p=0,005$) anlamlı olarak düşük saptandı. Grup T'de ilk gaz çıkışına kadar geçen süre kısa olmakla birlikte anlamlı değildi ($p=0,072$).
Sonuç: USG kılavuzluğunda uygulanan TAP bloğun sezaryen doğum sonrası multimodal analjeziye önemli katkı sağladığını ve hastaların erken mobilizasyonuna ve gastrointestinal fonksiyonların geri dönüşüne katkı sağlayabileceğini düşünüyoruz.
Anahtar kelimeler: Obstetrik analjezi; postoperatif ağrı; TAP blok; sezaryen doğum; erken ambulasyon; gastrointestinal motilite.

INTRODUCTION

Since most patients experience moderate to severe pain after cesarean delivery, it is essential to ensure pain control. Failure to provide pain palliation may affect the mother-infant bond, disrupt the care of the baby, and lead to difficulties in early breastfeeding. Although there are two components of postpartum pain: somatic (due to abdominal wall incision) and visceral (from the uterus), an essential component of pain originates from the abdominal wall incision (1). Difficulty in movement due to pain may delay mobilization and increase the risk of venous thromboembolism. Pulmonary complications may be triggered due to coughing due to pain and avoidance of deep breathing, and cardiac arrhythmias, hypertension, and myocardial ischemia may occur in patients exposed to severe pain for a long time (2,3).

Systemic or neuraxial opioids are frequently used to treat postoperative pain because they are effective against pain of both somatic and visceral origin. However, undesirable side effects such as nausea-vomiting, itching, decreased bowel movements or constipation, and respiratory and conscious depression may be observed (4). Enhanced recovery after surgery (ERAS) protocols emphasize transversus abdominis plane (TAP) block, which includes abdominal nerve block in multimodal analgesia, to reduce opioid consumption in providing postoperative pain control (5-7). Ultrasonography (USG) guided TAP block is a fascial plane block that can eliminate somatic pain by blocking the neural afferents of the abdominal wall between T6 and L1 between the internal oblique and transversus abdominis muscles and has become popular in postpartum patients as well (8-10).

This study investigates the effects of USG-guided bilateral TAP block on postoperative pain scores, analgesic consumption, mobilization time, and gastrointestinal functions in patients who had a cesarean delivery under spinal anesthesia.

MATERIAL AND METHODS

Study Design and Groups

After the approval of the Local Clinical Research Ethics Committee (date: 06.07.2022, no: 166) of the University of Health Sciences, Kanuni Sultan Süleyman Training and Research Hospital, this retrospective observational study was started following the principles of the Declaration of Helsinki.

Patients who had elective cesarean delivery under spinal anesthesia during the three months between March 1, 2022, and June 1, 2022, at the University of Health Sciences Turkey, Istanbul Kanuni Sultan Süleyman Training and Research Hospital, were retrospectively analyzed. All patients who met the inclusion criteria and had missing data were included in the study. Inclusion criteria for the study; American Society of Anesthesiologists (ASA) II physical status, 18 years of age and older, who underwent cesarean section with Pfannenstiel incision under spinal anesthesia. Exclusion criteria; Patients under 18 years of age, patients with intraoperative nausea and vomiting due to spinal anesthesia which do not go away in a short time, patients with severe preeclampsia or HELLP syndrome, patients with complicated diabetes who have ASA III physical status and need postoperative intensive care (Figure 1).

A total of 81 patients were divided into two groups the TAP block group (group T) and the control group without TAP block (group C). The visual analog scale (VAS) values of the patients at postoperative 2, 4, 6, 12, and 24 hours (0=no pain, 10=the most severe pain ever), diclofenac and tramadol requirements and consumption, postoperative nausea-vomiting (PONV, 0: none, 1: nausea without vomiting, 2: both nausea and vomiting), postoperative mobilization times and gastrointestinal motility were analyzed by accessing the time until passage of flatus from the patient follow-up forms.

In the gynecology and obstetrics clinic of our hospital, paracetamol (Rastamol 10 mg/mL vial, Haver Farma, Türkiye) 1 g 3x1 intravenous (iv) is administered as an analgesic in the postoperative period in all cesarean deliveries under general or spinal anesthesia. Diclofenac sodium (Dichloron 75 mg / 3 mL, Deva Farma, Türkiye) 75 mg intramuscularly 1 to 3 times is administered to patients who complain of pain despite paracetamol. If pain relief does not occur with diclofenac, tramadol hydrochloride (Tradolex 100 mg / 2 mL, Menta Farma, Türkiye) is infused 1 to 3 times iv in 100 ml of physiological saline. All patients included in the study were routinely administered 3x1 g paracetamol iv and diclofenac and tramadol were also administered according to pain complaints.

Spinal Anesthesia

In both groups, spinal anesthesia was performed by the same anesthesiologist and senior assistant using similar doses of drugs. Spinal anesthesia was performed with 20 mcg fentanyl and 8-10 mg 0.5% heavy bupivacaine, depending on the patient's height, using a 25 G Quincke spinal needle from the L3-L4 or L4-L5 interval in the sitting position. The surgical procedure was started when the sensory block level was T6 and the Bromage motor block level was 2-3.

Transversus Abdominis Plane (TAP) Block

TAP block was applied at the end of the operation to the patients who performed cesarean section under spinal anesthesia and accepted the block application. After skin antisepsis was achieved in the supine position, a linear

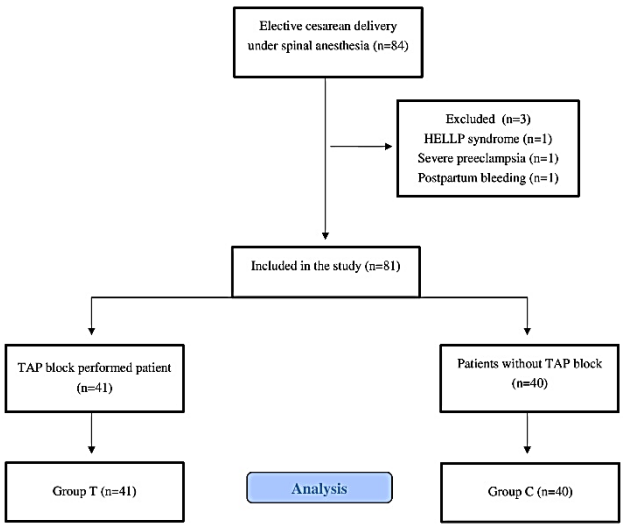

Figure 1. Flow chart of the study

probe was placed between the crista iliaca and coastal border under the guidance of USG (Esaote MyLabFive, Italy). The TAP was defined by providing the ideal image of the external oblique, internal oblique, and transversus abdominis muscles in the anterior part of the abdomen. With the in-plane technique, using a 100 mm 20 G block needle (Stimuplex Ultra 360, Braun, Japan), 20 ml of 0.25% bupivacaine (Bustesin 0.5% Vem Pharma, Türkiye) bilaterally on each side, the fascial plane between the internal oblique muscle and the transversus abdominis muscle injected. After the block, all patients were followed up in the recovery room for 45 minutes and transferred to inpatient services. No invasive procedure was applied to the patients in the control group at the end of the operation.

Postoperative Mobilization and Gastrointestinal Motility

Mobilization was defined as all activities in which the patient is out of bed, such as standing at the bedside, sitting in a chair, or taking a walk in the corridor (11). Motility is the ability to move, the power of movement, and mobility. Gastrointestinal motility is also explained as motility related to the stomach and intestines. The literature investigated the effect of different applications on gastrointestinal motility after cesarean delivery, and the time to first gassing was examined in many randomized controlled studies (12). Our study used the time until the passage of flatus recorded in inpatient wards to evaluate gastrointestinal motility. Similarly, standing, sitting in a chair, or walking was defined as mobilization.

Determination of Sample Size

A previous study was used to calculate the sample size (3). The authors determined that a 25% reduction in postoperative opioid consumption was significant in their pilot study, with a $p<0.05$ and study power of 0.8; they determined that should be 28 patients in each group. All patients who had a cesarean section under spinal anesthesia who met the inclusion criteria between the relevant dates and had no missing data were included in our study.

Statistical Analysis

IBM SPSS v.26.0 program was used to analyze the data. Descriptive data are expressed as number of patients, percentage, mean and standard deviation, and distribution range. The conformity of the variables to the normal distribution was evaluated analytically (Shapiro-Wilk test) and visually (histogram). An Independent sample t-test was used to analyze data with normal distribution. The Mann-Whitney U test was used to analyze data that did not show normal distribution. In the analysis of VAS scores within and between groups, repeated measures ANOVA test with Bonferroni post hoc comparisons was used. The Pearson chi-square and Fisher's exact tests were used to evaluate qualitative data. The significance level for all results was accepted as $p<0.05$.

RESULTS

Between March 2022 and June 2022, 84 patients who underwent elective cesarean section under spinal anesthesia and whose records were not missing were reached. Three patients transferred to the surgical intensive care unit after cesarean section diagnosed with HELLP syndrome, preeclampsia, and postpartum hemorrhage were excluded from the study. A total of 81 patients were included in the study. After a cesarean delivery, 41 patients who underwent USG-guided TAP block were classified as Group T, and 40 patients without TAP block were classified as Group C (Figure 1).

The mean age of all patients in the entire study group was 28.0±5.9 (range, 18-41) years, and all were in ASA II physical status. There was no statistically significant difference between the groups in terms of demographic characteristics.

POVN developed in 4.9% (n=2) of patients in group T and 27.5% (n=11) of patients in group C. Post-operative mobilization time was 5.7±0.9 (range, 4-8) hours and 6.5±1.2 (range, 4-9) hours in group T and group C, respectively. The mean time until the passage of flatus was 8.8±1.6 (range, 6-13) hours in group T, while it was 9.7±2.1 (range, 7-14) hours in group C (Table 1). The PONV rate was significantly lower, and post-operative mobilization time was considerably shorter in group T than in group C ($p=0.006$, and $p=0.005$, respectively). Although the mean time until the passage of flatus was found shorter in Group T than in Group C, it was not statistically significant ($p=0.072$).

When the VAS scores were analyzed, the 2nd-, 4th-, and 6th-hour VAS scores in Group T were significantly lower than in Group C ($p<0.001$). There was no significant difference between the groups' 12- and 24-hour VAS scores ($p=0.801$, and $p=0.859$, respectively). In the TAP group, the 2nd- and 4th-hour VAS scores were significantly lower than the 6th-, 12th-, and 24th-hour VAS scores. However, there was no statistically significant difference between VAS scores in the control group (Table 2, Figure 2).

Table 1. Demographic data and some clinical characteristics of the patients

	Group T (n=41)	Group C (n=40)	p	Overall (n=81)
Age (years), mean±SD (min-max)	27.8±6.1 (18-41)	28.3±5.8 (18-41)	0.710	28.0±5.9 (18-41)
Height (cm), mean±SD (min-max)	160.0±4.1 (152-167)	160.0±5.8 (150-175)	0.765	160.0±5.0 (150-175)
BMI (kg/m^2), median (min-max)	29.6 (24.1-40.0)	28.8 (24.6-45.2)	0.184	29.3 (24.1-45.2)
ASA II physical status, n %	41 (100)	40 (100)	-	81 (100)
PONV, n %	2 (4.9)	11 (27.5)	**0.006**	13 (16.1)
Mobilization (hour), mean±SD (min-max)	5.7±0.9 (4-8)	6.5±1.2 (4-9)	**0.005**	6.1±1.2 (4-9)
Time until passage of flatus (hour), mean±SD	8.8±1.6 (6-13)	9.7±2.1 (7-14)	0.072	9.3±1.9 (6-14)

SD: standard deviation, min: minimum, max: maximum, BMI: body mass index, ASA: American Society of Anesthesiologists, PONV: postoperative nausea and vomiting

Table 2. Visual analogue scale values of the groups

	Group T (n=41)				Group C (n=40)			
	Mean	**SD**	**SE**	**95% CI**	**Mean**	**SD**	**SE**	**95% CI**
2nd-hour	2.41	0.5	0.11	2.19-2.63	4.27	0.8	0.13	4.01-4.54
4th-hour	2.70	0.7	0.13	2.45-2.95	4.57	0.9	0.14	4.30-4.85
6th-hour	3.17	0.7	0.13	2.90-3.43	4.80	0.9	0.16	4.48-5.11
12th-hour	4.41	0.6	0.11	4.19-4.63	4.38	0.7	0.12	4.13-4.62
24th-hour	4.12	0.7	0.16	3.90-4.34	4.15	0.7	0.12	3.91-4.38

SD: standard deviation, SE: standard error, CI: confidence interval

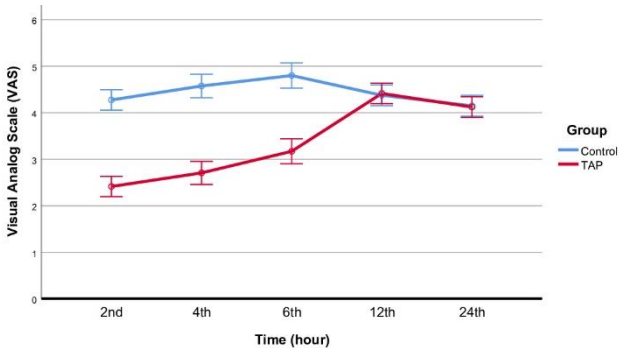

Figure 2. The mean visual analogue scale (VAS) scores of the groups in the first 24 hours.

Diclofenac was administered to 80.5% (n=33) of patients in group T and all of the patients in group C (p=0.005). Tramadol was required in 7.3% (n=3) of the patients in group T and 35% (n=14) in group C (p=0.002). The mean amount of diclofenac and tramadol administered in the first 24 hours postoperatively was 109.7±65.1 mg and 12.2±45.7 mg in group T, while it was 196.8±40.5 mg and 45.0±67.7 mg in group C, respectively. The amount of diclofenac (p<0.001) and tramadol (p=0.003) consumed postoperatively was significantly lower in group T. Local anesthetic systemic toxicity (LAST) was not observed in any patient after the TAP block.

DISCUSSION

In this study, we determined that a TAP block added to spinal anesthesia reduces the frequency of PONV, VAS scores in the first 6 hours postoperatively, mobilization times, and the need for opioids consumed in the postoperative period. In addition, the mean time until the passage of flatus was also lower in the TAP block group, although it was not significant.

It has been reported that more than 40% of patients in the postoperative period experience moderate or severe pain in the early period (13). ERAS protocols using evidence-based surgical principles have emphasized that TAP block is a simple and reliable analgesia technique in cesarean section, laparoscopic, and open abdominal operations to reduce opioid consumption as a part of multimodal analgesia (5,14). The widespread use of ultrasound and the clear visualization of the anatomy make the TAP block a safe and effective method for the multimodal postoperative analgesia technique in obstetric, urological, and lower abdominal surgery. Hebbard et al. (10) applied the ultrasound-guided TAP block technique for the first time. They reported that it has fewer potential complications than epidural analgesia and is an effective alternative analgesia technique. In another study, 20 mL of 0.25% bupivacaine and USG-guided TAP block was applied to women who had a cesarean section, and it was reported that the 24-hour morphine consumption in the TAP block group was significantly reduced compared to the control group (15). Cansiz et al. (16) reported that USG-guided TAP block in cesarean section patients significantly reduced pain scores and tramadol consumption. In some randomized controlled studies investigating the effectiveness of TAP block, a placebo control group was formed by administering saline to the TAP (17,18). However, USG-guided TAP block application is not a risk-free technique, although it is safe and has a low risk of complications. Therefore, the ethical legitimacy of using interventional placebo-controlled regional analgesia is controversial. In our study, while a bilateral TAP block was applied to the TAP block group with 20 mL of 0.25% bupivacaine under USG guidance, no intervention was applied to the control group. Following the literature, 2,4, and 6-hour VAS scores, nonsteroidal anti-inflammatory drug (NSAID), and tramadol requirement, NSAID, and tramadol consumption amount were significantly lower in patients who underwent TAP block.

Multimodal opioid-sparing analgesia approaches highlighted in ERAS protocols aim to reduce systemic opioid demand. Undesirable effects such as nausea, vomiting, constipation, delayed return of bowel movements, sedation, respiratory depression, hyperalgesia, and prolonged hospital discharge have increased the interest in analgesic modalities such as TAP block in postoperative analgesia (19-21). However, it has been reported that intrathecal morphine or opioid administration may contribute to postoperative analgesia and early mobilization by synergistic effect with local anesthetics in abdominal surgeries such as cesarean section and other surgeries (5,22). Zhang et al. (23) reported that preoperative TAP block with 0.6% concentration lidocaine in patients who underwent gynecological laparotomy reduced the incidence of perioperative opioid use and postoperative nausea, accelerated the recovery of bowel functions, shortened the hospital stay, and contributed to early postoperative mobilization. Another study emphasized that the incidence of PONV decreased significantly in patients who had a cesarean section because there was less need for opioids in the group that underwent TAP block. There was less need for antiemetics (24). Our study found that the mobilization time and incidence of PONV were significantly reduced in the group in which the TAP block was applied. The mean time until the passage of flatus we used to evaluate gastrointestinal motility was low in the TAP group, although

it was not significant (8.8±1.6 vs. 9.7±2.1 hours). In addition, we think that our 20 mcg intrathecal fentanyl administration to the patients in both groups positively affects postoperative analgesia and mobilization. Although patients who had a cesarean delivery in our clinic are requested to be mobilized at the sixth postoperative hour, mobilization times may be prolonged depending on the pain status of the patients or other factors. Consistent with the literature, we think significantly less opioid use and better pain palliation in the TAP block group may contribute to earlier mobilization and return of bowel movements.

USG-guided TAP block is generally safe and has a low complication rate. However, abdominal organ injuries, intraperitoneal injuries, intestinal hematoma, and LAST have been reported in TAP block performed both with the blind technique and USG guidance (25-27). It has been reported that the total dose should be 2-2.5 mg/kg in bupivacaine, and the maximum recommended dose in an adult patient is 175-225 mg (28). In our study, 100 mg of bupivacaine was used in the TAP block group, and LAST or other complications were not observed in any patient.

The major limitations of our study are its retrospective, single-center, and small sample size. The level of the sensory block before the TAP block was applied and the duration of the motor blockade after spinal anesthesia were not evaluated. Due to the insufficient number of Patient-controlled analgesia (PCA) devices used in our hospital, the amount of analgesic consumed in our study needed to be followed up with PCA. Therefore, pain assessment and analgesic consumption may not be optimal. The amount of analgesic consumed was determined according to the amount administered under a nurse's supervision and the patient's pain scores. Other conditions may affect patients' gastrointestinal motility (such as chewing gum). In addition, patient records cover the first 24 hours.

CONCLUSION

In conclusion, TAP block pain scores and analgesic requirements are reduced in pain control of patients who had a cesarean section under spinal anesthesia. In addition, we think it can contribute to the return of gastrointestinal motility with early mobilization, which is also emphasized in ERAS protocols because it contributes to pain palliation and reduces the amount of systemic opioids consumed.

Ethics Committee Approval: The study was approved by the Ethics Committee of Kanuni Sultan Süleyman Training and Research Hospital (06.07.2022, 166).

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The Relationship between Kappa Angle and Photic Phenomena after Trifocal Intraocular Lens Implantation

Trifokal Göziçi Lens İmplantasyonu Sonrasında Kappa Açısı ve Fotik Fenomenler Arasındaki İlişki

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ABSTRACT

Aim: This study aimed to investigate the relationship between photic phenomena and the kappa angle after trifocal lens implantations.
Material and Methods: Fifty eyes of 35 cases, 17 female and 18 male, were included in the study. The kappa angle was calculated with the Lenstar LS900 low-coherence interferometry device using the pupil barycenter parameter. It was also calculated by using the iris barycenter parameters. According to the calculations using the pupil barycenter distance, the patients were divided into two groups with the preoperative pupil barycenter distance below 0.4 mm and above 0.4 mm. A questionnaire was applied to the patients to evaluate complaints and satisfaction in the postoperative period.
Results: The mean preoperative pupil barycenter distance was 0.38±0.12 mm and 52.0% (n=26) of the measurements were below 0.40 mm, while the mean preoperative iris barycenter distance was 0.40±0.15 mm and 46.0% (n=23) of the measurements were below 0.40 mm. No significant correlation was found between the preoperative pupil barycenter distance and the preoperative iris barycenter distance (r_s=0.086, p=0.553). Additionally, there was no statistically significant difference between the two groups concerning symptoms such as halo and glare (p=0.948).
Conclusion: When considering a kappa angle upper limit of 0.6 mm, there is no discernible difference in the frequency of occurrence of photic phenomena. We believe that both iris barycenter parameters and pupil barycenter parameters, utilized for kappa angle calculations, can be effectively employed to determine the deviation distance.
Keywords: Iris barycenter; kappa angle; photic phenomena; pupil barycenter; trifocal intraocular lens.

ÖZ

Amaç: Bu çalışmanın amacı, trifokal lens implantasyonları sonrası fotik fenomen ile kappa açısı arasındaki ilişkiyi araştırmaktır.
Gereç ve Yöntemler: Çalışmaya 17 kadın ve 18 erkek olmak üzere 35 olgunun toplam 50 gözü dahil edildi. Kappa açısı, pupil barycenter parametresi kullanılarak Lenstar LS900 düşük koherens interferometri cihazı ile hesaplandı. Aynı zamanda iris barycenter parametreleri kullanılarak da hesaplandı. Pupil barycenter mesafesi kullanılarak yapılan hesaplamalara göre hastalar ameliyat öncesi pupil barycenter mesafesi 0,4 mm'nin altında olanlar ve 0,4 mm'nin üzerinde olanlar şeklinde iki gruba ayrıldı. Ameliyat sonrası dönemde şikayetleri ve memnuniyetleri değerlendirmek amacıyla hastalara anket uygulandı.
Bulgular: Ameliyat öncesi ortalama pupil barycenter mesafesi 0,38±0,12 mm ve ölçümlerin %52,0'si (n=26) 0,40 mm'nin altında iken, ameliyat öncesi ortalama iris barycenter mesafesi 0,40±0,15 mm ve ölçümlerin %46,0'sı (n=23) 0,40 mm'nin altındaydı. Ameliyat öncesi pupil barycenter mesafesi ile ameliyat öncesi iris barycenter mesafesi arasında istatistiksel olarak anlamlı bir korelasyon yoktu (r_s=0,086, p=0,553). Ek olarak, iki grup arasında halo ve kamaşma gibi semptomlar açısından da istatistiksel olarak anlamlı bir fark yoktu (p=0,948).
Sonuç: Kappa açısı için üst sınır 0,6 mm olarak dikkate alındığında fotik fenomenlerin meydana gelme sıklığında fark edilebilir bir fark yoktur. Kappa açısı hesaplamalarında kullanılan hem iris barycenter parametrelerinin hem de pupil barycenter parametrelerinin sapma mesafesini belirlemek için etkili bir şekilde kullanılabileceğine inanıyoruz.
Anahtar kelimeler: Iris barycenter; kappa açısı; fotik fenomen; pupil barycenter; trifokal intraoküler lens.

INTRODUCTION

After the widespread adoption of multifocal intraocular lenses (MIOLs) in cataract surgery, achieving perfection and flawless outcomes has become crucial. These lenses are commonly used for cataract and presbyopia surgeries, leading to rising expectations and demands. While many studies have reported positive results, certain issues persist (1). MIOLs have the ability to focus at various depths within the optical zone (2). They are designed to distribute light to different distances, using either refractive or diffractive optics (3). Trifocal intraocular lenses (IOLs), a new generation of MIOLs, possess a third focus that enhances intermediate vision while maintaining performance for near and far vision (4). Although MIOLs can provide spectacle-free vision, they may reduce contrast sensitivity and cause unwanted photic phenomena like glare and halos due to light passing through diffractive optics (5,6).

Studies in the past have pointed out various reasons for photic phenomena following MIOL implantations, including IOL decentralization, lens fragment residues, posterior capsule opacification, dry eye syndrome, uncorrected visual acuity, postoperative astigmatism, and postoperative ametropia (1,7,8). More recently, it has been suggested that MIOLs may induce higher aberrations, glare, and halos in patients with a high kappa angle (1).

The kappa angle represents the angle between the visual axis and the pupillary axis (9). It can be classified as positive (nasal light reflection) or negative (temporal light reflection). A positive kappa angle of up to 5° is considered physiological, whereas higher angles may result in pseudo-strabismus (10).

In this study, we aimed to examine the occurrence of photic phenomena in patients who have undergone trifocal lens implantation and investigate its relationship with the kappa angle.

MATERIAL AND METHODS

Study Design and Patients

This retrospective study was conducted at Kütahya Anadolu Hospital between 2017 and 2019, following the principles of the Helsinki Declaration. Approval for the study was obtained from the İstanbul Medipol University Ethics Committee (Date: 08.11.2019, Approval No: 61009), and written informed consent was obtained from all participating patients. The study included patients who were diagnosed with cataracts during their ophthalmologic examinations, and who willingly underwent cataract surgery with the desire for trifocal lens implantation. Each eye was treated as an individual case, and all examinations were conducted monocularly. Acrysof IQ PanOptix lenses (Alcon Laboratories, Inc.) were used for all patients in the study. The patients were categorized into two groups based on their preoperative kappa angle measurements: the first group included those with a preoperative kappa angle below 0.40 mm, while the second group comprised those with a measurement of 0.40 mm and above.

Inclusion and Exclusion Criteria

Patients with cataracts, corneal astigmatism of 1.00 D and below, and IOL strength between +16 D and +26.5 D were included in the study.

Patients with corneal astigmatism values above 1.00 D, irregular astigmatism, corneal dystrophy, dry eye syndrome, pupillary abnormality, glaucoma or intraocular inflammation history, macular disease, retinopathy, neuro-ophthalmic disease and patients with intraoperative or postoperative complications were not included in the study.

Acrysof IQ PanOptix

Acrysof IQ PanOptix lenses are non-apodized diffractive trifocal IOLs. In eyes with both small pupils and large pupils, it has the ability to distribute light to four focal points for near distance, intermediate distance, and far distance vision. The light passing through the lens is divided into two. Half fall to the distant focal point and the other half to the near to intermediate distance focal point. The lens has a diffraction zone of 4-5 mm. In this way, its performance is completely free from the size of the pupil. It is produced from hydrophobic acrylic material. The diameter of the optical body of IOL is 6 mm and has a total diameter of 13 mm (3).

Preoperative Assessment

All patients underwent a full ophthalmological examination preoperatively. Uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), manifest refractions, slit-lamp biomicroscopic examinations, non-contact tonometric examinations, and fundoscopic examinations were performed. IOL power was calculated using the SRK-II formula. The strength of all IOLs was calculated by targeting emmetropia. All values were obtained by Lenstar LS 900 (Haag-Streit AG, Koeniz, Switzerland) optical low-coherence reflectometry. This device does not automatically measure the angle of kappa. The distance between the corneal vertex and the center of the pupil (x and y Cartesian values) is measured by the Lenstar LS 900. After measuring the pupil barycenter with the device (x and y coordinates of pupil, dx, and dy), we calculated the kappa angle with the Pythagorean theorem. We called this deviation distance pupil barycenter distance (PBD). We also calculated the angle using the distance between the corneal vertex and the iris center using the same theorem and iris barycenter values. We called this deviation distance iris barycenter distance (IBD).

Surgical Procedure

All surgeries were performed under topical anesthesia by the same surgeon (HK). Surgical operations were completed without complications, and sutures, and were performed using a standard phacoemulsification technique with a superior corneal incision of 2.8 mm. All IOLs were implanted with an injector from the edge of the incision. As a postoperative medication, 0.5% moxifloxacin, 0.1% dexamethasone, 0.5% ketorolac, and lubricant drops were used when needed.

Postoperative Assessment

Postoperative examinations were performed on the 1st day, 1st week, 1st month, and 6th month. In the 6th month, manifest refraction, monocular and binocular UDVA from 6 m, CDVA, 40 cm, and 60 cm near vision, and intermediate distance visual acuity examinations were performed. Near and intermediate distance vision examinations were performed with N-type notation. At the postoperative 6th month, pupil barycenter distance and iris barycenter distance measurements were also determined in mm with the Lensstar LS 900 device. Patients were called and a questionnaire was applied to patients. When the

general satisfaction with the operation was questioned, it was evaluated as 5: excellent, 4: very good, 3: good, 2: not bad, 1: bad, and 0: very bad. Scoring according to the spectacle needs was evaluated as 3: having no need for spectacle, 2: needing spectacle during some activities, (such as reading, driving), and 1: constantly needing spectacle for daily activities. Preoperatively, patients were shown photic phenomena such as halo, glare, and starbursts with pictures, and they were told that these symptoms may occur after the operations. Scoring postoperatively for photic phenomena, 5: no symptoms, 4: no disturbing, mild symptoms, 3: symptoms that moderately disturb during some activities (such as driving, looking at light) but do not cause the activity to stop or change its tempo, 2: moderate symptoms that cause to change the tempo of the activity, requiring extra effort for the continuation of the activity, and 1: severe symptoms that would require avoiding or abandoning the activity completely.

Statistical Analysis

Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, minimum, maximum, frequency, and percentage) were used when evaluating the data. The suitability of quantitative data for normal distribution was tested by the Shapiro-Wilk test and graphical examinations. Student's t test was used for the comparison of the quantitative variables with normal distribution between the two groups, and the Mann-Whitney U test was used for the comparison of the quantitative variables without normal distribution between the two groups. Wilcoxon Signed Ranks test was used for preoperative and postoperative comparisons of variables that did not show normal distribution. In the comparison of qualitative data, the Pearson chi-square and Fisher-Freeman-Halton tests were used. Statistical significance was accepted as $p<0.05$.

RESULTS

The study included a total of 50 eyes of 35 cases, of which 48.6% (n=17) were female and 51.4% (n=18) were male. The ages of the cases ranged between 26 and 85, with a mean of 59.23±14.94 years. While 57.1% (n=20) of the cases were studied with only one eye, 42.9% (n=15) were included in the study with both eyes. The distribution of some preoperative and postoperative data of the cases were shown in Table 1.

The mean preoperative pupil barycenter distance was 0.38±0.12 mm, the measurement of 52.0% (n=26) of the cases was below the median value of 0.40 mm, and the measurement of 48.0% (n=24) was 0.40 mm or above. The mean postoperative pupil barycenter distance was 0.30±0.14 mm. The change in postoperative pupil barycenter distance measurement compared to the preoperative was statistically significant (p=0.001, Table 2).

The mean preoperative iris barycenter distance was 0.40±0.15 mm, the measurement of 46.0% (n=23) of the cases is below the median value of 0.40 mm, and the measurement of 54.0% (n=27) is 0.40 mm or above. The mean postoperative iris barycenter distance was 0.41±0.18 mm. The change in postoperative iris barycenter distance compared to the preoperative was not statistically significant (p=0.901, Table 3).

Table 1. Distribution of preoperative and postoperative data

Preoperative	**Mean±SD**	**Median (min-max)**
Axial length (mm)	23.44±1.07	23.5 (21.5 - 25.3)
Mean keratometry (D)	43.76±1.63	43.7 (38.7 - 47.6)
ACD (mm)	3.32±0.35	3.3 (2.6 - 4.2)
IOL power (D)	21.44±3.26	21 (16 - 29)
UDVA (logMAR)	0.49±0.28	0.5 (0.1 - 1.2)
SE (D)	-0.71±3.13	-0.1 (-9.9 - 3.5)
Corneal astigmatism (D)	0.35±0.63	0.6 (-1 - 1)
Postoperative	**Mean±SD**	**Median (min-max)**
SE (D)	0.40±0.48	0.4 (-1.5 - 1.4)
Corneal astigmatism (D)	0.56±0.43	0.5 (-0.5 - 1.4)
UNVA 40 cm (logMAR)	0.11±0.08	0.1 (0 - 0.4)
UIVA 60 cm (logMAR)	0.17±0.09	0.2 (0 - 0.4)
UDVA 4 m (logMAR)	0.13±0.19	0.1 (0 - 0.9)
CNVA 40 cm (logMAR)	0.05±0.06	0 (0 - 0.2)
CIVA 60 cm (logMAR)	0.11±0.09	0.1 (0 - 0.3)
CDVA 4 m (logMAR)	0.01±0.03	0 (0 - 0.2)

ACD: anterior chamber depth, IOL: intraocular lense, UDVA: uncorrected distance visual acuity, SE: spherical equivalent, UNVA: uncorrected near visual acuity, UIVA: uncorrected intermediate visual acuity, CNVA: corrected near visual acuity, CIVA: corrected intermediate visual acuity, CDVA: corrected distance visual acuity, D: dioptri, mm: millimeter, SD: standard deviation, min: minimum, max: maximum

Table 2. Evaluation of pupil barycenter measurements preoperative and postoperative

	Preoperative		**Postoperative**		**p**
	Mean±SD	**Median (min-max)**	**Mean±SD**	**Median (min-max)**	
Pupil barycenter X (mm)	-0.09±0.33	-0.3 (-0.5 - 0.6)	0.01±0.29	0 (-0.5 - 0.5)	**0.035**
Pupil barycenter Y (mm)	-0.03±0.20	0 (-0.5 - 0.3)	-0.09±0.15	-0.1 (-0.5 - 0.2)	**0.048**
Pupil barycenter distance (mm)	0.38±0.12	0.4 (0.1 - 0.6)	0.30±0.14	0.3 (0.1-0.6)	**0.001**
	<0.40	**≥0.40**	**<0.40**	**≥0.40**	
Pupil barycenter distance, n (%)	26 (52.0)	24 (48.0)	38 (76.0)	12 (24.0)	

Table 3. Evaluation of iris barycenter measurements preoperative and postoperative

	Preoperative		**Postoperative**		**p**
	Mean±SD	**Median (min-max)**	**Mean±SD**	**Median (min-max)**	
Iris barycenter X (mm)	-0.01±0.39	0.1 (-0.7 - 0.7)	0.03±0.40	0.1 (-0.7 - 0.8)	0.480
Iris barycenter Y (mm)	0.07±0.16	0.1 (-0.3 - 0.6)	0.02±0.20	0 (-0.3 - 0.5)	0.104
Iris barycenter distance (mm)	0.40±0.15	0.4 (0.1 - 0.7)	0.41±0.18	0.5 (0.1-0.8)	0.901
	<0.40	**≥0.40**	**<0.40**	**≥0.40**	
Iris barycenter distance, n (%)	23 (46.0)	27 (54.0)	20 (40.0)	30 (60.0)	

Table 4. The results of the questionnaire in terms of preoperative pupil barycenter distance (kappa angle)

	<0.40 mm (n=26)	**≥0.40 mm (n=24)**	**p**
General satisfaction, n (%)			
Very bad	1 (3.8)	0 (0.0)	0.224
Good	2 (7.7)	2 (8.3)	
Very good	11 (42.3)	16 (66.7)	
Perfect	12 (46.2)	6 (25.0)	
Spectacle requirement, n (%)			
Always	2 (7.7)	2 (8.3)	0.879
Sometimes	3 (11.5)	4 (16.7)	
Never	21 (80.8)	18 (75.0)	
The relationship between symptom and activity, n (%)			
Causes to change the tempo of activity, moderate symptom	1 (3.8)	1 (4.2)	0.948
Does not cause to change the tempo of activity, moderate symptom	1 (3.8)	2 (8.3)	
Mild symptom	15 (57.7)	14 (58.3)	
No symptom	9 (34.6)	7 (29.2)	

There was no statistically significant correlation between pupil barycenter distance and iris barycenter distance preoperatively (Spearman's rho, r_s=0.086 p=0.553), and postoperatively (r_s=0.266 p=0.062).

When the patients were asked about their general satisfaction after the surgery, 2.0% (n=1) responded very bad, 8.0% (n=4) good, 54.0% (n=27) very good, and 36.0% (n=18) perfect. While 8.0% (n=4) of the patients constantly need spectacle after surgery, 14.0% (n=7) do not need spectacle during some activities, and 78.0% (n=39) do not need spectacle at all. Moderate symptoms caused a change of activity tempo in 4.0% (n=2) of cases, moderate symptoms did not cause a change of activity tempo in 6.0% (n=3), and mild symptoms occurred in 58.0% (n=29), no symptoms were observed in 32.0% (n=16). According to the groups, overall satisfaction (p=0.224), spectacle need (p=0.879) and symptoms (p=0.948) do not differ statistically (Table 4).

DISCUSSION

With the development of new types of IOLs, the kappa angle has begun to be at the forefront among the subjects that cataract surgeons are interested in (1). We carried out this study over the kappa angle. We evaluated the angle of kappa both on the pupil center (pupil barycenter distance) and on the iris center (iris barycenter distance).

In eyes with a positive kappa angle, the pupillary axis is located temporally than the visual axis. In eyes with a negative kappa angle, the pupillary axis is located in the nasal relative to the visual axis. Thus, when an eye is fixed on any light source, the reflection on the surface of the cornea will not be in the center. It will be nasal in eyes with a positive kappa angle and temporal in eyes with a negative kappa angle (1).

Some studies have reported that if the angle of kappa is high (>0.6 mm), even if the IOL is centralized, halo and glare may occur (11). Therefore, it is important to evaluate the kappa angles before trifocal lens implantations. Devices such as Synoptophere, Orbscan II, Galilei, and OPD Scan II were used to detect the Kappa angle. Lenstar LS 900 device can be used for kappa angle calculations (11-13). This device does not automatically measure the kappa angle but can be calculated using the Pythagorean theorem after pupil barycenter values (x and y coordinates of pupil, dx, and dy) have been determined. With the same theorem, we calculated the angle on the iris barycenter.

MIOL designs have made significant progress since their introduction to the market. Patient satisfaction has increased significantly with these new models (14,15). Neuro-adaptation can play a very important role in some cases. Therefore, sufficient time should be provided before making a conclusion about the intensity of photic phenomena (16,17). Blurred vision and photic phenomena are the most common causes of patient dissatisfaction after MIOL implantation (18). The most important causes of dissatisfaction in patients with MIOL, causing the appearance of a halo, glare, and other negative photic phenomena, are ametropia and posterior capsule opacity. Qi et al. (19) stated that the incidence of glare and halo was associated with an increase in the kappa angle. In a study, it was reported that the diameter of the central region of the lens and biometric values may cause a high kappa angle, which may lead to the formation of negative photic phenomena (1).

Moderate photic phenomena caused the change of activity tempo in 4.0% of the cases included in our study, moderate photic phenomena did not cause the change of activity tempo in 6.0%, mild photic phenomena in 58.0%, and no symptoms were observed in 32.0%. 80% of the cases stated those did not experience any distress in terms of photic phenomena or those who experienced discomforts at a level that would not change the tempo of activity.

Some researchers have suggested that the light will pass through the center of the IOL and reach the center of the macula in small kappa-angled eyes. However, in wide kappa-angled eyes, the light can pass through diffractive rings, causing negative photic phenomena such as halo and glare (18). A wide kappa angle can cause misalignment between the MIOL center and the visual or optical axes. This can lead to the functional decentralization of the MIOL (19). Previous studies have reported that photic phenomena that occur after cataract surgery are associated with shifts in IOLs. In another study, it was reported that wide kappa angles can also cause halo and glare. They also reported that the intensity of the halo felt was correlated with the kappa angle and postoperative uncorrected visual activity. However, they also suggested that glare, halo, and other negative photic phenomena never appeared after surgery in some wide-angle patients (14).

The relation between the kappa angle and halo and glare is not fully understood (19). In a study using the standard ray-tracing technique, it has been reported that a shadow is formed between retinal images when a gap occurs between the rays that miss the IOL and the rays that are reflected from the IOL. In another study, it was suggested that if the kappa angle is wide, the light enters the eye through different diffraction rings, and thus negative photic phenomena can occur (20).

There was no statistically significant difference in terms of overall satisfaction, spectacle requirement, and photic phenomena according to the groups in our study. According to the kappa angle calculations made on the pupil barycenter, there is no significant difference in terms of photic phenomena between the group with a distance above 0.4 mm and the group below 0.4 mm, provided that the upper limit is 0.6 mm.

Kappa angle measurements using the pupil barycenter preoperatively are 0.38±0.12 mm. The results of 52.0% of the cases were below the median value of 0.40 mm, and the results of 48.0% were 0.40 mm and above. The mean kappa angle measurement results using the pupil barycenter postoperatively are 0.30±0.14 mm. Angle measurement results using the iris barycenter preoperatively were 0.40±0.15 mm. The results of 46.0% of the cases were below the median value of 0.40 mm, and the results of 54.0% were 0.40 mm and above. The mean postoperative iris barycenter was 0.41±0.18 mm. Since preoperative values are more important in terms of operation preparation, we see that the kappa angle values using the pupil barycenter and iris barycenter are close to each other.

Now more and more surgeons are paying attention to the kappa angle and the alpha angle. Interestingly, it was found that the alpha angle, defined as the intersection of the visual axis with the optical axis, is correlated with the IOL tilt, similar to the kappa angle (21). In eyes with a kappa or alpha angle less than 0.5 mm, the kappa angle has a greater effect in terms of postoperative visual quality parameters. Care should be taken in the use of trifocal lenses in eyes with a kappa or alpha angle greater than 0.5 mm (22).

Chord mu definition has started to be accepted as a new reference mark to be used in this context. Defines the displacement between the subject-fixated coaxially sighted corneal light reflex and the center of the pupil (23). In a study, it was reported that apparent chord mu values were higher in hyperopia compared to myopia (24).

We consider the fact that the number of cases we enrolled in our study is not too high and that we can operate on only one eye of some patients as factors that limit our study.

CONCLUSION

Regarding photic phenomena, a safe kappa angle limit of 0.6 mm can be considered. When the preoperative kappa angle value is below 0.6 mm, photic phenomena typically do not occur or do not significantly impact daily activities. Additionally, we believe that iris barycenter values, along with pupil barycenter values used for kappa angle determination, could serve as supplementary parameters. However, large sample studies are necessary to draw definitive conclusions.

Ethics Committee Approval: The study was approved by the Non-interventional Clinical Research Ethics Committee of Medipol University (08.11.2019, 61009).

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The Effects of Vasointestinal Peptide and Naringenin on Rotenone-Induced Experimental Model of Parkinson's Disease

Vazointestinal Peptid ve Naringeninin Rotenon Kaynaklı Deneysel Parkinson Hastalığı Modeli Üzerine Etkileri

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ABSTRACT

Aim: The aim of this study was to evaluate the intraperitoneal administration of naringenin and vasointestinal peptide (VIP), which are shown effective in various scientific studies, in terms of anti-Parkinsonian activity in rats.
Material and Methods: Forty-eight Wistar albino female rats were divided into 4 groups. No intervention was made in the control group, rotenone was given to the RT group, rotenone and VIP (25 ng/kg) to the RT+VIP group, and rotenone and naringenin (10 mg/kg) to the RT+NG group. All treatments were administered intraperitoneally for 14 days. The hole and board method was used to show the effects of the Parkinson's model on behavior. On the last day of the experiment, motor tests were carried out with the hole and board apparatus. After the study was completed, biochemical analyzes were performed from brain tissue samples.
Results: In comparison to the RT group, while the alpha-sync level in the RT+NG (p=0.023), malondialdehyde (MDA) levels both in the RT+VIP (p=0.039) and RT+NG (p=0.032), and superoxide dismutase (SOD) inhibition in the RT+VIP (p=0.042) groups decreased significantly, the 8-OHdG levels in the RT+VIP (p=0.042) and RT+NG (p=0.034) groups increased significantly. Statistically significant improvement was found both in biochemical and motor activities with the VIP and naringenin treatments applied.
Conclusion: According to the results obtained, the symptoms of Parkinson's disease were formed biochemically by rotenone application. The administration of VIP and naringenin treatments has shown positive effects experimentally and has been promising as an adjunct treatment element in the fight against Parkinson's disease.
Keywords: Parkinson's disease; rats; rotenone; vasointestinal peptide.

ÖZ

Amaç: Bu çalışmanın amacı, çeşitli bilimsel çalışmalar ile etkili oldukları gösterilmiş olan naringenin ve vazointestinal peptidin (VIP) intraperitoneal olarak uygulanmasının ratlarda anti-Parkinson aktivitesi açısından değerlendirilmesidir.
Gereç ve Yöntemler: Kırk sekiz adet Wistar albino dişi rat 4 gruba ayrıldı. Kontrol grubuna herhangi bir müdahale yapılmadı, RT grubuna rotenon verilirken, RT+VIP grubuna rotenon ve VIP (25 ng/kg) ve RT+NG grubuna ise rotenone ve naringenin (10 mg/kg) verildi. Tüm tedaviler 14 gün süreyle intraperitoneal yolla uygulandı. Parkinson modelinin davranış üzerindeki etkilerini göstermek için hole and board yöntemi kullanıldı. Deneyin son günü hole and board aparatı ile motor testleri yapıldı. Çalışma tamamlandıktan sonra alınan beyin dokusu örneklerinden biyokimyasal analizler yapıldı.
Bulgular: RT grubuyla karşılaştırıldığında, RT+NG (p=0,023) grubunda alfa senkronizasyon düzeyi, hem RT+VIP (p=0,039) hem de RT+NG (p=0,032) gruplarında malondialdehit (MDA) düzeyleri ve RT+VIP (p=0,042) grubunda süperoksit dismutaz (SOD) inhibisyonu anlamlı olarak azalırken, RT+VIP (p=0,042) ve RT+NG (p=0,034) gruplarında ise 8-OHdG seviyeleri anlamlı şekilde arttı. Uygulanan VIP ve naringenin tedavileri ile hem biyokimyasal ve hem de motor aktivitelerinde istatistiksel olarak anlamlı şekilde düzelme saptandı.
Sonuç: Elde edilen sonuçlara göre rotenon uygulaması ile Parkinson hastalığının semptomları biyokimyasal olarak oluşturulmuştur. VIP ve naringenin tedavilerinin uygulanması deneysel olarak olumlu etkiler göstermiştir ve Parkinson hastalığı ile mücadelede yardımcı bir tedavi unsuru olarak umut verici olmuştur.
Anahtar kelimeler: Parkinson hastalığı; rotenon; sıçanlar; vazointestinal peptit.

INTRODUCTION

Parkinson's disease is a multisystem progressive disease characterized by premotor and motor symptoms clinically due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) and denervation of dopaminergic pathways, in which intracytoplasmic inclusions are seen in the neurons affected in the central nervous system (1,2). A long period characterized by premotor symptoms, usually the appearance of motor symptoms, is referred to as the 'preclinical period' (3). When the disease is diagnosed, in other words, when clinically motor symptoms are observed, 50-70% of dopaminergic neurons in the substantia nigra pars compacta have degenerated. The reason is, it has been reported that it may be too late for neuroprotective applications that may slow down neurodegeneration or have an effect (4,5). Many experimental models have been developed to understand Parkinson's disease (3). One of the most widely used models is the rotenone model (6). Rotenone is a toxic substance used in pest control, a member of the rotenoid family, which is one of the natural cytotoxic compounds obtained from the extracts of tropical plants. It is lipophilic and can easily reach organs through circulation. Rotenone binds to the same site as MPP+ and inhibits mitochondrial complex I. Intravenous exposure to low-dose rotenone causes selective degeneration of nigrostriatal dopaminergic neurons with the formation of α-synuclein positive LC-like inclusions in rats. It has been reported that rotenone, which is lipophilic, easily crosses the blood-brain barrier. Rotenone accumulating in mitochondria blocks the complex-I unit of the electron transport chain (2,3).

Many of the features found in Parkinson's pathology include reactive oxygen species production, systemic mitochondrial degradation, microglial activation, α-synuclein phosphorylation, aggregation, and Lewy pathology, selective nigrostriatal dopaminergic degeneration, ubiquitin-proteasomal dysfunction, and L-3,4-dihydroxyphenylalanine (levodopa; L- many symptoms such as DOPA) responsive motor deficits, depletion of tyrosine hydroxylase immunoreactivity, oxidative damage are observed after rotenone administration (7-9). Mitochondrial complex I inhibition and reactive oxygen species production are key mechanisms for the degeneration of dopaminergic neurons. Increased intracellular oxidative stress also causes dysfunction of the ubiquitin proteasomal system, which provides degradation of misfolded protein aggregates (10). As a result, mitochondrial membrane potential decreases, intracellular calcium homeostasis is disturbed and mitochondria are destroyed (mitophagy), neurodegeneration is observed (11).

Vasointestinal peptide (VIP) is a peptide consisting of 28 amino acids, structurally belonging to the family of gastrointestinal tract peptide hormones such as glucagon, secretin, gastric inhibitory peptide (GIP), and growth hormone-releasing hormone (GHRH) (12,13). VIP has a wide range of biological activities and participates in the regulation of a wide range of physiological functions such as circulatory, respiratory, gastrointestinal, endocrine, and immune systems (12,14). VIP is the main neuropeptide in the brain with its neurotransmitter, neuromodulator, neurotrophic, anti-inflammatory, antioxidant, and antiapoptotic properties (15), it stimulates astrocyte mitosis and neuronal growth in the CNS (16), it provides neuronal vitality and prevents cell death against glutamate excitotoxicity (17) were reported. VIP is a molecule with tissue and cell protective properties. It has been shown in various studies to protect tissues against the undesirable damage of septic shock, Chron's disease, hemorrhagic shock, ischemia-reperfusion, and rheumatoid arthritis, and increase neuronal survival (14,18). It is suggested that VIP plays an important role in the protection and development of neurons in traumatic situations in the brain (19).

Naringenin is a flavonoid from a subclass of flavanones, which is found in various citrus fruits such as tomatoes and bergamot and can also be found in glycoside form (20). Naringenin, chemically named 2,3-dihido-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, indicates a molecular weight of 272.26 ($C_{15}H_{12}O$). This molecule is insoluble in water and dissolves in organic solvents such as alcohol (21). Flavonoids are phenolic compounds commonly found in fruits and vegetables that exhibit strong antioxidant activity and reduce the formation of free radicals (22). Naringenin exhibits antiatherogenic and anti-inflammatory effects, supporting carbohydrate metabolism, increasing antioxidant defense, scavenging reactive oxygen species, and modulating immune system activity, including reduction in lipid peroxidation biomarkers and protein carbonylation (23). It also promotes the oxidation of fatty acids and effectively disrupts the accumulation of plasma lipids and lipoproteins, which impairs the accumulation of lipids in the liver and prevents fatty liver. It has been reported to contribute significantly to modulating signaling pathways related to fatty acid metabolism (24).

This study aimed to investigate the neuroprotective effects of intraperitoneal use of VIP and naringenin agents in the experimental Parkinson's model created by rotenone administration.

MATERIAL AND METHODS

In this experimental study and the experimental interventions it contains, it has been approved that there is no ethical objection to the decision of Aydın Adnan Menderes University Animal Experiments Local Ethics Committee with the decision numbered 054, dated 21.05.2019. In the study, 48 Wistar albino male rats with an average weight of 300-350 grams, 12 weeks old, found in the Experimental Animals Laboratory of Aydın Adnan Menderes University were used. All rats were kept in rooms with 12 hours of dark and 12 hours of light circadian rhythm, 22±1°C temperature, and 40-60% relative humidity throughout the experiment. During the experiment, rats were fed with standard pellet feed ad libitum, and free-access city water was used as drinking water.

Rotenone (Sigma Aldrich) to be administered to rats was prepared by dissolving in dimethylsulfoxide (DMSO) (Sasol). It was administered intraperitoneally with an insulin injector at 1.25 mL/kg per rat. In the first 30 minutes after the application, some physical changes were observed in some of the rats. Mortality occurred when the rats remained in an inactive lying position after postural disorder and decreased breathing. When the second 45th minute was entered, this and similar situations were encountered in other groups as well, and the number of rats

starting with n=48 in total was n=36. The VIP, which was prepared in saline starting 1 hour after the Parkinson's model was created, was administered ip at 25 ng/kg, was applied as VIP treatment, and was continued every other day for 14 days (25). Naringenin was administered as 10 mg/kg in pure form on a repetitive day after the rotenone application (26). Experimental animals were randomly selected, 12 rats were in each group, and 4 groups were formed. The groups were; the Rotenone group (RT), the Rotenone+Vasointestinal Peptide Group (RT+VIP), and the Rotenone+Naringenin Group (RT+NG). Alpha-sync, 8-OHdG, malondialdehyde (MDA), and superoxide dismutase (SOD) kits were purchased to perform biochemical tests, and measurements were performed following the kit procedure.

Hole and Board Test

At the end of the 14th day, the Hole and board test setup was used to perform the motor analysis on the last day of the experiment. This arrangement is structured as follows: Perforated wooden apparatus, 68 cm x 68 cm, consisting of a wooden gray box, 40 cm high, with the front side open and the other three closed, and the hole diameter (may vary depending on the size of the experimental animal) is 9 cm, The box with 16 holes was raised 28 cm from the ground on a wooden stand (Figure 1). To ensure that the experimental animal is accustomed to the environment, it was put into the apparatus 3 times at different times. After the acclimatization period was completed, 5 minutes of shooting was taken for each rat with a fixed video camera that could fully see the experimental setup. Experimental animals performed hovering and exploratory movements on the apparatus. Parameters used in the Hole and board test; (i) Visit to the center: the animal moves from one area of the open area to another (all four claws must be placed on the floor of a new area), (ii) Head dipping: the animal places its head in one of the holes to a minimum depth with its ears flush with the floor of the device (the animal a new head tilt is recorded when he raises his head) a full movement is achieved when he pulls it out of the hole before continuing), (iii) Raising: the animal is fixed on its back paws and lifts its front paws off the ground, extending its body vertically, (iv) Number of holes: the animal extends and retracts its head without dipping its head into one of the holes.

Statistical Analysis

All statistical analyses were performed via IBM SPSS Statistics 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The distributions of measurements were evaluated by Shapiro-Wilk's test and skewness/kurtosis statistics. The homogeneity of the variances among groups was examined by Levene's test. Data were provided by mean±standard deviation. Groups were compared by ANOVA. Tukey's HSD test was performed, if necessary. A p-value <0.05 was accepted as statistically significant.

RESULTS

Biochemical Analysis Findings

In the results of the biochemical analysis performed on the samples taken from the striatum region, alpha-sync levels were found to be significantly lower in the control group than in the RT group (p=0.008). While no significant difference was found in the RT+VIP group (p=0.085), there was a significant decrease in the alpha-sync levels of the RT+NG group compared to the RT group (p=0.023).

8-OHdG levels in the striatum decreased significantly in the RT group compared to the control group (p=0.009) and increased closer to the control group in the RT+VIP and RT+NG groups. It was determined that 8-OHdG levels in the RT+VIP (p=0.042) and RT+NG (p=0.034) groups increased significantly compared to the RT group.

The MDA level in the RT group increased significantly compared to the control group (p=0.021), whereas it decreased significantly in the RT+VIP (p=0.039) and RT+NG (p=0.032) treatment groups.

While the SOD inhibition in the RT group increased significantly (p=0.038) compared to the control group, it was significantly lower in the RT+VIP (p=0.042) group compared to the RT group. The lowest inhibition was in the control group (Table 1).

Motor Test Findings

While the most head dipping movement was detected in the control group, this movement was significantly less in the RT group compared to the control group (p=0.007). Although there was a slight increase in the treatment groups, it was not statistically significant.

According to the number of holes visited, it was found that the group with the highest number of visits was the control group, and the lowest number was the RT group (p=0.019). There was an increase in both treatment groups compared to the RT group and the increase in the RT+VIP group was significant (p=0.031), while was not in the RT+NG group. The highest number of visits to the center was in the control group and the lowest was in the RT group (p=0.036). Although this number increased in the RT+VIP group, no significant difference was found (p=0.543). Also, there was no significant difference between the RT and RT+NG groups (p=0.961).

The highest number of rearing movements was found in the control group and the lowest in the RT group (p=0.037). Although this number increased in the RT+VIP (p=0.720) and RT+NG (p=0.994) treatment groups, no significant difference was found when compared to the RT group.

DISCUSSION

Creating an accessible model for Parkinson's disease studies is challenging. In the studies conducted by Sonia Angeline et al. (27) and Lapointe et al. (28), it was determined that the rotenone-induced Parkinson-like disease model causes neuronal damage in the important nigra and striatum, and leads to behavioral changes such as motor

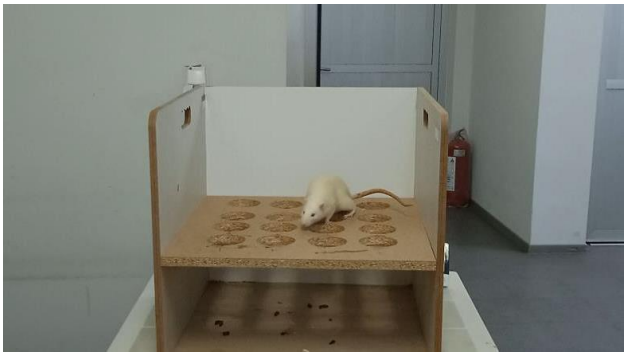

Figure 1. Hole and board assembly

Table 1. Biochemistry analysis results

	Control	RT	RT+VIP	RT+NG
Alfa-sync (pg/gr)	16.823±2.420	29.593±6.112	22.061±4.001	19.204±3.443
8-OHdG (µg/gr)	43.273±16.148	37.701±13.988	41.377±10.046	39.771±12.089
MDA (mU/ml)	1.392±0.305	1.952±0.361	1.584±0.034	1.510±0.694
SOD inhibition (%)	0.491±0.298	0.621±0.068	0.512±0.068	0.565±0.296

RT: rotenone, RT+VIP: rotenone+vasointestinal peptide, RT+NG: rotenone+naringenin, MDA: malondialdehyde, SOD: superoxide dismutase, Tukey HSD post hoc test results; Alfa-sync: RT vs Control: p=0.008, RT vs RT+VIP: p=0.085, RT vs RT+NG: p=0.023; 8-OHdG: RT vs Control: p=0.009; RT vs RT+VIP: p=0.042; RT vs RT+NG: p=0.034; MDA: RT vs Control: p=0.021; RT vs RT+VIP: p=0.039; RT vs RT+NG: p=0.032; SOD inhibition: RT vs Control: p=0.038; RT vs RT+VIP: p=0.042; RT vs RT+NG: p=0.055

Table 2. Motor test results

	Control	RT	RT+VIP	RT+NG
Head dip (score)	9.634±2.723	4.222±1.725	5.384±1.300	4.305±1.704
Hole visit (score)	8.250±3.451	4.558±1.591	7.126±1.134	5.200±1.140
Center visit (score)	2.000±0.816	1.250±0.500	1.500±0.554	1.200±0.425
Rampancy (score)	3.631±1.688	2.000±1.000	2.384±1.194	2.000±1.700

RT: rotenone, RT+VIP: rotenone+vasointestinal peptide, RT+NG: rotenone+naringenin, Tukey HSD post hoc test results; Head dip: RT vs Control: p=0.007; RT vs RT+VIP: p=0.087; RT vs RT+NG: p=0.063; Hole visit: RT vs Control: p=0.019; RT vs RT+VIP: p=0.031; RT vs RT+NG: p=0.074; Center visit: RT vs Control: p=0.036; RT vs RT+VIP: p=0.543; RT vs RT+NG: p=0.961; Rampancy: RT vs Control: p=0.037; RT vs RT+VIP: p=0.720; RT vs RT+NG: p=0.994

skill disorders and biochemical disorders. In our study, it was observed that the biochemistry and motor behavior results of the rotenone-applied group were impaired compared to the control group.

Experimental studies in this area reveal the promising role of flavonoids (29). It has been shown to improve cognitive function, reduce motor complications, and protect biochemically by protecting sensitive neurons (30). The study by Datla et al. (31) showed that these promising properties of flavonoids can be used as potent neuroprotectors for naringenin. The results we obtained in our study also showed that the application of naringenin caused improvements in both biochemical and motor activity results.

It was observed that the inhibition level of MDA and SOD decreased significantly after the treatment of rotenone-treated rats with VIP. While describing the neuroprotective effect of VIP, it is assumed that it increases astrocyte mitosis and stimulates the release of astrocyte-derived neurotrophic molecules such as ADNP (16,32). In their in vivo study by giving VIP antagonists in mice, it was shown that there was a dramatic loss of astrocyte in the neocortex, and neocortical astrogenesis occurred with VIP administration and this loss was reversed (33). The results of our study support these findings.

In the study conducted by Liu et al. (34), it was determined that the results obtained in the board and hole tests applied to rats with the Parkinson's model deteriorated significantly compared to the results in the control group. It was observed that rats with Parkinson's-like features had decreased ability to pass through, the number of rearing, and the number of holes visited. Wang et al. (35), on the other hand, applied behavioral tests on rats with Parkinson's-like effects in their experimental Parkinson's study and found a significant difference from the control group as a result of hole and board tests. In the study of Saleem et al. (36), the experimental results of Prunus armeniaca L. extract were investigated in rats with Parkinson's-like effects, and they determined that both biochemistry and motor activity results approached the control group. The results obtained in our study also support the findings obtained in these studies. Naringenin and VIP applications reduced the negative effects of the rotenone application.

In addition to nigrostriatal degeneration, when rotenone is administered by intraperitoneal injection, it causes behavioral deficits responsive to the dopamine agonist apomorphine, suggesting that these observed deficits are specific to dopamine loss. With these features, it has been determined that symptoms similar to Parkinson's disease in humans occur (6). Although it is known that the intrinsic pathway, that is, the mitochondrial pathway, is of great importance in models that occur with mitochondrial complex-1 inhibition, it is reported that the extrinsic pathway may be effective on this intrinsic pathway (1). The primary goal in the treatment of Parkinson's disease is to improve the patient's quality of life by treating motor and non-motor findings (37). The fact that only palliative treatment is possible with drugs aimed at increasing dopaminergic neurotransmission makes studies on potential drug candidates with neuroprotective effects in Parkinson's disease inevitable (3). The results revealed in our study showed that both naringenin and VIP have positive effects biochemically. When motor activity test findings are added to these results, it is thought that naringenin and VIP application will be effective elements in the Parkinson's treatment process in the future.

CONCLUSION

As a result, the results show that rotenone application negatively affects biochemical results and motor behaviors, and findings similar to Parkinson's disease are obtained. It was shown that the applied VIP and naringenin improved the negative results and changed the biochemical and motor test results similarly to the control group. The results show that VIP and naringenin, which did not cause any harm in the administration, are agents that can be added to the treatment of Parkinson's disease. Further molecular studies will reveal the pathways through which these two therapeutic agents act.

Ethics Committee Approval: The study was approved by the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (21.05.2019, 054).

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Predictive Role of Delta Neutrophil Index in Endometrial Cancer: A Promising Biomarker for Diagnosis

Endometriyal Kanserde Delta Nötrofil İndeksinin Öngörücü Potansiyeli: Tanı için Yeni Bir Biyobelirteç

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ABSTRACT

Aim: This study aimed to explore the potential of delta neutrophil index (DNI) as a predictive biomarker for the development of endometrial cancer (EC) in women with endometrial intraepithelial neoplasia (EIN).
Material and Methods: This retrospective study included 139 women diagnosed with EIN who underwent surgery between 2019 and 2022. Demographic data, medical history, and laboratory parameters, including DNI, were collected from the patients' medical records. Patients with other types of cancer, a history of steroid use, inflammatory, hematologic, or autoimmune diseases, or missing data were excluded. The patients' pathology reports were reviewed, and patients were divided into three groups by the final pathological diagnosis, benign (n=64), EIN (n=66), and EC (n=39).
Results: The mean DNI level of the EC group was found to be significantly higher than the EIN and benign groups (4.85±2.31, 2.31±0.89, 1.48±1.03, p<0.001, respectively). The optimal cut-off value of DNI was determined as 2.75% with 82.1% sensitivity and 73.8% specificity. DNI levels >2.75% were found to be associated with an 11.56-fold (95% CI: 4-59-29.09, p<0.001) increased risk of EC. Smoking and postmenopausal status were also identified as independent risk factors for EC. Patients with smoking had a 4.13-fold (95% CI: 1.54-11.01, p=0.005), and postmenopausal status had a 2.8-fold (95% CI: 1.87-9.04, p=0.034) increased risk of EC.
Conclusion: The results of this study suggest that DNI may be a useful biomarker for predicting the risk of EC. The results also confirm that smoking and postmenopausal status are independent risk factors for EC.
Keywords: Biomarker; endometrial cancer; endometrial hyperplasia.

ÖZ

Amaç: Bu çalışmanın amacı, endometriyal intraepitelyal neoplazi (EIN) tanısı almış olan kadınlarda delta nötrofil indeksi'nin (DNI) endometriyal kanser (endometrial cancer, EC) gelişimi için bir öngörücü biyobelirteç olarak potansiyelini değerlendirmektir.
Gereç ve Yöntemler: Bu retrospektif çalışmaya, 2019 ve 2022 yılları arasında EIN tanısı almış ve cerrahi geçirmiş olan 139 kadın dahil edilmiştir. Demografik veriler, tıbbi öykü ve DNI da dahil olmak üzere laboratuvar parametreleri hastaların tıbbi kayıtlarından toplanmıştır. Başka kanser türüne sahip olan hastalar, steroid kullanım öyküsü olanlar, enflamatuar, hematolojik veya otoimmün hastalığı olanlar ve eksik verisi olan hastalar çalışmadan çıkarılmıştır. Hastaların patoloji raporları incelenmiş ve hastalar nihai patoloji tanılarına göre benign (n=64), EIN (n=66) ve EC (n=39) olmak üzere üç gruba ayrılmıştır.
Bulgular: EC grubunun ortalama DNI düzeyi, EIN ve benign gruplarına göre anlamlı olarak daha yüksek bulundu (sırasıyla 4,85±2,31; 2,31±0,89 ve 1,48±1,03; p<0,001). DNI için optimal kesim değeri %82,1 sensitivite ve %73,8 spesifite ile %2,75 olarak belirlendi. DNI düzeyi >%2,75 olmanın 11,56 kat (95% GA: 4,59-29,09; p<0,001) artmış EC riski ile ilişkili olduğu bulundu. Sigara içmek ve postmenopozal durum da EC için bağımsız risk faktörleri olarak belirlendi. Sigara içen hastalarda 4,13 kat (95% GA: 1,54-11,01; p=0,005) ve postmenopozal durumda ise 2,8 kat (95% CI: 1,87-9,04; p=0,034) daha fazla EC riski vardı.
Sonuç: Bu çalışmanın sonuçları DNI'nin EC riskini öngörmede kullanılabilir bir biyobelirteç olabileceğini öne sürmektedir. Sonuçlar ayrıca sigara içmenin ve menopoz sonrası durumun da EC için bağımsız risk faktörleri olduğunu doğrulamaktadır.
Anahtar kelimeler: Biyobelirteç; endometriyal kanser; endometriyal hiperplazi.

INTRODUCTION

Endometrial cancer (EC) is one of the most common gynecologic cancers affecting women globally (1). The incidence of EC is increasing, and a significant number of deaths in women worldwide are due to this disease (2). Therefore, early diagnosis and accurate prognosis are crucial for the successful treatment of EC (3,4).

In recent years, researchers have been exploring the potential of biomarkers as a tool to improve the diagnosis and prognosis of EC. Biomarkers are measurable indicators that can be used to identify and diagnose a disease, predict its progression, and evaluate its response to treatment (5-7). One such biomarker that has shown promise in the diagnosis and prognosis of EC is delta neutrophil index (DNI) (8,9).

DNI is a laboratory parameter that measures the difference between the fraction of immature granulocytes and mature granulocytes in the bloodstream (8-10). DNI is a sensitive marker of systemic inflammation and infection and has been shown to be a promising prognostic biomarker in various cancers, including EC (10-14).

Furthermore, DNI can be used as a predictive biomarker for the development of EC in women with endometrial hyperplasia. In our study, it was aimed to explore the predictive role of DNI in EC.

MATERIAL AND METHODS

This retrospective study examined the data of 169 female patients diagnosed with endometrial intraepithelial neoplasia (EIN) who underwent surgery at Ankara Etlik Zübeyde Hanım Women's Health Training and Research Hospital between January 2019 and December 2022. Ethical approval was obtained from the local institutional review board of Ankara Etlik Zübeyde Hanım Women's Health Training and Research Hospital (10.08.2022, 103), and the study was conducted in accordance with the Declaration of Helsinki. Measures were taken to ensure patient confidentiality and privacy throughout the study. Demographic data, medical history, and laboratory parameters including DNI were collected from the medical files of the patients who underwent surgery for EIN. The DNI was measured from the complete blood count (CBC) test results prior to surgery to minimize any potential confounding factors. The DNI was calculated as the difference between the fraction of immature granulocytes (bands and metamyelocytes) and mature granulocytes (segmented neutrophils). Patients who had a history of cancer other than EC, a history of steroid use, inflammatory, hematologic, or autoimmune diseases, or had missing data in their medical records were excluded from the study. Patients' pathology reports were reviewed, and the final pathological diagnosis was classified as benign, EIN, or EC, based on the World Health Organization (WHO) criteria. Patients were divided into three groups as benign (n=64, 37.8%), EIN (n=66, 39%), and EC (n=39, 23.2%), according to the final pathologic findings.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics v.25.0 software (IBM Corporation, Armonk, NY, USA). The normality of the data was assessed visually by histogram and with Skewness and Kurtosis values. The mean and standard deviation (SD) were calculated for continuous variables, and the frequencies and percentages were calculated for categorical variables. The ANOVA test was used to examine group differences. Post-hoc multiple comparison tests, such as Bonferroni and Tukey correction, were conducted to identify specific group differences if a significant overall difference was found. The Kruskal-Wallis test and chi-square test were used to compare continuous and categorical variables, respectively. The receiver operating characteristic (ROC) curve was used to determine the optimal cut-off value, sensitivity, specificity, and the area under the curve (AUC) for DNI in predicting EC. Univariate logistic regression analysis was performed to determine the independent risk factors for EC. After univariate analysis, a model for multivariate logistic regression analysis was formed. 95% confidence interval (CI) and a p value of <0.05 were considered significant.

RESULTS

The comparison of demographic, clinical, and blood outcomes of the three groups was shown in Table 1. Age and body mass index (BMI) were found significantly different among the groups (p<0.001, and p=0.003, respectively). There was also a statistically significant difference in smoking status (p=0.001), and menopausal status (p=0.018).

Table 1. Comparison of demographic and clinical characteristics of the groups

	Benign (n=64)	EIN (n=66)	Malign (n=39)	p
Age (year), mean±SD	47.40±6.54[a]	48.72±8.00[a,b]	54.30±9.25[c]	**<0.001**
Body mass index (kg/m^2), mean±SD	29.65±3.68[a]	31.28±4.24[a,b]	32.76±5.70[b,c]	**0.003**
Gravidity, median (range)	3 (0-7)	3 (0-12)	3 (0-9)	0.059
Parity, median (range)	2 (0-6)	2 (0-7)	2 (0-7)	0.085
Endometrial thickness (mm), mean±SD	10.09±5.27	11.51±5.79	12.61±7.72	0.116
Preoperative leukocyte (x1/μL), mean±SD	7.55±2.16	7.64±2.53	7.35±1.96	0.817
Preoperative hemoglobin (x1/μL), mean±SD	12.03±1.81[a]	12.61±1.60[a,b]	13.00±1.89[b,c]	**0.020**
Preoperative platelet (x1/μL), mean±SD	291.92±82.92[a]	264.93±50.52[a,b]	327.69±63.11[c]	**<0.001**
Delta neutrophil index (%), mean±SD	1.48±1.03[a]	2.31±0.89[b]	4.85±2.31[c]	**<0.001**
Smoking, n (%)	5 (7.8)[a]	9 (13.6)[b]	14 (35.9)[c]	**0.001**
Postmenopausal, n (%)	13 (20.3)[a]	18 (27.3)[b]	18 (46.2)[c]	**0.018**

EIN: endometrial intraepithelial neoplasia, SD: standard deviation, [a,b,c]: groups with different letters are significantly different from each other

Preoperative hemoglobin (p=0.020), preoperative platelet count (p<0.001), and DNI levels (p<0.001) were significantly different among the groups. The highest values were observed in the cancer group and this difference was statistically significant (Table 1).

The optimal predictive value of DNI was calculated using ROC curve analysis (Figure 1). The cut-off value was calculated to be 2.75%, and AUC was 0.894 with 82.1% sensitivity and 73.8% specificity.

Patients were divided into two groups based on the cut-off value of DNI, median age, and BMI. Univariate logistic regression analyses revealed that age >48 years had 2.51 fold (95% CI: 1.22-5.17, p=0.012), DNI level >2.75% had 10.44 fold (95% CI: 4.52-24.12, p<0.001), being a smoker had 4.87 fold (95% CI: 2.07-11.43, p<0.001) and menopause had 2.67 fold (95% CI: 1.28-5.55, p=0.009) increased risk of having EC. After univariate logistic regression analyses, the multivariate logistic regression model was created. While patients with DNI >2.75% had an 11.56-fold (95% CI: 4.59-29.09, p<0.001) increased risk of having EC, patients who were postmenopausal had a 2.8-fold (95% CI: 1.87-9.04, p=0.034) and patients who smoke had a 4.13-fold (95% CI: 1.54-11.01, p=0.005) increased risk of having EC (Table 2).

DISCUSSION

In our study, we investigated the use of DNI as a potential biomarker for EC. The ability to diagnose EC is essential for timely and effective treatment. Our results showed that patients with high DNI levels had a significantly increased risk of EC, with an odds ratio of 11.5.

Our study also identified smoking status and menopausal status as independent risk factors for EC. These findings are consistent with previous research linking these factors to increased risk of EC and suggest that these factors should be considered when assessing a patient's risk of developing EC (15-18). Lou et al. (15) reported that preoperative-EIN patients who are postmenopausal or have an elevated level of CA125 may have a higher risk of concurrent EC. According to the Danish Gynecological Cancer Database (16), postmenopausal women have an almost 3-fold higher risk of final diagnosed EC compared with the premenopausal group, with 80% of postmenopausal women being affected. A systematic review that included 13 studies has shown women who were postmenopausal and smoking more than 15 cigarettes daily have higher circulating levels of estradiol than nonsmoker women (17). In contrast to these findings in the observational analysis, smoking was found associated with a low risk of EC (18).

The use of biomarkers, such as DNI, may have several clinical implications for the diagnosis and management of various cancers. According to Bozan et al. (13), DNI had a sensitivity of 86.5% and specificity of 80.4%, and the immature granulocyte (IG) count had 100% sensitivity and 82.6% specificity in detecting axillary metastasis of breast cancer. The study suggests that to detect axillary metastasis of breast cancer DNI and IG count may be helpful.

In another study, which evaluated the relationship between DNI and neutrophil-to-lymphocyte ratio (NLR) in the preoperative differentiation of benign and malign thyroid neoplasms, preoperative NLR, DNI, and IG count were found to be statistically significant between benign and malignant groups (19). Additionally, DNI levels were compared between patients with renal cell carcinoma and healthy individuals, and DNI and NLR were found to be predictors of renal cell carcinoma (14). It is important to note that different threshold values have been found for DNI in these studies, which can be attributed to the fact that different types of diseases have been examined. In our study, a DNI level >2.75% was the strongest independent risk factor for EC. Preoperative DNI may be used to identify patients who are at high risk of developing EC and who may benefit from more frequent screening or prophylactic measures such as chemoprevention. DNI may also be useful in monitoring the progression and response to treatment in patients with EC.

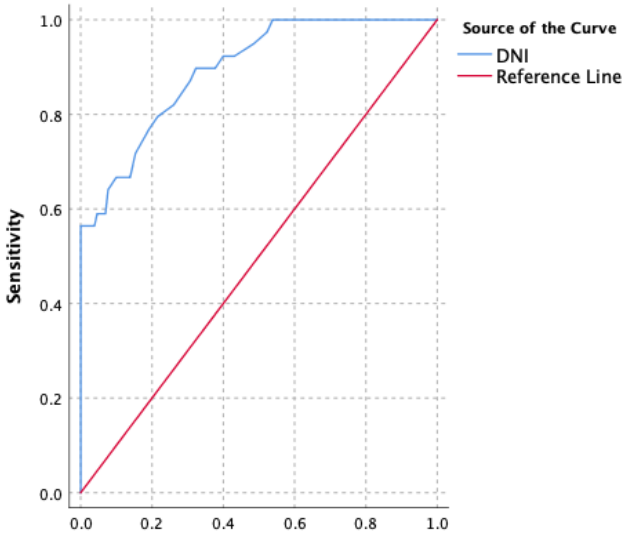

Figure 1. Receiver operating characteristics curve of delta neutrophil index for predictability of endometrial cancer

Table 2. Univariate and multivariate logistic regression analysis of the variables for endometrial cancer

	Univariate			Multivariate		
	OR	**95% CI**	**p**	**OR**	**95% CI**	**p**
Age (>48 years)	2.51	1.22-5.17	**0.012**	-		-
BMI (>30.82 kg/m^2)	1.87	0.91-3.82	0.085	-		-
DNI (>2.75%)	10.44	4.52-24.12	**<0.001**	11.56	4.59-29.09	**<0.001**
Smoking	4.87	2.07-11.43	**<0.001**	4.13	1.54-11.01	**0.005**
Menopause	2.67	1.28-5.55	**0.009**	2.80	1.87-9.04	**0.034**

BMI: body mass index, DNI: delta neutrophil index, OR: odds ratio, CI: confidence interval

Our study has several limitations, including the relatively small sample size and the retrospective nature of the study design. The inclusion of patients who underwent surgery for EIN may introduce selection bias and single-center conduction may limit generalizability. On the other hand, the study explores the potential of a novel biomarker (DNI) for the diagnosis of EC, which could have significant clinical implications.

CONCLUSION

DNI may be a useful biomarker for predicting the risk of EC. Our results also confirm smoking and postmenopausal status as independent risk factors for EC, consistent with previous research. Further studies are needed to support the use of DNI as a biomarker for EC.

Ethics Committee Approval: The study was approved by the Ethics Committee of the Etlik Zübeyde Hanım Women's Health Training and Research Hospital (10.08.2022, 103).

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The Intraoperative Use of Hypochlorous Acid in Infected Hip Arthroplasty Revision Surgery

Revizyon Yapılan Enfekte Kalça Artroplastilerinde İntraoperatif Hipokloröz Asit Kullanımı

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ABSTRACT

Aim: Arthroplasty infections are serious and difficult to treat complications. Hypochlorous acid (HOCl) is an oxidant produced endogenously in the body as a physiological part of the inflammatory process, with the aim of eliminating pathogens activated by neutrophils. The aim of this study was to evaluate the positive and negative effects on clinical results of HOCl used as an irrigation solution during surgical treatment.
Material and Methods: The study included 37 patients who underwent single- or two-stage revision surgery at the University of Health Sciences Şişli Hamidiye Etfal Training and Research Hospital Orthopedics and Traumatology Clinic between January 2017 and December 2021. Treatment was applied according to our standard protocol of irrigation with 450 cc HOCl following implant removal and an additional 50 cc HOCl to the subcutaneous tissue after closing the fascia. The patients were evaluated during follow-up in respect of infections.
Results: While single-stage revision surgery applied to 20 patients, two-stage revision surgery applied to 17 patients. 17 (45.9%) of the patients were male and 20 (54.1%) were female with a mean age of 72.8±11.1 years. The mean follow-up period was 25.8±14.1 months. Revision surgery was performed on one patient in each of the single and two-stage surgery groups. A success rate of 94.6% (n=37) was obtained when all patients were evaluated.
Conclusion: The HOCl solution can be considered to make a positive contribution to the eradication of infections in revision hip arthroplasty and can be an effective and safe alternative to other irrigation solutions.
Keywords: Arthroplasty; infection; hip; hypochlorous acid.

ÖZ

Amaç: Artroplasti enfeksiyonları ciddi ve tedavisi güç olan komplikasyonlardır. Hipokloröz asit (hypochlorous acid, HOCl), patojenleri ortadan kaldırmak amacıyla, nötrofiller tarafından aktive edilen inflamatuar sürecin fizyolojik kısmının bir parçası olarak vücutta endojen olarak üretilen bir oksidandır. Bu çalışmanın amacı, cerrahi tedavi sırasında irrigasyon solüsyonu olarak kullanılan HOCl'nin klinik sonuçlar üzerindeki olumlu ve olumsuz etkilerini değerlendirmektir.
Gereç ve Yöntemler: Çalışmaya Ocak 2017 ve Aralık 2021 tarihleri arasında Sağlık Bilimleri Üniversitesi Şişli Hamidiye Etfal Eğitim ve Araştırma Hastanesi Ortopedi ve Travmatoloji kliniğinde tek veya iki aşamalı kalça revizyon cerrahisi geçiren 37 hasta dahil edildi. Tedavi tüm implantlar çıkarıldıktan sonra standart protokolümüze göre 450 cc HOCl ile irrigasyon ve fasya kapatıldıktan sonra subkutan dokuya ek 50 cc HOCl uygulandı. Hastalar takip sırasında enfeksiyonlar açısından değerlendirildi.
Bulgular: 20 hastaya tek aşamalı revizyon cerrahisi uygulanırken, 17 hastaya ise iki aşamalı revizyon cerrahisi uygulandı. Hastaların 17'si (%45,9) erkek ve 20'si (%54,1) kadın olup ortalama yaşları 72,8±11,1 yıl idi. Ortalama takip süresi 25,8±14,1 ay idi. Tek ve iki aşamalı cerrahi uygulanan gruplardan ikisinde de birer hastaya revizyon cerrahisi uygulandı. Hastaların tamamı değerlendirildiğinde %94,6'lık (n=37) bir başarı oranı elde edildi.
Sonuç: HOCl solüsyonunun revizyon kalça artroplastilerinde enfeksiyonların eradikasyonu açısından olumlu katkılarının olduğu ve diğer irrigasyon solüsyonlarına alternatif olarak etkin ve güvenli bir yıkama solüsyonu olabileceğini öngörmekteyiz.
Anahtar kelimeler: Artroplasti; enfeksiyon; kalça; hipokloröz asit.

INTRODUCTION

Over recent years there has been an increase in the number of total hip arthroplasty (THA) operations as a result of longer implant life and increased social expectations of patients. Although there has been a decrease in periprosthetic hip infections (PHI) together with developments in the field of healthcare, infections occurring after THA are one of the most serious complications encountered (1). Over time there has been a great increase in both length of stay in hospital and costs associated with PHI (2).

The treatment to be selected after the occurrence of PHI can be separated into two forms, single-stage or two-stage revision surgical procedures. Although there is no clear consensus on which surgical treatment is to be selected, single- or two-stage revisions are preferred depending on the clinical status of the patient, the severity of the infection, and the organism causing the infection (3).

There are positive contributions to the use of wound antiseptics in surgical wound irrigation, but these solutions can also have a cytotoxic effect. The ideal antiseptic to be used in irrigations should have a bactericidal effect at low concentrations and the cytotoxic effect should be minimal (4,5).

Hypochlorous acid (HOCl) is an oxidant produced endogenously in the body as a physiological part of the inflammatory process, with the aim of eliminating pathogens activated by neutrophils. HOCl has been shown to have a rapid bactericidal effect against most pathogens responsible for surgical site infections. In addition to the fragmentation effect on biofilms, HCOl has a low cytotoxic effect and a neutral pH value. As a result of these properties, there has started to be an increase in studies related to the use of HCOl solution on the skin, as an oral antiseptic, and during the surgical treatment of open wounds and infections (6-15).

Whichever method of surgical treatment is selected, surgical success with respect to infection eradication is affected by numerous factors, including the facilities and characteristics of the center where the surgery occurs, the selection and application of the irrigation solution, and human factors.

The aim of this study was to evaluate the positive and negative effects of HOCl use as an irrigation solution during the surgical treatment of patients who developed chronic PHI following hip arthroplasty.

MATERIAL AND METHODS

A retrospective examination was made of 41 patients who underwent single-stage or two-stage revision surgery because of PHI between January 2017 and December 2021, with the use of HOCl as the irrigation solution during the surgical treatment. All the patients were given information about the medical and surgical treatments to be applied in the management of PHI, and about the HOCl irrigation solution that was planned to be used and informed consent was obtained from all patients. This study was performed in line with the principles of the Declaration of Helsinki. Ethics committee approval was granted by the Ethics Committee of Health Sciences University Şişli Hamidiye Etfal Training and Research Hospital (dated 18.04.2023, and numbered: 3881). The study exclusion criteria were defined as treatment with only anti-biotherapy or debridement irrigation and implant retention. Because four patients left the follow-up, the study was completed with 37 patients. A record was made for each patient of age, sex, body mass index (BMI), American Society of Anesthesiologists (ASA) score, operated side, and prophylactic antibiotics used.

The diagnosis of infection was made considering the criteria established by the Musculoskeletal Infection Society (MSIS) together with clinical, radiological, and laboratory findings such as fistulated discharge, elevated sedimentation and C-reactive protein (CRP), redness and increased temperature, and implant loosening observed on scintigraphy and radiographs (16).

All the patients were treated in a single center, and standard perioperative care was applied according to the rules of joint arthroplasty. Following sufficient debridement and the taking of a sample for culture, intravenous antibiotic prophylaxis in accordance with the national surgery guidelines was administered as 1-2 gr cefazolin or a single dose of 15-20 mg/kg vancomycin. Unless specific allergic reactions were reported, iodine-soaked drapes were used on all the patients. When there were no different indications related to comorbid diseases, low molecular weight heparin (once a day) was administered postoperatively.

Revision surgery was performed as single-stage to 20 patients and as two-stage to 17 patients. In both the single- and two-stage revision protocols, only isotonic and HCOl were used as irrigation solutions during debridement. During the single-stage revision, after the removal of the implant and surgical debridement, HCOl was used after mechanical debridement of the surgical site with physiological saline. In the first stage of the two-stage revision, the implant was removed, then following surgical and mechanical debridement with physiological saline, HCOl was used before the application of the spacer. In the second stage when clinical and hematological markers had returned to normal, the spacer was removed, then following surgical and mechanical debridement, and irrigation with HOCl, the revision implant was applied.

HOCl is produced from an inverse reaction of sodium hypochlorite and hydrogen peroxide (HP). The concentration used in this study was 200 ppm, pH 7.1, oxidation reduction potential (ORP) of 871 millivolt (mV), and stability for 24 months. For all patients, irrigation with 450 cc HCOl was applied to the surgical area for 5 minutes (Figure 1). After aspiration of the HCOl and closure of the fascia, irrigation was applied again with a 50 cc HOCl solution. The basic pathophysiological parameters of blood pressure, heart rate, and respiratory rate were recorded intraoperatively, before, during, and after the HCOl irrigation.

Standard postoperative follow-up in our institution includes follow-up examinations at 3 weeks, 6 weeks, 3 months, 6 months, and 1 year. The patients were followed up for at least 1 year. The sutures were removed after 21 days after checking wound healing. Anti-biotherapies were adjusted according to the recommendations of the Infectious Diseases Department according to the culture results. At the follow-up examinations, the presence of discharge, redness, or fistula was clinically evaluated.

Radiologically, it was examined whether or not there was loosening observed on the radiographs, and from blood samples, the infection parameters of sedimentation rate, white blood cell (WBC), and CRP were evaluated.

RESULTS

The evaluation was made of 37 patients operated on because of PHI, with single-stage revision surgery applied to 20 patients and two-stage revision surgery to 17. The patients comprised 17 (45.9%) males and 20 (54.1%) females with a mean age of 72.8±11.1 (range, 41-90) years. The operated hip was right side in 21 (56.8%) patients and left side in 16 (43.2%). The body weight of the patients was mean 76.0±7.8 (range, 60-100) kg, with mean BMI calculated as 27.9±3.6 kg/m^2. From the BMI measurements, 2 (5.4%) patients were classified as morbidly obese, 7 (18.9%) as obese, 21 (56.8%) as overweight, and 7 (18.9%) as normal weight. Additional diseases of the patients were determined as diabetes mellitus, hypertension, chronic renal failure, pulmonary diseases, history of cancer, cardiac pathologies, and cerebrovascular event (Table 1). The ASA scores were determined as ASA 1 in 4 (10.8%), ASA 2 in 12 (32.4%), ASA 3 in 17 (45.9%), and ASA 4 in 4 (10.8%) patients.

A partial hip prosthesis was present in 19 (51.4%) patients, a total hip prosthesis in 13 (35.1%), and a revision hip prosthesis in 5 (13.5%). In 1 patient for whom two-stage revision was planned, despite sufficient anti-biotherapy after the first stage, because of elevated infection values and discharge, debridement and spacer placement were repeated before the second stage.

As infection was not eradicated, revision surgery was performed in 1 patient who had undergone single-stage surgery and in 1 patient who had undergone two-stage surgery. A success rate of 94.6% (n=37) was obtained when all patients were evaluated together. When evaluated separately, the success rates were 95.2% (n=20) in the single-stage group and 94.4% (n=17) in the two-stage group.

Polymicrobial organisms were seen to be produced in the cultures of 5 patients. The organisms produced were shown in Table 2. Despite the infection parameters in 8 patients, no microbial agent was produced in the culture.

No data related to any allergic or unusual reaction having developed associated with the use of HCOl were obtained from any patient perioperatively or in the postoperative period. No complication or delay in wound healing was observed in any patient.

The mean follow-up period was 25.8±14.1 (range, 12-64) months. With the exception of the 2 patients who were operated on again, the clinical and laboratory parameters throughout the follow-up period showed a regression of infection and there was observed to be a decreased need for analgesia. No findings of loosening of the components were observed radiologically.

DISCUSSION

Together with the increased number of arthroplasty operations, there has been a relative increase in the development of PHI. Increased antibiotic resistance, which has become a significant problem in recent years, has made the use of irrigation solutions during surgical treatment of PHI more important (2-4,6).

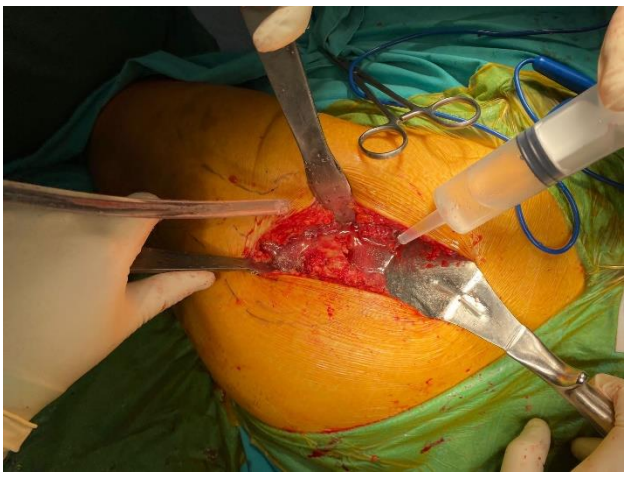

Figure 1. Hypochlorous acid application

Table 1. Additional diseases of the patients

Disease	n (%)
Diabetes mellitus	12 (32.4)
Hypertension	26 (70.3)
Chronic renal failure	6 (16.2)
Respiratory diseases	7 (18.9)
History of cancer	3 (8.1)
Heart diseases	11 (29.7)
Cerebrovascular event	3 (8.1)

Table 2. The organisms in the cultures of the patients

Pathogens
Escherichia coli
Pseudomonas aeruginosa
Staphylococcus epidermidis
Corynebacterium amycolatum
Proteus mirabilis
Candida albicans
Klebsiella pneumoniae
Enterobacter aerogenes
Streptococcus pyogenes
Staphylococcus aureus
Methicillin-resistant *Staphylococcus aureus* (MRSA)
Enterococcus faecium
Vancomycin-resistant *Enterococcus faecalis* (VRE)

HCOl is a small molecule produced by WBCs in the body in the oxidation process to kill pathogens. As it is small and neutral, it cannot be removed by bacteria membranes and thus has the effect of eliminating membrane components (15). In previous studies, HCOl has been shown to be extremely effective against antibiotic-resistant bacteria, those forming a biofilm, fungi, and viruses. All the micro-organisms produced in the cultures of the current study patients were seen to be micro-organisms against which HCOl has been shown to be effective in previous studies that have evaluated the bactericidal efficacy of HCOl (7,8,14).

The ideal irrigation solution should show destructive and bactericidal efficacy on biofilms even at low concentrations, and there should be a very low or no cytotoxic effect (4-6). Biofilm formation is accepted as a serious problem in chronic wound infections (17). HOCl has the characteristics of an ideal wound care solution in respect of showing a rapid micro-bactericidal effect against different micro-organism species within the biofilm. There is no negative effect on wound healing, and it has even been shown to make a positive contribution to wound healing as there are dose-related positive effects on fibroblast and keratinocyte migration (7,8). No wound healing problems and no delay in wound healing were observed in any of the current study patients.

Antiseptics that can be used as irrigation solutions during arthroplasty procedure include povidone-iodine (PI), chlorhexidine (CHG), acetic acid (AA), HP, sodium hypochlorite, and HOCl (6,18). In most clinical studies in the literature, PI and CHG have been used during arthroplasty (6). Hart et al. (19) used PI as the irrigation solution in a study of revision hip arthroplasty and reported revision because of infection in the first year at the rate of 5.2% after a 1-year follow-up period. In a study by Riesgo et al. (20), a success rate of 83.3% was reported with the use of PI together with vancomycin as the irrigation solution during implant change in infected hip and knee arthroplasties. Byren et al. (21) used CHG in implant change after PHI and reported a success rate of 86.5% (45/52).

Due to the low number of studies in literature, no consensus has yet been reached on the stage of determining the ideal irrigation solution. All the above-mentioned antiseptics have shown cytotoxic effects at different concentrations in studies in the literature (6,18). The cytotoxic effect of free iodine on chondrocytes, osteoblasts, and other normal host cells has been shown even at low concentrations (6,22,23). Compared with 1% PI, CHG, and 10% PI, HCOl at doses effective on the biofilm has been shown to have a less cytotoxic effect (9). As HCOl is not irritant to the skin, does not show a cytotoxic effect, has a neutral pH, and converts to salty water similar to tears by breaking down within minutes, it is not necessary to wash it from the wound and skin, unlike other wound cleaning solutions (24,25).

In the current study of patients operated on because of PHI, it was seen to be necessary to repeat the revision due to the infection in 2 patients, and thus a success rate of 94.6% (n=37) was obtained in total. In a review and meta-analysis by Kunutsor et al. (26), the total re-infection development rate was reported to be 8% in patients applied with single- or two-stage revision surgery because of PHI. The total re-infection rate in the current study was determined to be 5.4% (n=2). The results published in the literature of patients applied with single- and two-stage surgery because of PHI are shown in (Tables 3 and 4). When evaluated together with the literature, it can be seen that the results of the current study are at least as successful as those in the literature.

When the literature was scanned related to the duration of HCOl within the wound, it can be seen that no clear consensus has been reached. After use, after having encountered pathogens and the biological load, HCOl returns to non-reactive NaCl and H_2O within minutes (23). Although it has been reported that the destructive and bactericidal effect is shown within seconds against many bacteria and the biofilms formed, there are also data in the literature that an effect against fungi is formed within 5 minutes (6,7,10). Therefore, in the current study, irrigation was applied for 5 minutes.

There are reports in the literature that the use of HCOl in orthopedic implant-related infections can cause corrosion and wear on CoCr and Ti metals. In a review study by Siddiqi et al. (6), it was recommended that care should be taken in the use of HCOl in patients with an implant and in

Table 3. The results of single-stage arthroplasty in literature

Authors	Year	Number of patients	Type of arthroplasty	Infection control rate (%)	Follow-up (year)
Winkler et al. (27)	2008	37	one-stage	92	4.4
Rudelli et al. (28)	2008	32	one-stage	93.8	8.5
Yoo et al. (29)	2009	12	one-stage	83.4	7
Klouche et al. (30)	2012	38	one-stage	100	2
Choi et al. (31)	2013	17	one-stage	82	5.2
Hansen et al. (32)	2013	27	one-stage	70	4.2
Zeller et al. (33)	2014	157	one-stage	95	5

Table 4. The results of two-stage arthroplasty in literature

Authors	Year	Number of patients	Type of arthroplasty	Infection control rate (%)	Follow-up (year)
Masri et al. (34)	2007	29	two-stage	89.7	2
Biring et al. (35)	2009	99	two-stage	89	12
Oussedik et al. (36)	2010	39	two-stage	94.9	6.8
Engesæter et al. (37)	2011	283	two-stage	95	3
Choi et al. (31)	2013	44	two-stage	86.4	5.8
Ibrahim et al. (38)	2014	125	two-stage	96	5
Chen et al. (39)	2014	157	two-stage	91.7	9.7

those where the implant is not removed. Therefore, when an implant is present, the use of HCOl would be appropriate with a good benefit-harm calculation. As the HCOl was applied in the current study after the removal of the implant, the effect on implants was not evaluated (6,11).
There were some limitations to this study. These can be said to be the retrospective design, the lack of a control group, the relatively low number of patients, and the short follow-up period.

CONCLUSION

In the patients in this study with irrigation performed with HCOl, which is bactericidal and shows an effect on biofilms, no complications or allergic reactions were determined associated with the use of this solution. When compared with the results of other studies of revision surgery performed because of PHI, the current study results were seen to be at least as successful as the results of other studies. HOCl solution can be considered to make a positive contribution to the eradication of infections in revision hip arthroplasty and can be an effective and safe alternative to other irrigation solutions. Nevertheless, it would be appropriate to conduct further prospective studies with control groups and a longer follow-up period related to the use of HCOl.

Ethics Committee Approval: The study was approved by the Ethics Committee of Şişli Hamidiye Etfal Training and Research Hospital (18.04.2023, 3881).

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The Association between Inflammatory and Nutritional Markers and Survival in Elderly Patients Operated for Lung Cancer

Akciğer Kanseri Nedeniyle Ameliyat Edilen Yaşlı Hastalarda Sağkalım ile İnflamatuvar ve Beslenme Belirteçleri Arasındaki İlişki

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ABSTRACT

Aim: Today, there is an increase in cancer incidence and cancer-related deaths in the elderly population. This study aimed to evaluate the impact of neutrophil-to-lymphocyte ratio (NLR) and prognostic nutritional index (PNI) levels on the survival rate in elderly patients diagnosed with non-small cell lung cancer (NSCLC).

Material and Methods: A total of 73 patients (aged ≥70 years) who operated for NSCLC between 2012 and 2018 were included in this study. Patient records were analyzed retrospectively. The NLR value was calculated with the blood neutrophil count/lymphocyte count formula. The cut-off value for NLR was considered as 2.5. The PNI value was calculated as 10 x serum albumin (g/dL) + 0.005 × total lymphocyte count (per millimeter). PNI values lower than 50 were considered as low.

Results: The mean age of the patients was 74.15±3.23 years, 59 (80.8%) patients were male and 14 (19.2%) were female. No statistically significant relation of postoperative complication was noted with PNI (p=0.354) and NLR (p=0.483). Postoperative mortality was not significantly associated with PNI (p=0.188) and NLR (p=0.465). For patients with NLR <2.5, 1-, 3-, and 5-year survival rates were at 86.0%, 86.1%, and 78.3%, respectively, while these rates were at 76.9%, 65.2%, and 24.8%, respectively, for patients with NLR >2.5 (p=0.028).

Conclusion: Results revealed a significant association of NLR with survival rates in elderly patients with NSCLC. NLR can be considered inexpensive, easily measurable, and reproducible marker that can be incorporated into routine clinical practice for guiding and optimizing treatment decisions in patients with NSCLC.

Keywords: Lung cancer; elderly population; NLR; PNI; postoperative mortality.

ÖZ

Amaç: Günümüzde yaşlı nüfusta kanser insidansında ve kansere bağlı ölümlerde artış görülmektedir. Bu çalışmanın amacı küçük hücreli dışı akciğer kanseri (KHDAK) tanılı olan yaşlı hastalarda nötrofil lenfosit oranı (NLO) ve prognostik beslenme indeksi (prognostic nutritional index, PNI) düzeylerinin sağkalım oranı üzerindeki etkisinin değerlendirilmesidir.

Gereç ve Yöntemler: Bu çalışmaya 2012 ve 2018 yılları arasında KHDAK nedeniyle opere edilmiş olan toplam 73 hasta (≥70 yaş) dahil edildi. Hasta kayıtları geriye dönük olarak analiz edildi. NLO değeri, kan nötrofil sayısı/lenfosit sayısı formülü ile hesaplandı. NLR için kesim değeri 2,5 olarak kabul edildi. PNI değeri, 10 x serum albümini (g/dL) + 0,005 x toplam lenfosit sayısı (milimetre başına) şeklinde hesaplandı. 50'nin altındaki PNI değerleri düşük olarak kabul edildi.

Bulgular: Hastaların ortalama yaşı 74,15±3,23 yıl, 59 (%80,8) hasta erkek ve 14'ü (%19,2) hasta kadındı. PNI (p=0,354) ve NLO (p=0,483) ile postoperatif komplikasyon arasında istatistiksel olarak anlamlı bir ilişki saptanmadı. Postoperatif mortalite de PNI (p=0,188) ve NLO (p=0,465) ile anlamlı olarak ilişkili değildi. NLO <2,5 olan hastalarda 1-, 3- ve 5-yıllık sağkalım oranları sırasıyla %86,0, %86,1 ve %78,3 iken bu oranlar NLR >2,5 olan hastalar için sırasıyla %76,9, %65,2 ve %24,8 idi (p=0,028).

Sonuç: Sonuçlar KHDAK olan yaşlı hastalarda NLO ile sağkalım oranları arasında anlamlı bir ilişki olduğunu ortaya koydu. NLO, KHDAK hastalarında tedavi kararlarını yönlendirmek ve optimize etmek için rutin klinik uygulamaya dahil edilebilecek ucuz, kolay ölçülebilir ve tekrarlanabilir bir belirteç olarak kabul edilebilir.

Anahtar kelimeler: Akciğer kanseri; yaşlı nüfus; NLO; PNI; postoperatif mortalite.

INTRODUCTION

Lung cancer is the leading cause of cancer deaths worldwide, with high mortality, resulting in approximately 1.7 million deaths yearly (1). Lung cancers include two main histological types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), based on the natural course of the disease and treatment strategies. About 85% of lung cancers are NSCLC; the most common subtypes are squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (2).

There is an increase in cancer incidence and cancer-related deaths in the elderly population, along with rising average life expectancy and cancer occurrence. Approximately 50% of all cancers and 70% of cancer-related deaths occur in elderly persons aged ≥65 years. Hence, given the growing importance of cancer diagnosis, treatment, and follow-up in the elderly population, identifying effective biomarkers of prognosis and improved clinical outcomes is considered critical (3-5).

Inflammation and tumor microenvironment are associated with cancer development and progression. The cells that play a crucial role in inflammation can be involved in inflammation-induced cancer development and may affect tumor progression and life expectancy in cancer patients. Although there is increasing evidence for the roles of local immune response and the systemic inflammatory response in the formation and progression of cancer cells in recent studies, it is still unclear which inflammatory parameter is a better indicator of cancer prognosis (6,7).

The neutrophil-to-lymphocyte ratio (NLR) is a reliable and predictive inflammatory marker of the prognosis of cancer and inflammatory diseases. Current evidence indicates that high NLR has a prognostic value in various cancers, including lung cancer (8).

The prognostic nutritional index (PNI) is one of the immune nutritional parameters calculated based on serum albumin and the total lymphocyte count in peripheral blood. Although its use for stratification of operative risk and evaluation of perioperative nutritional and immunological conditions was recommended (9,10), increasing evidence has shown that its preoperative nutritional and immunological status not only affects the rapid postoperative complications but are also thoroughly related to the long-term oncological outcomes (11).

In this study, we investigated the relationship between NLR and PNI biomarkers and survival and postoperative mortality incidence in elderly lung cancer patients.

MATERIAL AND METHODS

A total of 73 elderly patients (aged ≥70 years of age) with operated NSCLC performed between 2012 and 2018 were included in this study. Patient medical records and hospitalization data were analyzed retrospectively in this study. The NLR level was assessed using the formula of blood neutrophil count/lymphocyte count. The cut-off value for NLR was considered as 2.5 (12). In addition, the PNI value was calculated as 10 x serum albumin (g/dL) + 0.005 × total lymphocyte count (per millimeter). PNI values lower than 50 were considered as low (10).

The study was approved by the Ministry of Health and Local Ethics Committee (Başkent University Local Ethic Committee's approval document dated 18.11.2020 and numbered KA20/410).

Statistical Analysis

Categorical and continuous measurements were expressed in numbers and percentages, and in mean and standard deviation values, respectively. The chi-square test or Fisher's test was used to compare categorical variables. Distributions were analyzed with the Kolmogorov-Smirnov test to compare continuous measurements between the groups. The Student's t-test was used for two groups and the one-way ANOVA for three or more groups. Survival curves for relapse and mortality were created using the Kaplan-Meier analysis and compared by log-rank test. All statistical analyzes were performed using the IBM SPSS software package v.25.0, and all p-values of <0.05 were considered statistically significant.

RESULTS

A total of 73 patients, 59 (80.8%) males and 14 (19.2%) females, participated in the study. The mean age of the patients was 74.15±3.23 (range, 70-83) years. Anatomical and wedge resections were performed in 53 (72.6%) and 20 (27.4%) patients, respectively. The number of patients in stage 1A was 14 (19.2%), and in stage 4 was 1 (1.4%). The demographic and clinical characteristics of the patients were summarized in Table 1.

No statistically significant relation in PNI (p=0.354) and NLR (p=0.483) was noted with postoperative complication. Postoperative mortality was also not significantly associated with PNI (p=0.188) and NLR (p=0.465). The comparison of NLR and PNI values according to the characteristics of the patients were shown in Table 2.

Table 1. General characteristics of the patients

Characteristics	n (%)
Gender	
Male	59 (80.8)
Female	14 (19.2)
Smoking	
Present	63 (86.3)
Absent	10 (13.7)
Localization	
Superior lobe	46 (63.0)
Inferior lobe	19 (26.0)
Middle lobe	3 (4.1)
Hiler	5 (6.9)
Resection	
Anatomical resection	53 (72.6)
Wedge resection	20 (27.4)
Surgery	
Lobectomy	43 (58.9)
Pneumonectomy	4 (5.5)
Lung-thoracic wall resection	4 (5.5)
Segmentectomy	2 (2.7)
VATS-wedge resection	20 (27.4)
Cell type	
Squamous cell	24 (32.9)
Adenocarcinoma	42 (57.5)
Others	7 (9.6)
Stage	
1A	14 (19.2)
1B	19 (26.0)
2A	19 (26.0)
2B	5 (6.8)
3A	15 (20.6)
4	1 (1.4)

Table 2. Comparison of NLR and PNI values according to the pre- and post-operative characteristics of the patients

	Smoking History		p
	Present (n=63)	**Absent (n=10)**	
NLR, mean±SD	3.26±1.32	2.03±1.02	**0.013**
PNI, mean±SD	49.45±6.83	52.60±7.79	0.231
	Lymph Node		**p**
	Present (n=9)	**Absent (n=64)**	
NLR, mean±SD	3.02±1.55	3.14±1.32	0.815
PNI, mean±SD	47.27±9.19	50.11±6.69	0.310
	Cell Type		**p**
	Squamous Cell (n=24)	**Adenocarcinoma (n=42)**	**Others (n=7)**
NLR, mean±SD	3.36±1.53	2.99±1.29	3.14±0.91	0.557
PNI, mean±SD	49.99±5.75	50.43±7.53	45.68±6.16	0.248
	Postoperative Complication		**p**
	Present (n=24)	**Absent (n=49)**	
NLR, mean±SD	2.96±1.20	3.20±1.41	0.483
PNI, mean±SD	48.71±6.77	50.37±7.06	0.354
	Postoperative Mortality		**p**
	Present (n=6)	**Absent (n=67)**	
NLR, mean±SD	3.51±1.72	3.09±1.31	0.465
PNI, mean±SD	46.23±5.89	50.16±6.99	0.188

NLR: neutrophil-to-lymphocyte ratio, PNI: prognostic nutritional index, SD: standard deviation

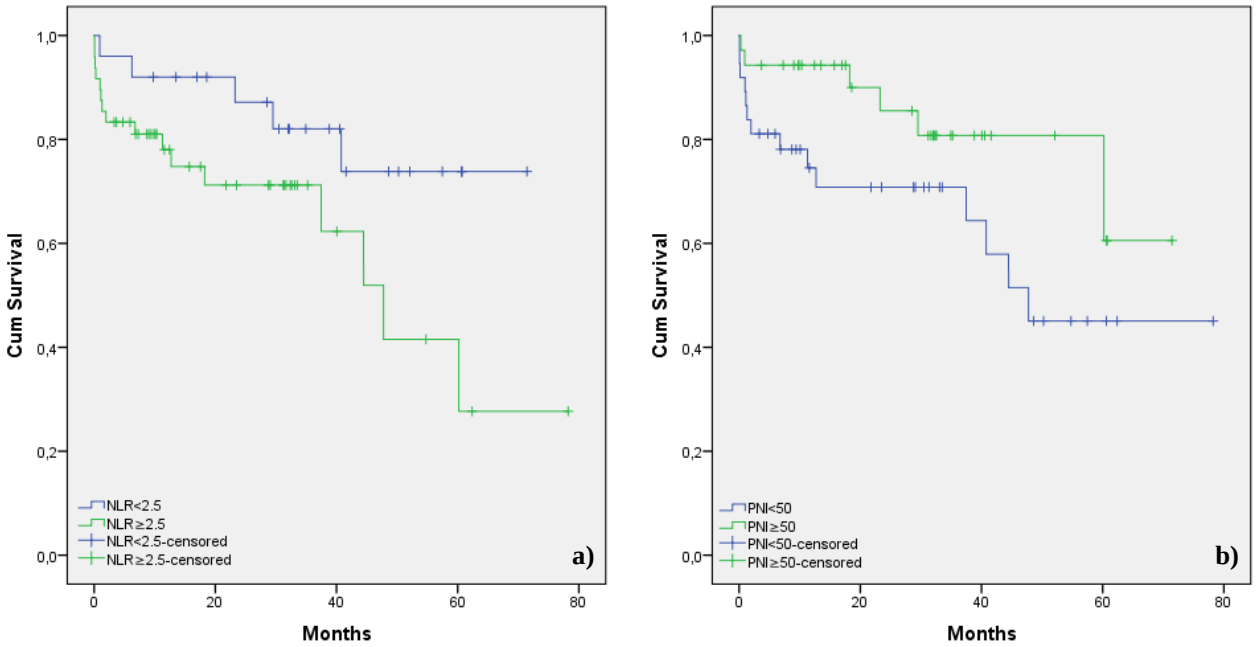

Figure 1. Kaplan-Meier curves for **a)** NLR, and **b)** PNI, NLR: neutrophil-to-lymphocyte ratio, PNI: prognostic nutritional index

For patients with NLR <2.5, 1-, 3-, and 5-year survival rates were at 86.0%, 86.1%, and 78.3%, respectively, while for patients with NLR >2.5, 1-, 3-, and 5-year survival rates were at 76.9%, 65.2%, and 24.8%, respectively (p=0.028, Figure 1a). While in patients with PNI <50, 1-, 3-, and 5-year survival rates were at 73.8%, 63.8%, and 44.6%, respectively, 1-, 3-, and 5-year survival rates were at 92.1%, 86.1%, and 61.2%, respectively, in patients with PNI >50 (p=0.065, Figure 1b).

DISCUSSION

There is growing evidence showing that the patient's nutritional and immunological status is strictly related to the long-standing outcomes of malignant tumors. Both NLR and PNI biomarkers can be calculated with simple blood analysis and can be used as prognostic indicators in determining survival and prognosis in elderly patients. Our findings revealed no significant association both for NLR and PNI values, with neither postoperative complications nor postoperative mortality. A statistically significant

association of NLR value was found with survival rate, while it was not found for PNI.

Albumin is the main protein component of plasma that maintains colloid osmotic pressure, reflecting nutritional status, and its levels tend to decline with age (13,14). Hypoalbuminemia is partly an indicator of the suppressed immune system. Therefore, a low albumin level can be associated with a weakened immune system and low survival. It has been shown that preoperative chronic inflammation and malnutrition could generate a microenvironment favorable to tumor recurrence. Considering elderly patients, chronic inflammation, malnutrition, and comorbidities often accompany the clinical picture (15). A low level of PNI is associated with a lower survival rate in several malignancies (15,16). Lymphocyte count also reflects the activation of the immune system and the inflammatory process, and low levels indicate a weakened immune system (17). The amount of lymph tissue decreases with age, consequently, the number and percentage of lymphocytes are known to decrease (18). Given its inhibitory effect on cytokine production and cytotoxic cell death, low lymphocyte counts can be associated with forming a weak immune reaction against tumor cell destruction (14-19).

In a recent meta-analysis, a low level of PNI indicated a lower survival rate in lung cancer, especially among NSCLC patients (13). In our study, the PNI value had no significant impact on the survival rate of 1-, 3-, or 5-year.

In a study by Kang et al. (20), the assessment of preoperative PNI values of 324 renal cell cancer patients, preoperative PNI values were found associated with both overall survival rate and cancer-specific survival.

In a meta-analysis study by Yang et al. (21), the authors concluded that PNI is a predictive indicator of survival rate and postoperative complications in gastric cancer patients. Albumin, the main element of serum plasma proteins, may reflect nutritional status (22). PNI reflects the host's nutritional and immunological status and can be suggested as a simple, inexpensive, easily calculated measure to predict postoperative complications and survival. In our study, no statistically significant association was observed between PNI values and postoperative complications.

Lymphocytes play an essential role in immunity against cancer, and higher lymphocyte levels are associated with better clinical outcomes. Neutrophils also play an essential role in tumor formation triggered by inflammation, and high neutrophil levels are associated with poor clinical outcomes. An increase in neutrophils inhibits the activity of lymphocytes and other immune cells (7,23,24). It is known that the leukocyte and neutrophil counts are also higher in smokers (25). Smoking is the main risk factor for lung cancer (26) and in our study, we found that NLR was significantly higher in smokers.

In a study among 171 patients diagnosed with stage 4 NSCLC, a high NLR ratio was reported negatively correlated with survival rate. The NLR ratio was suggested to be a simple, inexpensive, and reproducible survival marker (27). Similarly, our findings also revealed the significant association of NLR value with survival rate.

In a study conducted on 388 chemo-naive patients with stage IIIB or IV NSCLC, the authors reported that the pretreatment neutrophil count was significantly associated with overall and progression-free survival rates. A multivariate analysis observed a linear association between pretreatment elevated neutrophil count and shorter overall and progression-free survival. However, the relationship between NLR and overall survival was weak and non-linear (24).

Due to the retrospective single-center design of the study and the relatively small sample size, the potential lack of generalizability is an important limitation of the current study.

CONCLUSION

Our findings revealed a significant association of NLR with survival rate in elderly NSCLC patients. Several parameters are required to perform a stage-independent assessment of the surgical treatment in advanced-age cancer patients. NLR can be considered inexpensive, easily measurable, and reproducible markers that can be incorporated into routine clinical practice for guiding and optimizing treatment decisions in NSCLC patients.

Ethics Committee Approval: The study was approved by the Ethics Committee of Başkent University Institutional Review Board (18.11.2020, KA20/410).

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Evaluation of Nodular Goiter and Papillary Thyroid Cancer Coincidence in Patients with Primary Hyperparathyroidism

Primer Hiperparatiroidili Hastalarda Nodüler Guatr ve Papiller Tiroid Kanseri Birlikteliğinin Değerlendirilmesi

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ABSTRACT

Aim: Primary hyperparathyroidism and differentiated thyroid carcinoma are the most common endocrinological diseases. Since its first definition in the 1950s, nodular goiter and differentiated thyroid carcinoma in patients with primary hyperparathyroidism have been examined in many studies and an increase in cancer incidence has been found. In this study, we aimed to investigate the co-incidence of nodular goiter and differentiated thyroid cancer in patients with primary hyperparathyroidism.

Material and Methods: One hundred seventy-two patients who underwent parathyroid surgery in our hospital between 2012 and 2015 were included in this study. Demographic, clinic, and surgical data of the patients were reviewed retrospectively.

Results: The mean age of the patients was 54.3±11.3 years and 85.5% (n=147) of them were female. Nodular goiter was observed at a rate of 61.0% (n=105) in preoperative evaluation. Parathyroidectomy was performed in 125 (72.7%) and simultaneous total thyroidectomy was performed in 32 (18.6%) and lobectomy in 15 (8.7%) of the patients. Histopathologically, 94.8% (n=163) were interpreted as adenoma, 2.9% (n=5) as parathyroid hyperplasia, and 2.3% (n=4) as parathyroid carcinoma. Patients with papillary thyroid carcinoma (n=30) and benign (n=17) histopathologically were compared in terms of clinical and laboratory characteristics, and no significant difference was observed in any parameter.

Conclusion: There was no significant increase in thyroid nodularity, but a significant increase was found in differentiated thyroid carcinoma incidence in patients with primary hyperparathyroidism in this study in accordance with the literature. This finding highlights the importance of preoperative thyroid evaluation in this patient group.

Keywords: Primary hyperparathyroidism; nodular goiter; thyroid cancer.

ÖZ

Amaç: Primer hiperparatiroidi ve diferansiye tiroid karsinomu en sık görülen endokrinolojik hastalıklardır. İlk tanımlanmış olduğu 1950'li yıllardan bu yana primer hiperparatiroidili hastalarda nodüler guatr ve diferansiye tiroid karsinomu varlığı birçok çalışmada incelenmiş ve kanser insidansında bir artış olduğu saptanmıştır. Bu çalışmada, primer hiperparatiroidili hastalardaki nodüler guatr ve diferansiye tiroid kanseri birlikteliğinin araştırılması amaçlandı.

Gereç ve Yöntemler: Bu çalışmaya hastanemizde 2012 ve 2015 yılları arasında paratiroid cerrahisi yapılmış olan toplam 172 hasta dahil edildi. Hastaların demografik, klinik ve cerrahi verileri geriye dönük olarak incelendi.

Bulgular: Hastaların yaş ortalaması 54,3±11,3 yıl olup %85,5'i (n=147) kadın idi. Hastaların preoperatif değerlendirmesinde %61,0 (n=105) oranında nodüler guatr izlenmiştir. Hastaların 125'ine (%72,7) sadece paratiroidektomi uygulanırken 32'sine (%18,6) eş zamanlı total tiroidektomi ve 15'ine (%8,7) ise eş zamanlı lobektomi uygulanmıştır. Histopatolojik olarak değerlendirilen paratiroid dokularının %94,8'i (n=163) adenom olarak yorumlanırken, %2,9'u (n=5) paratiroid hiperplazisi ve %2,3'ü (n=4) paratiroid karsinomu olarak yorumlanmıştır. Histopatolojik olarak tiroid karsinomu saptanan (n=30) hastalar ile benign saptanan (n=17) hastalar klinik ve laboratuvar özellikleri yönünden karşılaştırılmış olup hiçbir parametrede anlamlı bir farklılık izlenmemiştir.

Sonuç: Primer hiperparatiroidili hastalarda tiroid nodülaritesinde anlamlı bir artış olmamakla birlikte, bu çalışmada literatürle uyumlu şekilde diferansiye tiroid kanseri insidansında anlamlı bir artış saptanmıştır. Bu bulgu da bu hasta grubunda preoperatif olarak yapılan tiroid dokusu değerlendirmesinin önemini vurgulamaktadır.

Anahtar kelimeler: Primer hiperparatiroidi; nodüler guatr; tiroid kanseri.

INTRODUCTION

Primary hyperparathyroidism is one of the most common endocrinological diseases currently. Its frequency in the population varies between 0.1-0.4%. The frequency increases with age and is most commonly detected in the fifth decade, and much more common in females than males. The cause is a single adenoma in 80-85% of patients and its treatment is surgical excision (1-4).

In the past, more invasive explorative surgeries were preferred as surgical approaches. However, nowadays, minimally invasive approaches have come to the fore, as the rate of single adenoma is significantly higher, the incidence of parathyroid cancer is very rare, and adenomas can be detected at a much higher rate with the development of preoperative imaging techniques. Therefore, the most common method preferred in parathyroid surgery today is minimally invasive parathyroid surgery. With the minimally invasive approach, the rates of neurological complications such as loss of voice have been greatly reduced (5-7).

One of the foremost and widely used imaging methods in preoperative evaluation is neck ultrasound (US). Neck US performed in experienced hands is very sufficient to accurately detect the localization of parathyroid pathologies, it can also detect accompanying thyroid pathologies early and accurately (8,9).

The rate of concomitant thyroid nodularity in primary hyperparathyroidism varies between 15-75% according to the literature (3,10). This frequency rate is similar to the nodular goiter seen in the normal population. Today, with the widespread use of neck US, the rate of nodule detection in the thyroid has increased to 70% (11). However, according to studies the incidence of thyroid malignancy in primary hyperparathyroidism is between 2-29.8% and it's higher than the normal population (3,10,12). There is a known association between primary hyperparathyroidism and medullary thyroid cancer in Multiple Endocrine Neoplasia-2 (MEN-2) Syndrome. However, the most common thyroid malignancy in patients with sporadic primary hyperparathyroidism is differentiated thyroid cancer (12).

Differentiated thyroid carcinoma is one of the most common malignancies and about 80-85% of the cases consist of papillary thyroid carcinoma (PTC). In recent years, with the increase in the accessibility and quality of neck US and other imaging modalities, there has been an increase in the rate of diagnosis of occult thyroid carcinoma, but also there has been a parallel increase in the general incidence and mortality of thyroid cancer (11,13,14).

The association of primary hyperparathyroidism and differentiated thyroid cancer was first described by Ogburn and Black in 1956 (15). Since then, many studies have been conducted on primary hyperparathyroidism and differentiated thyroid carcinoma, and in these studies, it has been found that primary hyperparathyroidism changes the frequency and characteristics of differentiated thyroid cancer.

In this study, we aimed to investigate the rates of concomitant benign and malignant thyroid nodules and differentiated thyroid carcinoma in our patients with primary hyperparathyroidism who underwent parathyroid surgery in our center.

MATERIAL AND METHODS

Patients with primary hyperparathyroidism, over the age of 18, who underwent surgery in our center between January 2012 and 2015 were included in the study. The data of the patients were scanned retrospectively from the online data system of our center. The diagnosis of primary hyperparathyroidism was made by high serum calcium (Ca) values accompanied by high or inappropriately normal parathyroid hormone (PTH) levels. After scanning the demographic data, routine laboratory tests and imaging required by their diseases were performed. Fine-needle aspiration biopsy (FNAB) was performed on appropriate nodules from patients with thyroid nodularity according to neck US results. In line with the examination and FNAB results, the patients with parathyroid surgery indications were evaluated and subjected to a surgical procedure suitable for accompanying thyroid pathologies.

Patients with any syndromic condition such as MEN-2 syndrome that will affect the frequency of thyroid nodules or cancer, with a history of radiotherapy to the neck, a familial history of thyroid carcinoma, chronic renal failure, and active thyroid cancer or any other active malignancy and pregnant women were excluded from the study.

Laboratory Analysis and Imaging

Blood was taken from the patients by venous route in the morning after an overnight fast for laboratory analysis. Ca and phosphorus (P) levels were studied with the colorimetric method, thyroid stimulating hormone (TSH) and anti-thyroid peroxidase (anti-TPO) levels were studied with chemiluminescence microparticle immunological method, PTH levels were studied with intact chemiluminescence immunoassay method and 25-OH-D3 levels were studied with radioimmunoassay method in Abbott Architect I 2000 SR® device. Twenty-four-hour urinary Ca levels were studied and recorded by atomic absorption spectrophotometry method.

Neck US was performed by experienced Endocrinology and Metabolic Diseases Specialists with a 13 mHz linear probe B Mode High-Resolution USG device (Logic 9, General Electric USA®). In ultrasonographic evaluation; the number of parathyroid adenoma, its detailed location, and dimensions in all three planes, borders, echogenicity, Doppler blood supply, and relationship with surrounding tissues were defined. In addition; the size, echogenicity, Doppler blood supply of the thyroid tissue in all three planes, detailed location, diameters, borders, echogenicity, and ultrasonographic features (cystic content, spongioform appearance, microcalcification, macrocalcification foci, etc.) and Doppler blood supply of the nodules were specified. Planar images of 99mTc-MIBI sestamibi (methoxy-isobutyl-isonitrile) were obtained using a single-headed Siemens E-Cam gamma camera with a low-energy high-resolution collimator positioned as close to the neck region as possible. Early images were taken 15 minutes after MIBI application from anterior, left, and right anterior oblique angles (Thyroidal phase). Late images were obtained between 90-120 minutes (Parathyroidal phase). Cases that could not be visualized with the two basic imaging methods were referred to further imaging methods.

Fine-Needle Aspiration Biopsy and Surgical Procedure

Indications for performing FNAB were determined according to the American Thyroid Association's 2015 guidelines and the pathologic evaluations and classifications

were made according to the Bethesda system (11,16). Surgical procedures were performed by our experienced surgeons in the General Surgery Department of our center. Parathyroid surgery indication was determined according to the Fourth International Workshop 2014 Endocrine Society Guidelines criteria (17).

Video-assisted minimally invasive parathyroid surgery was applied as a standard procedure to the patients whose adenoma was localized preoperatively and no additional thyroid pathology that would require surgery was detected. Simultaneous thyroid surgery together with parathyroid surgery was planned for the patients with thyroid nodularity detected in preoperative imaging and FNAB results with Bethesda class III and above, those with nodules that are unsuitable for sampling but may be risky for malignancy, and those with plunging and retrosternal goiter. Lobectomy with adenomectomy was performed in unilateral suspicious nodules, and total thyroidectomy with adenomectomy procedure was performed in patients with bilateral multinodular goiter.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics software, Version 22. The normality of the distribution of continuous variables was determined using the Kolmogorov-Smirnov test. Categorical variables were described as frequencies and percentages. Categorical features and relationships between groups were assessed using an appropriate chi-square test. The continuous variables were expressed as mean and standard deviation or median, interquartile range, and minimum-maximum, depending on the normality of their distribution. The Mann-Whitney U test was used to compare the variables that were not normally distributed. On the other hand, the Student's t test was used to compare the variables with normal distribution. To determine if predictor factors, such as particular test results and demographic information, substantially predicted thyroid cancer, logistic regression was used. The statistically significant two-tailed p-value was considered as <0.05.

RESULTS

A total of 172 patients, all of whom underwent parathyroidectomy were included in the study. The mean age was 54.3±11.3 years and 147 (85.5%) of the patients were female. It was observed that parathyroid adenoma was located most frequently in the lower right (n=71, 41.3%) and lower left (n=68, 39.5%) locations, respectively. According to the preoperative US, goiter was observed in 105 (61.0%) of the patients, and the mean thyroid volume was found to be 14.4±10.5 cm^3. Mean serum Ca and P levels were 11.1±0.7 mg/dL, and 2.6±0.5 mg/dL, and the median PTH level was 179 pg/dL, respectively (Table 1).

Minimally invasive parathyroidectomy was performed in 125 (72.7%) of the patients. However simultaneous parathyroidectomy and total thyroidectomy were performed in 32 (18.6%) and simultaneous parathyroidectomy and unilateral lobectomy were performed in 15 (8.7%) patients. Histopathologically 94.8% (n=163) were interpreted as parathyroid adenoma, 2.9% (n=5) as parathyroid hyperplasia, and 2.3% (n=4) as parathyroid carcinoma. PTC was detected in 30 (63.8%) of 47 patients who underwent thyroidectomy, corresponding to 17.4% of the total patient group (Table 2).

According to tumor localizations, it was determined that 46.7% (n=14) was single-focus left lobe, 23.3% (n=7) single-focus right lobe, 20.0% (n=6) bilateral, 6.7% (n=2) multifocal right lobe, and 3.3 % (n=1) multifocal left lobe. All of the corresponding nodules (100%) were of solid hypoechoic nature in the preoperative US in the cases with thyroid carcinoma. In FNAB, 22.2% (n=6) of the nodules were reported as malignant cytology, 18.5% (n=5) were benign, 48.2% (n=13) were atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), and 3.7% (n=1) were as follicular neoplasia (Table 3).

Table 1. Baseline clinical and laboratory characteristics of the patients (n=172)

Age (years)	54.3±11.3
Gender, n (%)	
Female	147 (85.5)
Male	25 (14.5)
Body mass index (kg/m^2)	30.8±6.5
Smoking, n (%)	30 (17.4)
Tumor localization, n (%)	
Right superior	7 (4.1)
Right inferior	71 (41.3)
Left superior	12 (7.0)
Left inferior	68 (39.5)
Double adenoma	4 (2.3)
Others (Ectopic)	10 (5.8)
Goiter, n (%)	105 (61.0)
Thyroid volume (cm^3)	14.4±10.5
Adenoma volume (cm^3)	0.49 (0.24-0.98) [0.03-17.70]
TSH (mIU/L)	1.84±1.24
Anti-TPO positivity, n (%)	28 (16.3)
Serum creatinine (mg/dL)	0.7 (0.6-0.8) [0.5-1.5]
Albumin (gr/L)	4.5±0.3
Preoperative serum iPTH (pg/dL)	179 (126-252) [68-1727]
Corrected calcium (mg/dL)	11.1±0.7
Urinary calcium (mg/24-hour)	370±188
Phosphorus (mg/dL)	2.6±0.5
25-hydroxy vitamin D3 (ng/ml)	10 (6-17) [4-150]
Renal stone, n (%)	58 (33.7)
Osteoporosis, n (%)	82 (47.7)

TSH: thyroid stimulating hormone, Anti-TPO: anti-thyroid peroxidase, iPTH: intact parathyroid hormone, descriptive statistics for continuous variables were expressed as mean±standard deviation or as median (interquartile range, 25^{th}-75^{th} percentile) [minimum-maximum] depending on the normality of their distribution

Table 2. Surgery and histological results (n=172)

Surgery, n (%)	
Parathyroidectomy	125 (72.7)
Parathyroidectomy+total thyroidectomy	32 (18.6)
Parathyroidectomy+lobectomy	15 (8.7)
Histopathological findings for thyroid, n (%)	
Benign	142 (82.6)
Malignant	30 (17.4)
Histopathological findings for parathyroid, n (%)	
Adenoma	163 (94.8)
Hyperplasia	5 (2.9)
Carcinoma	4 (2.3)

Table 3. Preoperative fine needle aspiration results of cases with malignant pathology results (n=27)

Fine needle aspiration, n (%)	
Nondiagnostic	2 (7.4)
Benign	5 (18.5)
AUS/FLUS	13 (48.2)
Follicular neoplasm or suspicious	1 (3.7)
Malignant	6 (22.2)

AUS/FLUS: atypia of undetermined significance/follicular lesion of undetermined significance

Patients with thyroid carcinoma (n=30) and benign pathology (n=17) were compared in terms of clinical and laboratory characteristics, and no significant difference was observed in any of the parameters (Table 4). Age, gender, thyroid volume, parathyroid adenoma volume, urinary Ca level, serum Ca, and serum 25-OH-D3 levels which may have an impact on the development of thyroid cancer in patients with parathyroid adenoma, were analyzed by univariate regression analysis. It was determined that only thyroid volume had a significant effect (OR: 1.06, 95% CI: 1.016-1.101, p=0.006).

DISCUSSION

Primary hyperparathyroidism is the third most common endocrinological disease after thyroid diseases and diabetes mellitus (1,12). Differentiated thyroid carcinoma is also one of the most common malignancies currently and the most common thyroid malignancy in patients with primary hyperparathyroidism is PTC (12). Given the worldwide high prevalence of both diseases, the possibility of one inducing the other and increasing its frequency is of interest.

In our study, 85.5% (n=147) of the patients who underwent parathyroidectomy were female and the mean age was 54.3±11.3 years. In our patient group, we found nodular goiter in 61.0% (n=105) and PTC in 17.4% (n=30).

The female/male ratio and mean age in our study are similar to the studies in the literature (3,12,18,19). Although differentiated thyroid carcinoma can be seen at younger ages, the age of occurrence of sporadic primary hyperparathyroidism is mostly in the fifth decade, and the average female-to-male ratio is around 7-8/1 (1,3). Our study is compatible with the literature in this respect.

Since its first definition in 1956, there have been numerous studies in the literature examining the frequency and characteristics of nodular thyroid disease and differentiated thyroid cancer in primary hyperparathyroidism. According to the general results of these studies, the incidence of nodular goiter in primary hyperparathyroidism was roughly the same as in the general population, while the frequency of PTC was found to be increased. In the study of Vargas-Ortega et al. (3) the rate of PTC in 59 patients who underwent simultaneous parathyroid and thyroid surgery was found to be 20.3%, similar to our study. Also in the study of Hu et al. (20), the PTC rate was found 22% compatible with our study. In the study of Liu et al. (12) the rate of nodular goiter in 304 patients with hyperparathyroidism was found to be 61.5%, similar to ours, but the rate of PTC was found to be 29.9%, slightly higher than ours. In two studies conducted in Türkiye, the rates of concomitant nodular goiter in patients with hyperparathyroidism were found to be 42% and 55.6%, and PTC rates of 11.3% and 10.2% (18,21). In another study, the nodularity rate was found to be 53.6%, and the PTC rate was 13.6% (2). In our study, the frequency of nodular goiter and papillary cancer in patients with primary hyperparathyroidism is consistent with the literature.

When we compared the patients with PTC with those who were with benign cytology, we found no difference in terms of demographic characteristics and clinical parameters. In three studies, two of which were conducted in Türkiye, no significant difference was found between benign and malignant groups in patients with hyperparathyroidism in terms of age, gender, preoperative PTH, Ca, P, 25-OH-D3, 24-hour urinary Ca levels (3,21,22) compatible to our study. In the study of Celik et al. (22) also anti-TPO levels were compared and no significant difference was found similar to our study. In

Table 4. Comparison of clinical and laboratory characteristics of patients with and without thyroid carcinoma

	Thyroid Carcinoma		**p**
	Yes (n=30)	**No (n=17)**	
Age (years)	54.5±10.2	58.5±12.2	0.227
Gender, female, n (%)	25 (83.3)	15 (88.2)	0.650
Smoking, n (%)	6 (20.0)	2 (11.8)	0.470
Body mass index (kg/m^2)	30.5±6.0	30.9±4.0	0.793
Thyroid volume (cm^3)	20.3±10.4	20.8±22.2	0.912
Adenoma volume (cm^3)	0.48 (0.28-0.81) [0.08-3.49]	0.57 (0.38-3.12) [0.04-8.21]	0.285
TSH (mIU/L)	1.65±1.12	1.27±0.86	0.239
Anti-TPO positivity, n (%)	4 (13.3)	1 (5.9)	0.426
Serum creatinine (mg/dL)	0.60 (0.57-0.80) [0.50-1.50]	0.70 (0.60-0.90) [0.50-1.40]	0.370
Preoperative serum iPTH (pg/dL)	195 (122-289) [74-830]	205 (130-420) [94-645]	0.580
Corrected calcium (mg/dL)	11.0±0.7	11.3±0.8	0.132
Urinary calcium (mg/24-hour)	339±175	366±138	0.609
25-hydroxy vitamin D3 (ng/ml)	8.5 (7.0-20.8) [4-63]	12.0 (6.5-17.0) [4-20]	0.764

TSH: thyroid stimulating hormone, Anti-TPO: anti-thyroid peroxidase, iPTH: intact parathyroid hormone, descriptive statistics for continuous variables were expressed as mean±standard deviation or as median (interquartile range, 25^{th}-75^{th} percentile) [minimum-maximum] depending on the normality of their distribution

the other two studies, lower preoperative Ca and PTH levels were found in the PTC group compared to the benign group (12,20). In a study conducted in Korea, 25-OH-D3 levels were found to be significantly lower in patients with hyperparathyroidism accompanied by PTC compared to those with only hyperparathyroidism (23).

In our study, the rate of patients who underwent simultaneous parathyroid-thyroid surgery was similar to other studies. Preoperative FNAB was performed in 57% of patients scheduled for simultaneous thyroid parathyroid surgery. Our preoperative FNAB and simultaneous thyroid surgery rates are similar to the study of Haciyanli et al (21). In this study, 26.3% of the patients were found with malignant cytology by preoperative FNAB, while this rate remained at 12.7% in our study. An average of 5% of thyroid nodules are malignant. Preoperative FNAB is the best method to detect differentiated thyroid cancer, but it is known in the literature that the false-negative rate can reach up to 40% in patients with primary hyperparathyroidism (19,21,24).

The rate of thyroid nodularity in patients with primary hyperparathyroidism was similar to the general population in the literature but it was observed that differentiated thyroid cancer rate is increased in these patients. Although it is thought that incidentally detected occult tumors may increase this frequency while imaging related to primary hyperparathyroidism is performed, some recent studies have also found an increase in invasiveness in PTC in patients with primary hyperparathyroidism (12,25,26).

The reason why the risk of differentiated thyroid cancer is increased in patients with primary hyperparathyroidism has not yet been fully clarified. Common embryological origin of the thyroid and parathyroid tissue and the commonly shared effect of numerous genes and transcription factors come to the fore. In addition, it is speculated that chronically high PTH and Ca, and low 25-OH-D3 levels increase the levels of many growth factors such as insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF), and thus have a mitogenic effect on thyroid follicular cells (18,25-29). Larger studies are needed to understand the definite effect of hyperparathyroidism on differentiated thyroid cancer.

Our study has some limitations. It is a retrospective and single-center study with a limited number of patients, and there is no separate PTC control group comparing papillary tumor characteristics. Nevertheless, in our study, we investigated the frequency of nodular goiter and differentiated thyroid cancer in our primary hyperparathyroidia patients operated in our tertiary center. In accordance with the literature, the rate of nodularity was found to be 61.0% and the rate of PTC was found to be 17.4%. As a tertiary center where selected patients are referred and operated on, we think that our study, together with a few studies from Türkiye, contributes to the literature.

CONCLUSION

Although there is no increased rate of thyroid nodularity in patients with primary hyperparathyroidism compared to the population, the frequency of differentiated thyroid cancer is defined as 2-29% in the literature. In our study, the nodularity rate was found to be close to the norm at 61.0%, but the frequency of papillary tumors was found to be 17.4%, more common than the normal population, but consistent with the literature. The pathogenetic mechanisms by which hyperparathyroidism increases the frequency of differentiated thyroid cancer are not yet certain, but it is now certain that it does. Therefore, it is important to make appropriate preoperative thyroid imaging and sampling in patients with hyperparathyroidism and to plan appropriate simultaneous thyroid surgeries correctly both in terms of early detection of thyroid cancer and in protecting the patient from unnecessary re-surgeries.

Ethics Committee Approval: The study was approved by the Ethics Committee of Dışkapı Yıldırım Beyazıt Training and Research Hospital (23.03.2015, 21/03).

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Management of Perineal Epidermoid Cyst in a 20-year-old Female

20 Yaşında Bir Kadında Perineal Epidermoid Kist Tedavisi

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ABSTRACT

Epidermoid cysts are cutaneous or subcutaneous masses caused by the implantation of epidermal elements into the dermis. This case report presented an epidermal cyst of the perineum clinically mimicking endometriosis in a female patient. A 20-year-old virgo female patient was admitted to our clinic with complaints of severe pain, especially in the right leg, and postmenstrual bleeding for three years. In the transrectal ultrasound, a 54*39 mm cystic lesion with dense internal echo was detected in the medial neighborhood of the pelvic floor right obturator internus muscle. Since the cystic lesion detected in the contrast-enhanced pelvic MRI was evaluated as an epidermoid cyst and no significant endometrioma or endometriosis focus was detected, an operation was planned. Surgical excision should be performed not only for symptom relief and diagnosis but also to exclude rare malignancy. However, it is vital to master the pelvic and perineal anatomy along with careful surgery.
Keywords: Epidermoid cyst; perineum; surgery.

ÖZ

Epidermoid kistler, epidermal elemanların dermise implantasyonundan kaynaklanan deri veya deri altı kitlelerdir. Bu vaka raporunda, bir kadın hastada, klinik olarak endometriozisi taklit eden bir perine epidermal kisti sunulmaktadır. 20 yaşında virgo kadın hasta kliniğimize üç yıldır devam eden özellikle sağ bacakta şiddetli ağrı ve adet sonrası kanama şikayeti ile başvurdu. Transrektal ultrasonda, pelvik taban sağ obturator internus kas medial komşuluğunda 54*39 mm boyutlarında yoğun internal ekolu kistik lezyon saptandı. Kontrastlı pelvik MRG'de saptanan kistik lezyonun ön planda epidermoid kist olarak değerlendirilmesi ve belirgin bir endometrioma veya endometriozis odağı saptanmaması üzerine operasyon planlandı. Cerrahi eksizyon sadece semptomların giderilmesi ve tanı için değil, aynı zamanda nadir görülen maligniteyi dışlamak için de yapılmalıdır. Ancak, dikkatli cerrahi ile birlikte pelvik ve perineal anatomiye hâkim olmak hayati önem taşır.
Anahtar kelimeler: Epidermoid kist; perine; cerrahi.

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INTRODUCTION

Epidermoid cysts are cutaneous or subcutaneous masses caused by the implantation of epidermal elements into the dermis (1). The cysts are usually caused by the invagination of the keratinized squamous epithelium within the dermis. Epidermoid cysts are typically slow growing and solitary. Their size can vary from a few millimeters to several centimeters (2). These cysts are usually located on the face, scalp, neck, and trunk, and formations on the perineum are rare. Epidermoid cysts are much rarer in women than in men (3). In the differential diagnosis of perineal cysts,

abscess, pilonidal cyst, Gardner canal cysts, trauma, perianal dermatoses including anal canal cysts, benign teratomas, teratomas with malignant transformation, skin cancer, and endometriosis should be considered (4). This case report presented an epidermal cyst of the perineum clinically mimicking endometriosis in a female patient.

CASE REPORT

A 20-year-old virgo female patient was admitted to our clinic with complaints of severe pain, especially in the right leg, and postmenstrual bleeding for three years. In the transabdominal ultrasound performed on the patient, who was started on dienogest (Visanne) and later on estradiol valerate and dienogest combination (Qlarista) with a preliminary diagnosis of endometriosis in an external center, the uterus and ovaries were observed in a natural appearance. In the transrectal ultrasound performed with the patient's consent, a cystic lesion measuring 54*39 mm with intense internal echo was detected on the pelvic floor, in the medial neighborhood of the obturator internus muscle on the right. When the patient's CA125 value was 10.3 and CA19-9 value was 8.46, the cystic lesion identified in the contrast-enhanced pelvic magnetic resonance imaging (MRI) was evaluated as an epidermoid cyst in the foreground, and no significant endometrioma or endometriosis focus was detected (Figure 1). The operation was planned for the patient with the decision of the council consisting of the gynecology, general surgery, and radiology teams. Necessary consent was obtained from the patient before the operation.

After cleaning the abdomen and perineum, the patient was prepared in the lithotomy position and covered with sterile drapes. In the vaginal examination, a cyst of approximately 4-5 cm in size was palpated on the right lateral wall of the vagina. In the rectal examination performed by the general surgeon, it was determined that the cyst was distant from the rectum and anal sphincter and was not related. Therefore, it was decided to remove the cyst through the vaginal route. A longitudinal incision was made from the right lateral wall of the vagina. The cyst wall was reached by cutting the vaginal mucosa and paravaginal fascia, respectively. The cyst was separated from the surrounding tissues by blunt and sharp dissections. The cystic tissue was removed completely without any rupture (Figure 2). There was no active bleeding area in the cyst bed in the observation. The operation was terminated by primary suturing of the paravaginal fascia and vaginal mucosa.

The patient was discharged on the first postoperative day, as her general condition was good.

In the examination made by the pathologists, it was stated that when the cyst material with a size of 6*5*3 cm, gray-brown, with soft elastic consistency, was opened, the gray-colored mash-like material was discharged. The final pathology result was reported as an epithelial inclusion cyst (keratinous cyst) (Figure 3).

Written informed consent was obtained from the patient for publication and images.

DISCUSSION

Epidermoid cysts are benign, slow-growing lesions that develop from the epidermal layer of the skin, which are much rarer in women than in men. They are most common in the third and fourth decades of life. The cysts usually occur on the face, scalp, neck, and trunk but are rarely reported in the perineum. Mechanical pressure or minor trauma can cause epidermoid cyst development (5). The cyst can become inflamed or infected, causing pain, tenderness, and a foul-smelling cheese-like discharge. However, in a rare case of malignancy, rapid growth and

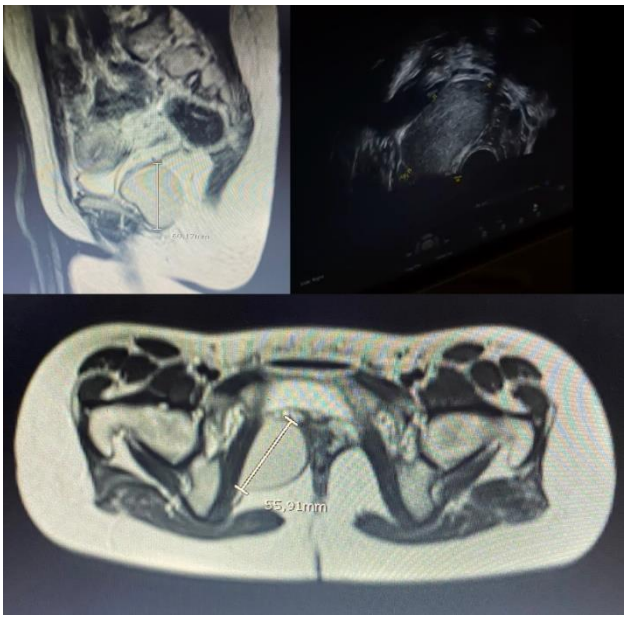

Figure 1. Transrectal ultrasound and magnetic resonance imaging of the epidermoid cyst

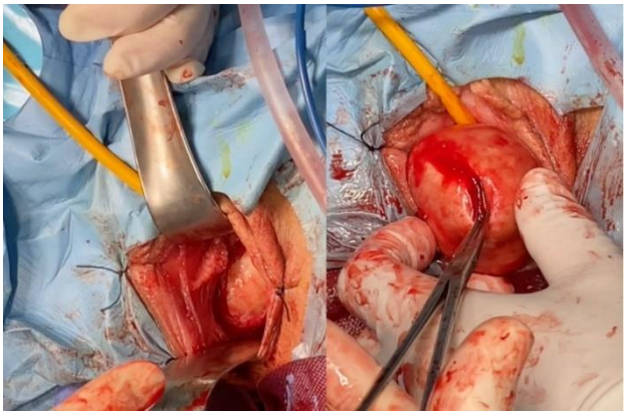

Figure 2. Surgical excision of the epidermoid cyst in the perineal region

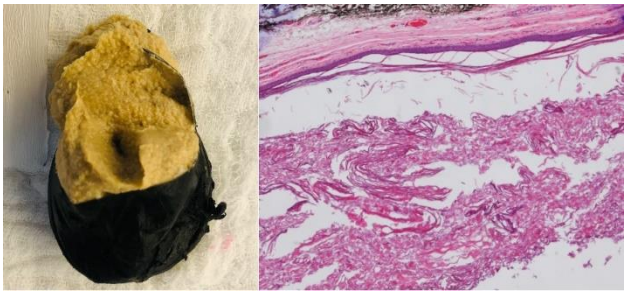

Figure 3. Macroscopic and microscopic view of epidermoid cyst

bleeding have also been reported (6). In female patients, epidermal cysts in the perineum may displace the anus and vagina and extend into the pelvic cavity adjacent to the rectum (7). Ultrasound, MRI, and CT can be used as imaging modalities in these patients. Imaging provides an idea about the relationship of this cyst in the perineum with neighboring anatomical structures and is very effective in managing the operation. Treatment of perineal epidermoid cyst is surgical excision with careful and meticulous dissection, taking precautions to prevent spillage of the contents and also not to damage nearby vital structures such as the perineal urethra and anal canal (3). Epidermoid cyst of the perineum has been reported rarely in a female patient in the literature (8-10). These patients are elderly female patients. In the literature, a case of clitoral epidermoid cyst has been reported in 2 patients aged 16 and 22 years (11,12). Epidermoid cysts can occur as vulvar lesions on the labia major and labia minora, and often large cases without a history of trauma have been reported. An 11 cm epidermoid cyst in the labia majora was presented in a 17-year-old girl with no history of trauma who complained of a painful and palpable mass (13). Gardner's duct cysts should also be considered in the differential diagnosis of perineal epidermoid cysts. Most of the time, the basic treatment for these cysts, which can be easily diagnosed by pelvic examination, is excision, which includes the complete removal of the cyst capsule. There are case series involving benign vaginal cyst excisions in the literature (14).

To the best of our knowledge, no case of epidermoid cyst has been reported in a young patient with a localization similar to our case. Surgical excision should be performed not only for symptom relief and diagnosis but also to exclude rare malignancy. However, it is vital to master the pelvic and perineal anatomy along with careful surgery. In this case, we presented our own surgical management of a rare epidermoid cyst of the perineum.

Informed Consent: Written informed consent was obtained from the patient for publication and accompanying images.

Conflict of Interest: None declared by the authors.

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Author Contributions: Idea/Concept: OŞ; Design: GK; Data Collection/Processing: FBT; Analysis/Interpretation: ÇÇ; Literature Review: FBT; Drafting/Writing: FBT, BG; Critical Review: OŞ.

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Yamaguchi Syndrome: A Difficult Diagnosis in the Differential Diagnosis of Acute Coronary Syndrome

Yamaguchi Sendromu: Akut Koroner Sendrom Ayırıcı Tanısında Zor Bir Tanı

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ABSTRACT

Apical hypertrophic cardiomyopathy (ApHCM) (Yamaguchi Syndrome) with hypertrophy of the ventricular apex constitutes 8% of the hypertrophic cardiomyopathies (HCMs). ApHCM can cause ventricular malignant dysrhythmias, atrial fibrillation, and ischemic chest pain. Definitive diagnosis is made by electrocardiography (ECG) and transthoracic echocardiography. A 73-year-old male patient was admitted to the emergency department with chest pain. The patient's vital signs were within the normal range. In the ECG, there were giant negative T wave in leads V4-5-6, 0.5 mm ST segment depression, and left ventricular hypertrophy in the inferior derivations. The left ventricular apex thickness was measured as 14 mm (reference range: 6-11). Although the HEART score was 4, the preliminary diagnosis of the patient was determined as ApHCM. Beta-blocker and antiplatelet therapy were started. The mortality and morbidity rates of ApHCM are higher among HCMs. Clinicians should be aware of such ECG and echocardiography findings to prevent possible morbidity and mortality.
Keywords: Apical; hypertrophic cardiomyopathy; Yamaguchi syndrome; acute coronary syndrome; echocardiography.

ÖZ

Ventriküler apeksin hipertrofisi ile seyreden apikal hipertrofik kardiyomiyopati (ApHKM) (Yamaguchi Sendromu) hipertrofik kardiyomiyopati (HKM)'lerin %8'ini oluşturmaktadır. ApHKM ventriküler malign disritmilere, atriyal fibrilasyona ve iskemik göğüs ağrısına neden olabilir. Kesin tanı elektrokardiyografi (EKG) ve transtorasik ekokardiyografi ile konur. 73 yaşında bir erkek hasta göğüs ağrısı şikayetiyle acil servise başvurdu. Hastanın vital bulguları normal sınırlardaydı. EKG'de V4-5-6 derivasyonlarında dev negatif T dalgası, 0,5 mm ST segment depresyonu ve inferior derivasyonlarda sol ventrikül hipertrofisi vardı. Sol ventrikül apeks kalınlığı 14 mm (referans aralığı: 6-11) olarak ölçüldü. HEART skoru 4 olmasına rağmen hastanın ön tanısı ApHKM olarak belirlendi. Beta-bloker ve antiplatelet tedavi başlandı. HKM'ler arasında ApHKM'nin mortalite oranları daha yüksektir. Klinisyenler olası morbidite ve mortaliteyi önlemek için bu tür EKG ve ekokardiyografi bulgularının farkında olmalıdır.
Anahtar kelimeler: Apikal; hipertrofik kardiyomiyopati; Yamaguchi sendromu; akut koroner sendrom; ekokardiyografi.

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease that occurs as a result of mutation of sarcomere proteins in the myocardium (1). Unlike classical HCM, cardiomyopathies with hypertrophy of the ventricular apex are called apical HCM (ApHCM). ApHCM constitutes 8% of all HCMs (2). In the Asian population, up

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to 25% of all HCMs are ApHCM, while it is 1-10% in the non-Asian population (3). ApHCM's typical features were first described by Sakamoto and Yamaguchi in 1976 (2). ApHCM, also known as Yamaguchi Syndrome, is clinically associated with widespread giant negative T waves (≥1 mV, ≥10 mm) and high voltage QRS complex in the precordial derivations (4). In definitive diagnosis, the apical wall thickness is expected to be ≥15 mm and the ratio of maximal apical wall thickness to the posterior wall thickness is expected to be ≥1.5 (2). In recent studies, apical wall thickness ≥13 mm was accepted as a diagnostic criterion (5). Its echocardiographic findings are hypertrophy in the apical myocardium and a "spadelike" configuration of the left ventricular cavity at the end of diastole (5). Compared with classical HCM, ApHCM causes atrial fibrillation more frequently and the sudden cardiac death rate is higher, 0.5-4% vs 1.3% (3,6). For this reason, early diagnosis of ApHCM will prevent possible mortality and morbidity. ApHCM has different clinical presentations. The most common symptom is chest pain. It causes an acute coronary syndrome-like condition in which there is T wave negativity in the precordial derivations accompanied by chest pain. In the preliminary diagnosis of ApHCM, a thorough evaluation of the patient by a cardiologist and a comprehensive echocardiography can confirm the diagnosis and prevent potential morbidity and mortality.

CASE REPORT

A 73-year-old male was admitted to the emergency department with the complaint of intermittent chest pain that started 3 hours before admission and the pain was increased with breathing. The patient did not have any accompanying disease in his past medical history and had a 60-pack-year smoking history. The vital signs of the patient were in the normal range at admission (blood pressure: 140/80 mm Hg, pulse: 75 beats/min, respiratory rate: 20/min, temperature: 36.7 ºC, saturation: 94%). There was not any pathological finding in the cardiac examination of the patient. In 12-lead electrocardiography (ECG), there were signs of giant negative T wave in leads V4-5-6, 0.5 mm ST segment depression, and left ventricular hypertrophy in the inferior derivations (Figure 1). The serum troponin I values at the beginning, 3rd and 6th hour were 11.1, 10.1, and 15.6 ng/L (reference range: 14-42.9), and the CK-MB values were 2.7, 2.8, 2.5 µg/L (reference range: 0.6-6.3), respectively. The HEART score was calculated as 4 (History:0, ECG:1, Age:2, Risk factors:1, Troponin:0). In bedside echocardiography performed by an emergency physician, left ventricular apex thickness was measured as 14 mm (reference range: 6-11) and the ejection fraction was within normal values. (Figure 2). The ECG and echocardiography findings were found to be significant in terms of "Yamaguchi Syndrome". The patient was consulted by the cardiologist. Antiplatelet and beta-blocker therapy was started as a medical treatment and medical follow-up was recommended by the cardiologist. During the outpatient follow up cardiac magnetic resonance imaging was performed, and it revealed hypertrophy at the apex of the left ventricle, and the diagnosis was confirmed (Figure 3).

The patient consented to the use of his medical data for scientific purposes.

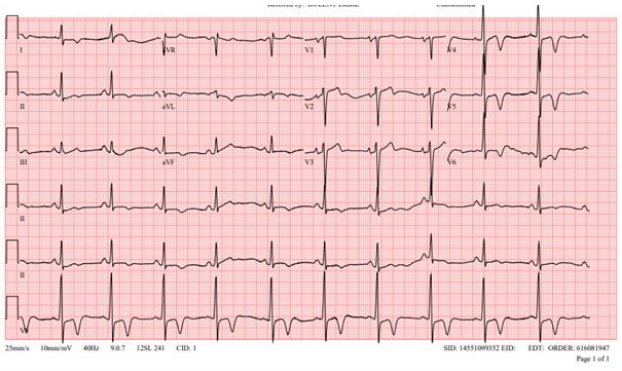

Figure 1. Giant negative T waves in Yamaguchi syndrome

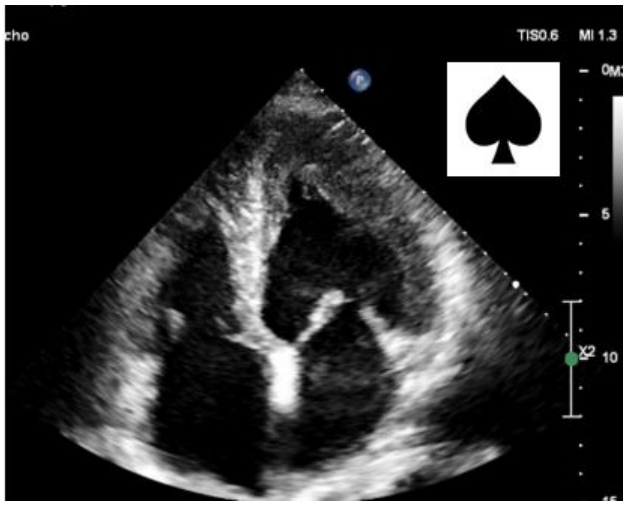

Figure 2. "spadelike" configuration of the left ventriculi in Yamaguchi syndrome

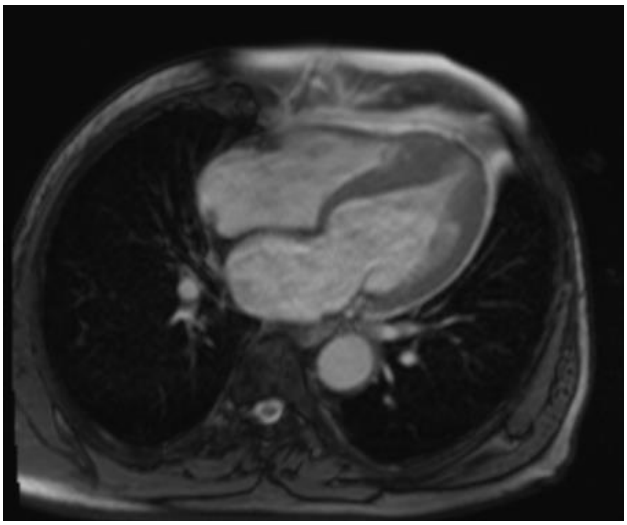

Figure 3. Magnetic resonance imaging of Yamaguchi syndrome

DISCUSSION

In contrast to the diffuse hypertrophy seen in HCM, wall thickening in ApHCM is located distal to the papillary muscle and occurs at the apex. It has been shown that the mortality and morbidity rates of ApHCM are higher than the other HCMs and it causes death more frequently,

especially in female patients (3,7,8). It may cause especially malignant ventricular dysrhythmias and apical aneurysms (8).

Continuation of contraction of the apical region in early-mid diastole in ApHCM can be seen as the cause of small vessel occlusion (9). Small vessel occlusions cause regional perfusion defects and chest pain occurs. Increased myocardial tissue, small vessel disease, and impaired vasodilator reserve have been suggested as possible causes of myocardial ischemia. T wave inversion (93%), giant T wave negativity (47%) and LVH (65%) accompanying ApHCM clinical findings cause cases to be confused with acute coronary syndrome (4). However, in most cases, coronary arteries are found to be normal on coronary angiography. Patients who had early repolarization in the anterolateral derivations in previous ECGs should be carefully evaluated for the diagnosis of ApHCM. Transthoracic echocardiography is the first choice for the evaluation of these patients. However, bedside echocardiography is not always sufficient due to the difficult evaluation of the apical region, artifacts, and poor echogenicity. In such cases, it is necessary to resort to other diagnostic methods such as magnetic resonance imaging and nuclear scintigraphy (10,11).

CONCLUSION

Apical-type hypertrophic cardiomyopathy, which can mimic acute coronary syndrome, is often miss diagnosed or undiagnosed, as it is a rare case, especially in non-Asian populations. When a giant negative T wave in the anterior derivations and signs of left ventricular hypertrophy are seen in the ECG, especially in low-risk patients for acute coronary syndrome, it should definitely be considered as a differential diagnosis and early referral should be made. Thus, some complications such as malignant ventricular dysrhythmias, myocardial infarction, and sudden cardiac death that may occur due to ApHCM (Yamaguchi Syndrome) can be prevented.

Informed Consent: Written informed consent was obtained from the patient for publication and accompanying images.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

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Author Contributions: Idea/Concept: AB, HCS; Design: AB, HCS, AK; Data Collection/Processing: HCS, AK; Analysis/Interpretation: AB, BE; Literature Review: AB, HCS, AK; Drafting/Writing: AB, BE; Critical Review: AB, BE.

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Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE (not exceeding 40 characters), ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES

ABSTRACTS in both languages (English and Turkish) must be fully compatible with each other, and each should be between 150 and 200 words.

ABSTRACT should be unstructured.

Case Report
TITLE (English and Turkish), SHORT TITLE (not exceeding 40 characters), ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACTS in both languages (English and Turkish) must be fully compatible with each other, and each should be between 100 and 150 words.
ABSTRACT should be unstructured.

Other
The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except for these three basic types of articles. There is no title or abstract section in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution, and address of the author should be included at the end of the writing. The answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

WRITING RULES
- Manuscripts should be prepared as Microsoft Word® documents.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed in the bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12-point Times New Roman font.

KEYWORDS
- Number of keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in English must be given in accordance with Medical Subject Headings (MESH, http://www.nlm.nih.gov/mesh/MBrowser.html), and keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT, http://www.bilimterimleri.com).

STATISTICAL METHODS
- All research articles should be assessed in terms of biostatistics and indicated with the appropriate plan, analysis, and report. In these manuscripts, the last subtitle of the MATERIAL AND METHODS section should be "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, and package programs and versions used for statistical analysis should be specified.
- All p values should be reported in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can be obtained from www.icmje.org.

ABBREVIATIONS
- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES
- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each is to be on a separate page.
- The table headings should be written at top of the table (Table 1. Table heading), and the figure captions should be written below the figure (Figure 1. Figure caption) as their first letters being upper case.
- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
- The figures and photographs should be uploaded as separate files in .png, .jpg, etc. format, and at least 300 dpi resolution.
- Captions of figure and photograph should be given on a separate page respectively, after the page including the last table.
- If a figure, picture, table, graphic, etc. which has been published before is used, written permission must be taken and it should be stated in the explanation of the figure, picture, table, or graphic. The legal responsibility in this regard belongs to the authors.

ACKNOWLEDGEMENT
- If any conflict of interest, financial support, donation, and another editorial (English/Turkish evaluation) and/or technical support, it must be stated in this section before the REFERENCES section.

REFERENCES
- References should be numbered according to the order of use and stated with numbers in parentheses as (1) or (1,2) or (3-5) at the end of the relevant sentence in the text.
- Reference list should be formed according to the reference order used in the text.
- If the number of authors is 6 or less, all authors should be specified, if there are 7 or more, "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses, and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:
Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Book:
Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:
Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

Makale Göndermek için ve ayrıca Yazar Yönergeleri, Etik İlkeler ve Yayın Politikası ve Değerlendirme Süreci hakkında ayrıntılı bilgi için lütfen web sayfasını ziyaret edin (https://dergipark.org.tr/en/pub/dtfd).

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörleri Komitesi (ICMJE, http://www.icmje.org/recommendations/), Dünya Tıbbi Editörler Birliği (WAME, https://www.wame.org/policies) ve Yayın Etiği Komitesi (COPE, https://publicationethics.org/guidance/Guidelines) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerin araştırma ve yayın etiğine uygun olması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Gönderilecek makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uyulması zorunludur. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- İnsan öğesini içeren tüm araştırmalar için, Helsinki Deklarasyonu Prensipleri'ne (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/) uygun olarak tüm katılımcılardan (veya 18 yaşından küçük çocuklar ve yerel mevzuat uyarınca reşit olmayan kabul edilen hastalar söz konusu olduğunda bir ebeveyn/yasal vasiden) çalışmaya katılım veya dokularının kullanımı için bilgilendirilmiş olur alındığı belirtilmelidir. Tüp bebek çalışmalarında gamet donörleri de dahil olmak üzere kök hücre araştırması ve translasyonu için biyomateryallerin tedariği için de onam gereklidir. Zorlama veya istismar potansiyeli olan (örneğin, mahkumlar veya bilinci kapalı hastalar) veya rızanın tam olarak bilgilendirilemeyebileceği (örneğin, bir dil engeli nedeniyle) savunmasız/hassas grupları (yani, kötü muamele veya zarar görme riski daha yüksek olan bireyler) içeren çalışmalar, istisnai durumlarda Editörün takdirine bağlı olarak değerlendirilecektir. Savunmasız/hassas grupları içeren bilimsel araştırmalar, yalnızca amaçları ve kapsamı bu gruplara fayda sağlıyorsa ve onların özel ihtiyaçlarını karşılıyorsa gerçekleştirilebilir ve yazarlar, makalelerinin yayınlanmak üzere değerlendirilmesi için bunu gösterebilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren bir kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili olabilecek tüm kişisel ve finansal ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından yazar sıralamasına uygun şekilde imzalanmış olmalıdır. Tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu olmayan başvurular değerlendirme sürecine alınmaz.

Benzerlik Raporu: Yazarların iThenticate vb. intihal programlarından elde ettikleri benzerlik raporunu başvuru sırasında sisteme yüklemeleri gerekmektedir. İntihal saptanan durumlarda, benzerlik oranına bağlı olarak editörlerin makaleyi reddetme ve/veya yazarlardan düzeltme isteme hakkı saklıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekiller yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK (40 karakteri geçmeyen), ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

Her iki dildeki (İngilizce ve Türkçe) ÖZ birbiriyle tam uyumlu ve her biri 200 ile 250 kelime arasında olmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK (40 karakteri geçmeyen), ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

Her iki dildeki (İngilizce ve Türkçe) ÖZ birbiriyle tam uyumlu ve her biri 150 ile 200 kelime arasında olmalıdır.

ÖZ yapılandırılmamış olmalıdır.

Olgu Sunumu
BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK (40 karakteri geçmeyen), ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR
Her iki dildeki (İngilizce ve Türkçe) ÖZ birbiriyle tam uyumlu ve her biri 100 ile 150 kelime arasında olmalıdır.
ÖZ yapılandırılmamış olmalıdır.

Diğer
Bu üç temel makale türü dışındaki (editöre mektup, editöryal yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık veya öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya ithaf olunan makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- İngilizce anahtar kelimeler Medical Subject Headings (MESH, http://www.nlm.nih.gov/mesh/MBrowser.html) ve Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT, http://www.bilimterimleri.com) ile uyumlu olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- Tüm p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığı yerde parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve bu durum şekil, resim, tablo veya grafik açıklamasında belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması, finansal destek, bağış ve diğer bütün editöryal (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

Makale:
Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Kitap:
Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:
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