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Earthquake and the Lung

Deprem ve Akciğer

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ABSTRACT

Natural disasters cause extensive damage to nature and impact on large numbers of people throughout the world. Recently, earthquakes have caused a large number of death, injured, missing, and displaced people. More than a million earthquakes occur worldwide every year, which equates to roughly two earthquakes per minute. Lung problems in earthquakes are one of the main causes of morbidity and mortality. Many pulmonary complications that occur after earthquakes are a direct result of the disaster itself. Pulmonary complications such as inhalation of dust and particulates from collapsed buildings in earthquakes, aspiration of water and pathogens due to tsunami after earthquakes, pulmonary thromboembolism, psychological effects caused by respiratory symptoms, infectious respiratory diseases and chest traumas such as pneumothorax, rib fracture, hemothorax, hemopneumothorax, diaphragmatic tear can develop after earthquakes. People in the earthquake area, search and rescue teams, and those involved in demolition and debris removal activities are at risk for asbestos exposure. The most effective solution against the lung effects of earthquakes is to take preventive and protective measures. It is very important for disaster preparedness and response teams to be aware of these problems. Respiratory problems that may arise in earthquakes were discussed in this review.

Keywords: Earthquake; lung; respiratory.

ÖZ

Doğal afetler, doğaya büyük zarar vermekte ve dünya çapında çok sayıda insanı etkilemektedir. Yakın zamanda, depremler çok sayıda can kaybına, yaralıya, kayıplara ve insanların yaşadıkları yerden ayrılmak zorunda kalmalarına neden olmuştur. Her yıl dünya çapında bir milyondan fazla deprem olmakta, bu da yaklaşık dakikada iki depreme denk gelmektedir. Depremlerdeki akciğer sorunları, morbidite ve mortalitenin ana nedenlerinden biridir. Depremlerden sonra meydana gelen pek çok pulmoner komplikasyon, felaketin kendisinin doğrudan bir sonucudur. Depremlerde yıkılan binalardan çıkan toz ve partiküllerin solunması, deprem sonrası oluşan tsunamiye bağlı su ve patojenlerin aspirasyonu, pulmoner tromboemboli, solunum semptomlarının neden olduğu psikolojik etkiler, depremlerden sonra ortaya çıkan bulaşıcı solunum yolu hastalıkları ve pnömotoraks, kaburga kırığı, hemotoraks, hemopnömotoraks, diyafragma yırtığı gibi göğüs travmaları gibi birçok pulmoner komplikasyon gelişebilmektedir. Deprem bölgesindeki insanlar, arama kurtarma ekipleri, yıkım ve enkaz kaldırma faaliyetlerinde bulunanlar asbeste maruz kalma riski altındadır. Depremlerin akciğerlere olan etkilerine karşı en etkili çözüm önleyici ve koruyucu tedbirlerin alınmasıdır. Afete hazırlık ve müdahale ekiplerinin bu sorunların farkında olması çok önemlidir. Bu derlemede depremlerde ortaya çıkabilecek solunumsal sorunlar ele alınmıştır.

Anahtar kelimeler: Deprem; akciğer; solunum.

INTRODUCTION

Natural disasters cause extensive damage to nature and impact on large numbers of people throughout the world. The victims of disasters are not just those killed and injured but those displaced by the disaster and those whose access to health care is vastly compromised. Survivors of disasters are at risk of very serious lung diseases. Such risks may be directly related to the disaster or respiratory infections caused by overcrowding after the disaster (1,2). Earthquakes are some of the most catastrophic natural disasters to affect mankind. Recently, earthquakes have caused a large number of death. Also, earthquakes have caused a large number of injured, missing, and displaced people. More than a million earthquakes occur worldwide every year, which equates to roughly two earthquakes per minute (3,4). Many pulmonary complications that occur after earthquakes are a direct result of the disaster itself. Pulmonary complications are inhalation of dust and particulates, aspiration of water and water-borne pathogens, chest traumas, pulmonary thromboembolism, psychological effects caused by respiratory symptoms, and infectious respiratory diseases (1,5,6). Respiratory problems that may arise in earthquakes were discussed in this review.

Inhalation of Dust and Particulates

Although particles larger than 10 µm are usually filtered by the upper respiratory tract, particles larger than this size have been detected in the lungs of firefighters exposed to the dust cloud after the destruction of the World Trade Center in New York (7). It has been thought to be likely to occur as a result of exposure to high concentrations of particles as well as mouth breathing bypassing the nasal filtration system of the airways (8).

Studies conducted on rescue teams after the destruction of the World Trade Center have shown that short-term intense exposure can cause acute and chronic health effects. Acute bronchospasm, chronic rhinosinusitis, chronic cough, persistent bronchial hypersensitivity, and decreases in respiratory function test values were observed at high levels in rescue teams (8-10).

Asbestos is a known carcinogen that can cause mesothelioma or asbestosis. With the collapse of many buildings because of the earthquake, it is likely to have released asbestos fibers into the air putting disaster victims and rescue teams at risk of inhalation. Appropriate safety measures should be taken on how to control the risk of clean-up in earthquake-affected areas and how to safely dispose of asbestos waste (11,12).

Aspiration of Water and Water-Borne Pathogens

Earthquake-induced tsunamis cause mass casualties in a very short time, often due to temporary paralysis of local emergency response and health care services (13). Sudden increases in water levels that occur during a tsunami, hurricane, or flood are more likely to cause drowning, aspiration, and traumatic injuries than predictable increases (14). Water aspiration can lead to infection, loss of alveolar surfactant, pulmonary edema, and acute respiratory distress syndrome (ARDS) (15). Pulmonary edema is more common in salt water aspirations. Vomiting of swallowed water can also lead to aspiration of stomach contents due to unconsciousness and inhibition of airway protective reflexes. Significant aspiration findings are

usually clinically detectable. Namely, if the physical examination, arterial blood gas, and chest X-ray are normal in patients at the admission stage and there are no signs of aspiration, treatment is required, and the probability of developing pulmonary edema or pneumonia is very low (16). After the tsunami in Sri Lanka in 2004, drowning and trauma accounted for the majority of post-disaster morbidity (17). Aspiration pneumonia developed in half of 37 patients who aspirated soil-contaminated salt water, reported by a medial group after the tsunami, and ARDS was observed in 8 patients. Different organisms may predominate in fresh water and salt water aspirations, but aerobic gram-negative bacteria, including pseudomonas and pseudomonas-like species, are the most commonly reported (15,18).

Antibiotic therapy should include those effective against pseudomonas and locally common organisms. Antibiotics should be started in patients with fever, pulmonary infiltrates, and/or signs of systemic toxicity (18). The fungal infection may be present in individuals with acute post-aspiration pneumonia, brain abscess, or meningitis who do not respond to antibacterial therapy. In this case, it has been reported that both *Pseudallescheria boydii* and aspergillus species were isolated as agents (18-20).

Pneumonia caused by aspirated sludge and bacteria and contaminated salt water due to tsunami is known as tsunami lung (21). A tsunami lung is thought to occur with the combination of bacterial and chemical pneumonia. Chest radiographs of two female patients, aged 88 and 37, who developed tsunami lung in the tsunami after the Great East Japan Earthquake, were shown in Figure 1 (22).

Chest Traumas

Trauma is an important cause of morbidity and mortality in earthquakes and is the most common cause of earthquake-related hospital admissions in the first 24 hours (23). The very young and the very old patients have a higher risk of earthquake-related mortality (24). Approximately 10% of those who applied to the hospital during the earthquake had chest traumas (25,26).

Chest injuries are often accompanied by injury to other organs, and multiple injuries are associated with increased mortality. In the 1999 Marmara earthquake, in 19 patients with chest trauma evaluated by Toker et al. (27), pneumothorax was 37%, rib fracture 26%, hemothorax and hemopneumothorax 11%, diaphragmatic tear 11%, subcutaneous emphysema 11%, and cervical tracheal damage 5%. Ozdoğan et al. (25) reported that in the Düzce and İzmit earthquakes in 1999, among the total of 356

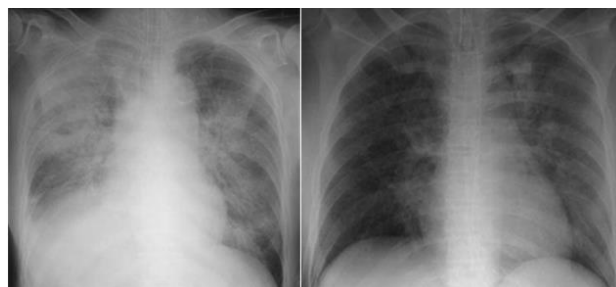


Figure 1. Tsunami lung of different two patients (22)

hospitalized patients, 21 (9.7%) in the İzmit earthquake, and 6 (7.6%) in the Düzce earthquake had thorax and lung injuries. Pneumothorax and rib fractures were the two most frequent pathologies and accounted for 50% and 33.3% of the injuries, respectively. They suggested that approximately 10% of the casualties of a great earthquake may be expected to have thorax and lung injuries, and traumatic chest diseases should be considered in planning the medical response strategies (25). In the 2011 Van earthquakes, pneumothorax and rib fractures were reported as the most common thoracic injuries (26). In the 1999 Marmara earthquake, the most common surgical procedures performed by Bulut et al. (23) in patients with chest trauma were fasciotomy (38%) and tube thoracostomy (13%).

Pulmonary Thromboembolism

Cardiovascular events are often reported following disasters. Among these, venous thromboembolism is deemed serious and thus should be taken into consideration. Indeed, its risk has been demonstrated to increase following earthquakes, floods, burns, and intoxications. Proper venous risk assessment and guideline application have been determined to be essential in disaster management, which can increase the risk for venous thrombotic diseases (28). Dehydration, prolonged immobility, and earthquake-related injuries are thought to be the causes of the high prevalence of deep vein thrombosis in evacuees from earthquakes (29).

In a study evaluating risk factors for acute pulmonary embolism (APE) after the 2004 Mid-Niigata Prefecture earthquake in Japan, a rate of 5% and higher for settlements with a high post-earthquake evacuee rate, and <5% for an area with a low evacuee rate was found. The incidence of APE in the first month after the earthquake was high in the evacuated area and was also higher in women. All patients in the high evacuee area had stayed there in their automobiles for long periods of time, but none had done so in the low evacuee rate area (6). Also, Watanabe et al. (30), reported the possible role of automobile sheltering in the increased incidence of pulmonary embolism during the 2004 Niigata earthquake. Another study from Japan, it was confirmed that automobile sheltering and oral contraceptives are potential risk factors for pulmonary embolism (31).

Psychological Effects Caused by Respiratory Symptoms

Natural disasters create also great psychological stress. Post-disaster post-traumatic stress disorder and depression-like symptoms may occur. Respiratory complaints can cause anxiety syndromes. The psychological impact can also be seen in rescue and aid workers, victims, and survivors (32,33).

Infectious Respiratory Diseases

All infectious diseases, including those of the lung, usually occur in the aftermath of disasters. This is due to population displacement, unsafe water and sanitation facilities, non-functional health services in overcrowded and affected areas, and the poor and low immunization status of the state (34). Having to live in an overcrowded environment after a natural disaster is a common problem and can facilitate the transmission of infectious diseases,

especially respiratory and gastrointestinal diseases. Due to the loss of housing, people are forced to live in emergency shelters and tents with limited daily life support. Respiratory tract infections are the main cause of morbidity and mortality in the first 3-5 days in populations who have to relocate after an emergency (1). The World Health Organization reported that the victims of the 2004 Aceh tsunami and the 2015 Pakistan earthquake had a large number of deaths due to acute respiratory infections (35,36).

Viral acute respiratory infections spread rapidly in crowded populations of victims. Overcrowding is a risk for lung infection for healthy survivors, especially children, and the injured. This risk of infection can be avoided by adequate ventilation to ensure good airflow and prevent increased concentrations of respiratory particles (1,37).

Rescuers and healthcare workers may also be at increased risk for acute respiratory infections. Kun et al. (38) reported acute upper respiratory tract infections (13.2%) were the most common disease in rescue teams living in shelters in the city of Beichuan after the 2008 China earthquake (38).

Pulmonary tuberculosis contagion also increases in populations displaced by natural disasters. Contagion is facilitated by the recirculation of external air, the length of contact with the tuberculosis patient, insufficient ultraviolet light, and malnutrition (39). Transmission rates increase as a result of staying away from tuberculosis treatment programs, especially due to population movements (40). Balbay et al. (41) published a study on whether 112 tuberculosis patients in Düzce were affected by the consequences of the earthquake after the Marmara earthquakes. According to this study, they reported that the treatment and follow-up of tuberculosis patients in the three periods before the earthquake, during the earthquake, and after the earthquake did not differ significantly. However, due to the small number of patients examined, it was specifically stated that it cannot be generalized to the entire Marmara region, which is a limitation of the study. Acquired drug resistance may occur due to non-compliance with treatment, inappropriate treatment regimens, irregular drug support and use, and poor drug quality in the periods after disasters. In these patients; precautions should be taken to provide medication for 2 weeks or 30 days, to issue a card containing the contact information of the personnel in charge of tuberculosis control, to send patient lists to national tuberculosis control units to be sent to other states, and finally to determine a reference center (1,42).

CONCLUSION

After an earthquake, lung diseases can be seen both directly and indirectly. Lung problems in earthquakes are one of the main causes of morbidity and mortality. The most effective solution against the lung effects of earthquakes is to take preventive and protective measures. It is very important for disaster preparedness and response teams to be aware of these problems. Strong disaster preparedness plans should be established and characterized by appropriate and adequate environmental planning and resilient health facilities. Early diagnosis, surveillance, and control of earthquake-related lung diseases are very important in reducing mortality.

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The Earthquake Disaster in Türkiye: A Review from Child and Adolescent Psychiatry Perspective

Türkiye'de Deprem Felaketi: Çocuk ve Ergen Psikiyatrisi Perspektifinden Bir Değerlendirme

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ABSTRACT

The earthquake has long-lasting various mental and behavioral effects on children and adolescents. The aim of this review was to discuss the nature and extent of psychiatric problems, management options, and the process of organizing psychological interventions for affected children. Individuals show a range of physically, emotionally, and cognitively healthy responses that can help them cope with the aftermath of a disaster. Psychiatric symptoms such as acute stress reactions, post-traumatic stress disorder, depression, anxiety disorder, increased risk of suicide, sleep disorders, substance use disorders, and psychotic disorders may develop in some children. Comorbidities and sub-clinical syndromes are also common. There are many risk factors and protective factors in the development of mental disorders. Close follow-up of children at high risk and interventions for psychosocial support may prevent the development of mental disorders. It is very important to start the intervention at the earliest period. The psychological impacts of young disaster victims can be addressed by skilled local volunteers, medical professionals, and educators in primary health care programs. With the nation's overall social and economic recovery, children can recover more quickly from traumatic experiences.

Keywords: Earthquake; disaster; child and adolescent psychiatry.

ÖZ

Depremi çocuk ve ergenler üzerinde uzun süre devam eden çeşitli zihinsel ve davranışsal etkileri bulunmaktadır. Bu gözden geçirmenin amacı, psikiyatrik sorunların doğası ve kapsamını, yönetim seçeneklerini ve etkilenen çocuklar için psikolojik müdahaleleri organize etme sürecini tartışmaktır. Bireyler afet sonrasında başa çıkmalarına yardımcı olabilecek bir dizi fiziksel, duygusal ve bilişsel olarak sağlıklı tepkiler gösterir. Bazı çocuklarda ise travma sonrası akut stres reaksiyonları, travma sonrası stres bozukluğu, depresyon, anksiyete bozukluğu, artmış suisid riski, uyku bozuklukları, madde kullanım bozuklukları ve psikotik bozukluklar gibi psikiyatrik belirtiler gelişebilmektedir. Komorbiditeler ve sub-klinik sendromlar da yaygındır. Ruhsal bozuklukların gelişimde çok sayıda risk faktörü ve koruyucu faktör bulunmaktadır. Yüksek risk altındaki çocukların yakın takibi ile psikosozal destek müdahalelerinde bulunulması ruhsal bozuklukların gelişimini engelleyebilir. Müdahaleye en erken dönemde başlamak çok önemlidir. Genç afet kurbanlarının psikolojik etkileri, vasıflı yerel gönüllüler, tıp uzmanları ve temel sağlık hizmetleri programlarındaki eğitmenler tarafından ele alınabilir. Ulusun genel sosyal ve ekonomik hızlı iyileşmesi ile çocuklar travmatik deneyimlerden daha çabuk kurtulabilir.

Anahtar kelimeler: Deprem; afet; çocuk ve ergen psikiyatrisi.

INTRODUCTION

In February 2023, a series of devastating earthquakes occurred in an area encompassing eleven provinces of Türkiye and affecting more than 13.5 million people. The magnitude of the first earthquake was measured at 7.8 Mw, and the earthquake that followed in the afternoon of the same day was measured at 7.7 Mw. The first one was the second-strongest earthquake in Türkiye, following the 1668 North Anatolia Earthquake (1). After two major earthquakes, unexpectedly, other significant earthquakes occurred in different regions throughout the country. Thousands of aftershocks continue to follow these earthquakes.

The earthquake caused a lot of damage in a large area. A significant portion of Türkiye's population has been affected and 1.5 million people have been displaced and left homeless (2). Many buildings were damaged and some were destroyed. Thousands of people were left under the rubble when the buildings collapsed. Damaged roads, winter storms, and communication disruptions hampered rescue and relief efforts in earthquake zones. All these were disappointing and important issues to be dealt with in the acute period besides the earthquake. Unfortunately, people trapped under the debris died from hypothermia in sub-zero temperatures. During rescue efforts, body parts were often found in the rubble. Hundreds of children who lost their parents or could not reach their families because the hospitals in the area became inoperative, were left orphaned. When we consider what has happened, it is an indisputable fact how compelling it is for the mental health of individuals of all ages.

This earthquake is supposed to lead to material damages amounting to at least 84 billion dollars according to the United Nations Development Program Türkiye Office (3). Housing damage constitutes the majority of this loss. The International Organization for Migration (IOM) prepared a report titled '2023 Earthquakes Displacement', which stated that the disaster resulted in 2.7 million people leaving the affected region. The lives of people who migrated from the earthquake area have suddenly undergone great changes. The survivors, who lost many relatives, were unemployed in different cities, in unfamiliar homes, and had to deal with many fears for the future. The survivors who had to stay in the region continue to have problems with shelter, heating, and clean water. Another important risk is epidemic diseases. Problems such as corpses that cannot be removed from the rubble, insufficient toilets, and the inability of earthquake victims to use clean water increase the risk of epidemics (4). Meeting the basic care of all earthquake victims in terms of both physical and mental health should be the primary goal. Without meeting basic care, an individual cannot attain mental well-being.

Natural disasters can cause traumatic effects on both survivors and witnesses. To reduce mental health issues, prompt psychosocial support is crucial after meeting basic care, with ongoing follow-up. Delayed care-seeking after disasters increase the risk of psychological problems, particularly in children (5).

Türkiye is a developing country and has a substantial child population with a rate of 26.9% according to the Turkish Statistical Institute (Türkiye İstatistik Kurumu, TÜİK)'s 2021 data. Most of the earthquake-affected provinces

have a higher percentage of children than the national average (6). Although Türkiye is prone to earthquakes and has a significant child population, scientific research on the developmental and psychological effects of earthquakes on this vulnerable population is limited. Comprehensive researches are needed to recognize the psychological reflections and develop effective mental health programs for early diagnosis of psychopathology in young people affected by natural disasters.

In this context, the aim of this review was to compile the short and long-term psychological effects of the earthquake on children and adolescents, based on the current literature with the goal of minimizing these effects and reviewing protective measures. The articles in this review were selected by searching Pubmed, Web of Science, and Google Scholar with the keywords "child and adolescent psychiatry, trauma, disaster, earthquake". Related articles referenced in the articles were also reviewed and included.

The Psychological Impact of the Earthquake on Children and Adolescents

The earthquake not only results in fatalities, injuries, destruction of buildings, and loss of livelihoods and materials but also severe and maybe permanent psychological consequences on the people living through the trauma. Common psychiatric problems following a disaster include acute stress reactions, post-traumatic stress disorder (PTSD), anxiety disorder, depression, panic disorders, and phobias in children and adolescents (7-10).

Healthy Human Responses to Earthquake

Healthy human responses to earthquakes involve a range of physical, emotional, and cognitive reactions that can help individuals cope with the aftermath of the disaster. Some of the healthy human responses to earthquakes include seeking social support, practicing self-care, maintaining a positive attitude, and also engaging in problem-solving activities. These responses can help individuals maintain a sense of control, reduce feelings of isolation and promote resilience in the face of adversity. Another normal reaction is to grieve. Normal grief typically lasts a few months and survivors should be viewed as normal people in abnormal situations. A prolonged, intense, and disabling form of grief that interferes with an individual's ability to function in daily life is called complicated grief (11). It is important to distinguish between normal and complicated grief because complicated grief requires specialized treatment.

Acute Stress Reactions/Disorder (ASD)

After a natural disaster, children may develop stress reactions to cope with the event, which can differ based on age, developmental stage, severity and duration of the event, and family and environmental conditions (12). Symptoms of acute stress disorder (ASD) can include intrusive thoughts, memories, and images of the traumatic event; avoidance of the reminders of the event; negative changes in mood; hyperarousal and dissociative symptoms. These symptoms typically appear within a month of the traumatic event and last for a minimum of three days and a maximum of one month (11).

After the earthquake, trauma and disaster outpatient clinics were established in various cities throughout Türkiye in a very short amount of time. During the first days and weeks after the earthquake, many families sought help for their children's stress reactions at polyclinics. Post-disaster psychological reactions in children can be cognitive, emotional, behavioral, and somatic-physiological. Symptoms can vary but typically include anxiety, fear, and behavioral changes. Fear of staying alone, fear of bad things happening to loved ones, fear of recurrence of the earthquake, avoidance behaviors, sleep disturbances, irritability, agitation, difficulty in concentration, and psychical symptoms such as headaches, stomachaches, and muscle tension are the most common symptoms we encounter in the outpatient clinic.

The most common diagnosis in children and adolescents two weeks after the 1999 Marmara earthquake was ASD, with a rate of 74.5% (13). A study conducted within a month of the Wenchuan earthquake in China found that the incidence of ASD was 54.3%. The study also showed a significant difference between genders, with a higher rate of 63.6% in females compared to 44.0% in males (14). We know that girls are at a higher risk for developing ASD than boys depending on socio-cultural, biological, and environmental factors (14-16). Studies indicated that the strongest predictors for ASD were being trapped or injured under rubble, amputation of body parts, injury to parents or relatives, and loss of home (14,15). The impact of acute stress symptoms on duration, severity, and functionality should be evaluated by experts, and interventions for children in the risk group should be planned in the early period, which may prevent the development of mental disorders in the long term (17).

Post-Traumatic Stress Disorder (PTSD)

Post-traumatic stress disorder (PTSD) is the most prevalent negative psychological reaction experienced by survivors after an earthquake (18). Symptoms of PTSD can include re-experiencing the trauma through nightmares or flashbacks, avoidance of reminders, negative moods like depression and anxiety, increased arousal, irritability, and difficulty sleeping and concentrating in children and adolescents (11).

In children, the prevalence of PTSD after an earthquake varied a wide range between 2.5% and 60.0% depending on age, gender, the severity of the trauma, medical history, cultural factors, measurements, and evaluation time (19,20). A study in Türkiye three years after the 1999 Marmara earthquake found 56% of children and adolescents had severe PTSD symptoms (21). Another study following the 2011 Van earthquake in Türkiye found 40.69% of participants had severe PTSD symptoms six months after the earthquake (22).

Considering the high rates of PTSD, a question arises as to "Why do some children and adolescents develop PTSD after an earthquake while others do not?"

The risk factors of developing PTSD after an earthquake are heightened by various factors, such as the severity of the earthquake, extensive exposure to trauma, pre-existing mental health issues, history of trauma, loss of a family member, inadequate social support, and limited access to resources (19,22-24). Age, developmental stage, and

personality characteristics such as obsessive-compulsive traits are important factors too (25). Younger children may have difficulty understanding what has happened and may have a harder time expressing their feelings and emotions. Adolescents, on the other hand, may have a greater awareness of the event and its impact, which can lead to feelings of helplessness and hopelessness. It is necessary to assess each patient separately based on their characteristics. A recent meta-analysis has indicated that the absence of psychological support in areas affected by trauma significantly heightens the risk of PTSD among survivors (26). Children with a history of trauma were at a higher risk of developing PTSD after an earthquake (27). The exact mechanism of this process is not fully understood. Previous trauma may lead to alterations in brain structure and function, particularly in areas involved in stress regulation and emotional processing. The alterations may make individuals more susceptible to PTSD in response to subsequent traumatic events (28). Prior to the earthquakes, as in the whole world the coronavirus disease 2019 (COVID-19) pandemic had already caused trauma to children and adolescents in Türkiye. The pandemic brought about the fear of infection and death, loss of loved ones, limited communication with peers, quarantine measures, social isolation, disruptions to education, and ongoing exams for high school and university admission. As the COVID-19 pandemic continues to cause psychological distress, the recent earthquakes have become a secondary source of trauma for these young individuals. In Türkiye, the youths have been faced with numerous psychological stress factors. These include the ongoing refugee crisis that began in 2011, as well as past natural disasters such as earthquakes, floods, and fires. Political instability and the failed coup attempt of 2016 have also contributed to feelings of insecurity and fear. Economic challenges including high unemployment and inflation have added to the stress. Furthermore, the highly competitive nature of the education system and academic pressure have also impacted the mental health of youths. We must be aware of and follow the proper process in clinical practice, as we anticipate encountering mental disorders that are both frequent and severe.

Anxiety Disorders, Depression, and Suicide

Children affected by disasters may develop increased fear, anxiety, and phobia against stimuli directly related to the disaster (29). Common fears among those who experienced a traumatic event include darkness, being alone, death, and the possibility of re-experiencing the event or new trauma. Reminders of the traumatic event can evoke distressing thoughts or images, leading to feelings of helplessness, hopelessness, and fear (11). Grief reactions are seen intensely in children who suddenly lose their homes, schools, friends, and relatives, and they usually manifest themselves as crying, sadness, depression, separation anxiety, and restlessness. Children, in particular, may experience intense feelings of guilt and shame if their traumatic event involves the death of others and their survival (30,31).

Post-disaster depression and anxiety symptoms are common. Three years after the Wenchuan Earthquake, a study found that rates of PTSD, depression, and anxiety

were correspondingly 29.6%, 44.8%, and 37.6% (32). The prevalence of PTSD, depression, and anxiety was found to be 13.1%, 19.8%, and 37.3%, respectively, in the study, which included 6132 teenagers who lived three years after the 2013 Ya'an earthquake. Participants with PTSD also had 71.5% anxiety and 49.7% depression (33).

Among subjects with PTSD, co-morbidity rates of depression range from 21% to 94% and anxiety from 39% to 97% (34). Moreover, substance use disorders and psychotic-like experiences are common in post-earthquake adolescents, although to a lesser extent than depression and anxiety (35,36). The various prevalence rates for mental diseases in the research may be due to a variety of factors, including the locations, the type of disaster, the duration of the disaster, cultural factors, and available social support.

Researches suggests that natural disasters including earthquakes can increase the risk of suicide in both adults and children/adolescents. The risk of suicide can be increased in many mental disorders including PTSD, depression, anxiety, and hopelessness. Depression symptoms were shown as the biggest predictor of suicidal ideation after the earthquake (37). Suicide risk may increase if one is exposed to a family member's death or a friend's or family member's suicide (38). Children who are protected from suicide by high-quality parenting before the disaster may increase the risk of suicide if the quality of parenting declines after the disaster (39). There are reports of the risk of suicide in earthquake survivors decreased (40), increased (41), and remained the same over time (42).

Parental mental health can have a significant impact on the mental well-being of children after an earthquake. After an earthquake, parents with mental health conditions may struggle to cope, creating a challenging environment for children that negatively affects their mental health. Children with a genetic predisposition to mental health conditions may be more likely to develop issues. It is crucial for healthcare providers and caregivers to monitor children and adolescents for depression symptoms after an earthquake and to provide appropriate support and resources to help them cope with the aftermath of the disaster.

Sleeping Disorders

Sleep problems are common in children and adolescents after the earthquake. Short sleep duration, daytime dysfunction, difficulty falling asleep, difficulty in maintaining sleep, and recurrent nightmares after natural disasters are common symptoms of sleep disorders in adolescents (12). Half of the young people with mental health problems after the earthquake experience sleep disorders (33). Additionally, sleep problems can impact the symptoms and severity of diseases. While sleep disorder increases the risk of PTSD and depressive symptoms, it also causes the symptoms to persist (14). Insufficient sleep is associated with anxiety and depression. Nightmares and difficulty falling asleep can be associated with PTSD (33). Sleep disorders after the earthquake can worsen mood and increase suicidal ideation (15). A study revealed that short sleep duration in children aged 4-6 and oversleeping in children aged 7-15

years pose a higher risk for mental health (13). Adolescent earthquake survivors often and persistently experience sleep difficulties. The increased risk of sleep problems may be associated with a variety of demographic, psychological, and earthquake-related factors (12). It's important for parents and caregivers to be aware of these potential issues and seek help from a mental health professional if necessary.

What about Children and Adolescents with Developmental Disorders? How Are They Affected?

Children and adolescents with developmental disorders are particularly vulnerable in the aftermath of earthquakes, as they may have difficulty understanding what is happening and communicating their needs. They may also struggle with disruptions to their routines and environments, which can exacerbate their existing symptoms and cause new problems.

The earthquake experience in Central Italy, for example, showed that individuals with autism struggled with communication, daily living, social interaction, and motor skills during the first few months following the earthquake. However, with prompt and intensive intervention, there can be some improvement in their adaptive functioning (43). A study about the experiences of Japanese mothers caring for children with special needs after two earthquakes revealed that these mothers faced negative social interactions including discrimination and stigmatization. They also adopted various coping behaviors (44).

Children with special needs may require specialized medical care which can be challenging in emergency settings where resources are limited and access to medical professionals is restricted. Overall, it is important for caregivers, educators, and emergency responders to have a plan in place to address the unique needs of these children. Schools and teachers play a significant role in the recovery of children with disabilities after providing tailored support can help ensure their well-being during times of crisis.

Risk Factors and Protective Factors

Childhood and adolescence are known to be vulnerable periods for post-disaster psychological disorders (24,33). Risk factors for the development of PTSD in children are female gender and older age. Further research is necessary to examine the underlying psychosocial and biological mechanisms of older girls at greater risk for PTSD after earthquakes. Additionally, adults with lower levels of education and socioeconomic status have a higher likelihood of developing PTSD after earthquakes, while higher education levels in children are associated with a greater risk of PTSD. Child and adolescent psychiatrists work with a high-risk population, so it is important for them to know the risk and protective factors and to monitor them during clinical assessments.

Final-year students due to their heavier academic workload may also experience a higher level of stress, leading to a greater prevalence of PTSD (24). Despite all kinds of disasters, life goes on, which creates additional stress, especially for children and families preparing for university and high school entrance exams. This can lead to many mental health problems, including exam anxiety,

depression, and somatic complaints, in addition to PTSD. At our trauma and disaster outpatient clinic, we have also observed a significant number of complaints related to the inability to focus on studies and prepare for exams in its applications, which cannot be underestimated in this regard.

There are various additional factors that can increase the risk of developing PTSD, such as the severity of exposure to traumatic events (34-36), the extent of family members' loss (34), parental distress linked to trauma, short-term proximity to traumatic events (36), experiencing multiple stressors, previous exposure to stressful events, and coexisting adverse circumstances (37).

Parental psychopathology has different effects on children's psychopathology. The father's traumatic stress predicted the traumatic stress of earthquake survivors while the mother's level of depression predicted the depression experienced by their children (38). When fathers experience symptoms of PTSD such as irritability and detachment after an earthquake, their symptoms have a greater impact on their children (39). Working with children and adolescents cannot be separated from their family members and family dynamics, as they are all interconnected. Therefore, it is important to evaluate the mental health of parents who

have experienced a disaster, review their risk factors, closely monitor them, and collaborate with adult psychiatrists when necessary.

Untreated adolescents who experienced severe trauma are at risk of developing chronic PTSD and depressive symptoms (40). Loss of either both parents or just the father is a significant risk factor for depression, but not as much for PTSD (41). In risky situations and following traumatic exposures, many features of parent-child interaction are visible as modulating the underlying causes of anxiety disorders in children. These characteristics, which aggravate children's nervous and avoidant behaviors, may include the reciprocation of avoidance reactions, parental criticism, and parental constraint (42). Current risk factors and protective factors are summarized in Table 1.

Intervention

Planning for disasters should include mental health intervention training. The need of talking to children about trauma and the importance of emotional first aid are both topics that disaster personnel participating in rescue and relief efforts need to be well-trained in preparation. It is preferable to combine mental health care with other disaster relief activities than to provide it

Table 1. Risk factors and protective factors for post-disaster mental health problems in children and adolescents

	Risk Factors	Protective Factors
Demographic variables	<ul style="list-style-type: none"> • Older age • Female gender • Only child • Higher education • Low father education • Low mother education • Rural 	<ul style="list-style-type: none"> • Male gender • Urban • Higher education level of parents
Pre-trauma factors	<ul style="list-style-type: none"> • Physical illness • Low self-esteem • Negative life events • Prior trauma • Parents with mental health disorders 	<ul style="list-style-type: none"> • High self-esteem • Mentally healthy parents
Objective trauma characteristics	<ul style="list-style-type: none"> • Bereavement (Family Member) • Family member injured • Other injured/killed (e.g., friend) • Witnessed other injury/death • Separated from family • Personal injury • Hospitalization/surgery/amputation • Trapped/buried • House damage • Loss of property (excluding home) 	<ul style="list-style-type: none"> • Get together with family • Good housing high standard of living
Subjective trauma characteristics	<ul style="list-style-type: none"> • High-severity trauma • Perceived threat/fear • Negative coping • PTSD-Anxiety-Depression 	<ul style="list-style-type: none"> • Positive coping
Post-trauma environmental factors	<ul style="list-style-type: none"> • Displacement • Poor social support • Delayed care-seeking • Family violence (e.g., corporal punishment) • Meeting basic care needs 	<ul style="list-style-type: none"> • Early return to routine life • Utilization of mental health services • Adequate social support • Family communication • Lack of basic care needs • Positive school climate

alone (45). Young catastrophe victims can have their psychological effects addressed by skilled local volunteers, medical professionals, and instructors in basic health care programs. Mental health specialists' duties include assisting children with complicated psychiatric symptoms who have been sent to them, providing support for disaster workers whose own emotions may make recovery difficult, and training disaster workers (46). Early detection of children's mental health issues is crucial, as are supportive actions taken at home, at school, and in the community (47).

Children's post-disaster treatments are divided into three time frames: acute, short-term, and long-term. These time frames are arranged around the psychological responses that occur following catastrophes. It is believed that psychological first aid is the most successful intervention since the immediate aftermath of a traumatic incident frequently entails uncertainty and dread and victims exhibit high emotional reactions (48). The fundamental aspects of post-disaster psychotherapy for children are to listen, explain, promote attachment connections, enable symbolic expression in play and art, and to encourage the capacity to envisage healing (49). A lot of kids want to express how they and their families are experiencing. It's crucial to support kids in finding language for their emotions and in understanding them. It is advised to take the child's lead, refrain from prying, just reply to the things the youngster has introduced on their own, and encourage the containment of intense emotions. Facilitating the child's desire to reflect on and communicate about their deceased loved one is necessary after a parent or other family member has passed away (50).

Parent participation in evaluation and intervention is crucial. The management of post-disaster mental health sequels benefits greatly from psychoeducation of the family members and the affected children on the symptoms. Parents also require assistance in comprehending and accepting the fact that their child's puzzling and distressing emotions and actions in the aftermath of a tragedy are "normal" for such an "abnormal" scenario (49). Parents must be more approachable, giving youngsters the knowledge they require while also immediately and honestly responding to their inquiries. Parents ought to stop watching television and refrain from subjecting them to unending replays of horrific pictures (51). The family should be urged to make an effort to resume regular daily activities and accustomed schedules as soon as feasible. In the early aftermath of trauma, children should be near their families since tight mother-child, family, and related interactions are crucial to the healing process. Adopting orphaned children by relatives or foster homes may be very beneficial (52).

The post-acute stage consists of embracing the occurrence, assisting with emotion awareness and processing, acquiring coping skills that may be a source of strength, and concentrating on future adaption (48). To achieve these goals, school-based mental health programs can offer accessible services to children affected by disasters, reduce traumatic psychopathology, and place an emphasis on normalization. These programs are typically carried out in children's and

adolescents' own schools and classrooms without interfering with their routines (53,54).

Many government institutions and non-governmental organizations come together and carry out studies in the field of psychosocial intervention. With the psychosocial support tents set up in the earthquake-affected areas, an area where children can play and spend time with educational activities is created (55). Many children and families with mental special needs became more helpless after the earthquake. In this process, it can continue to receive expert support from the experts in tent cities and the Special Child Support System application developed by the Ministry of Health of the Republic of Türkiye (56). In a variety of circumstances, children should be referred to secondary care by mental health specialists for psychiatric examination and treatment. This needs to be covered in the training of emergency personnel. Very severe symptoms of any type, symptoms that continue despite emotional support, suicidal thoughts or actions, psychotic symptoms, disruptive behavior, drug abuse issues, and children with additional life stresses or little social support are typical referral criteria (57). In order to give children a relaxing and encouraging setting where they can adapt and recover more quickly, it may occasionally be necessary to remove them from a stressful situation. The overall social and economic recovery of the community or nation has an impact on how quickly children may recover from traumatic experiences. A thorough disaster recovery program must involve public mental health strategies, such as systematic screening and trauma/grief-focused therapies (58).

CONCLUSION

Available information shows that children may face many psychosocial difficulties after a disaster. It is very important to start the intervention at the earliest period. These interventions should be community-based, multi-level and comprehensive, and long-term involving relevant institutions such as health, education, and local and national governments. An integrated approach using psycho-socio-educational and clinical interventions is expected to yield better results than any single approach. There is a need for more systematization and further research on interventions to be made in post-disaster children and adolescents.

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Therapeutic Plasma Exchange in Adult Patients with COVID-19 and Severe Pneumonia: Single Center Experience of Eighty Patients

Ağır Pnömonili Erişkin COVID-19 Hastalarında Terapötik Plazma Değişimi: Seksen Hastada Tek Merkez Deneyimi

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ABSTRACT

Aim: Therapeutic plasma exchange (TPE) is a frequently discussed treatment modality in severe coronavirus disease 2019 (COVID-19) patients. It requires an apheresis device and experienced personnel for the application. In this study, we aimed to reveal the characteristics and clinical outcomes of adult patients with COVID-19 who experienced TPE.

Material and Methods: Adult patients who had undergone TPE in our apheresis unit were retrospectively analyzed and COVID-19-positive cases were included in the study. All the medical information about the cases was obtained from the electronic database and technical details of the procedures were gathered from apheresis unit records.

Results: A total of 80 patients with a median age of 60 (19-85) years were included in the study. Severe pneumonia was present in 98.8% (n=79) of the cases. More than three-quarters of the patients had lymphopenia, critically elevated C-reactive protein (CRP), and D-dimer, and 41.0% (n=32) had high ferritin. The median length of stay in the intensive care unit was 26 (5-124) days. The mortality rate observed on the 14th and 28th days following the TPE procedure was 51.3% (n=41) and 75.0% (n=60), respectively. High ferritin level, multiple organ failure (MOF), and intubation were parameters found to be associated with mortality in the multivariate analysis.

Conclusion: The mortality rate observed in patients with COVID-19 who underwent TPE in our study was similar to the cases in the literature without the procedure, while it has been shown that high ferritin levels, intubation, and the presence of MOF increase the risk of mortality.

Keywords: Therapeutic plasma exchange; pneumonia; COVID-19.

ÖZ

Amaç: Terapötik plazma değişimi (TPD), ağır düzey koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) hastalarında sıklıkla tartışılmakta olan bir tedavi şeklidir. Uygulanması için deneyimli personele ve aferez cihazına gereksinim vardır. Bu çalışmada, TPD uygulanmış olan erişkin COVID-19 hastalarının özellikleri ve klinik sonuçlarının ortaya konulması amaçlanmıştır.

Gereç ve Yöntemler: Aferez ünitemizde TPD uygulanmış olan erişkin hastalar geriye dönük olarak incelenmiş ve COVID-19 pozitif olan vakalar bu çalışmaya dahil edilmiştir. Olgulara ait olan tüm tıbbi bilgiler elektronik veri tabanından alınmış ve aferez işlemlerine ait teknik detaylar ise aferez ünitesi kayıtlarından elde edilmiştir.

Bulgular: Bu çalışmaya, ortanca yaşı 60 (19-85) yıl olan toplam 80 hasta dahil edildi. Olguların %98,8 (n=79)'inde ağır pnömoni varlığı söz konusu idi. Tüm olguların dörtte üçünden daha fazlasında lenfopeni, kritik düzeyde C-reaktif protein (CRP) ve D-dimer yüksekliği ile %41,0 (n=32)'inde ise ferritin yüksekliği mevcuttu. Yoğun bakım ünitesinde ortanca kalış süresi 26 (5-124) gündü. TPD işlemini takip eden 14. ve 28. günlerde gözlenen mortalite oranları sırasıyla %51,3 (n=41) ve %75,0 (n=60) idi. Çok değişkenli analizlerde ferritin yüksekliği, çoklu organ yetmezliği (multiple organ failure, MOF) varlığı ve entübasyon ihtiyacı mortalite ile ilişkili olarak bulundu.

Sonuç: Araştırmamızda TPD uygulanan COVID-19'lu hastalar için gözlenen mortalite oranı literatürdeki işlem yapılmayan olgularla benzer bulunurken, bu hastalarda ferritin yüksekliği, entübasyon ve MOF varlığının mortalite riskini artırdığı gösterilmiştir.

Anahtar kelimeler: Terapötik plazma değişimi; pnömoni; COVID-19.

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INTRODUCTION

The new type of coronavirus disease 2019 (COVID-19) is an infectious disease that first appeared in China at the end of 2019 and quickly spread to all continents, causing it to be declared as a global epidemic by the World Health Organization (WHO). The causative agent of the disease has been identified as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which manifests itself with respiratory symptoms such as fever, cough, and shortness of breath (1). Since the outbreak of the pandemic, many promising treatment options have been tested, but only a few have proven effective to date (2,3). Mortality rates in critically ill COVID-19 cases, on the other hand, are still very high (4,5).

Therapeutic plasma exchange (TPE) is a treatment modality based on separating the plasma from whole blood extracorporeally with the help of an apheresis device and returning the cellular blood components to the patient together with the replacement fluid (6). It is possible to remove the pathological substances from the body and replace missing plasma components with this method. TPE is a well-known treatment modality and it is currently used for many diseases (7).

During the pandemic, TPE has frequently been discussed for COVID-19 patients as a supportive non-drug treatment approach, and especially in severe cases requiring admission to the intensive care unit, it has been used as a remedy for cytokine storm (8-10). However, the literature on the subject is still limited and additional studies are needed.

This study aimed to examine the adult patients with COVID-19 who had undergone TPE in our apheresis unit and to determine the characteristics and clinical outcomes of these patients.

MATERIAL AND METHODS

We retrospectively searched the records of Adana City Training and Research Hospital's Apheresis Unit for adult patients aged 18 years and older who had TPE between Aug 01, 2020, and Sep 01, 2021. Then the medical records of these patients were examined and the cases whose COVID-19 positivity was confirmed by polymerase chain reaction (PCR) test were included in the study. Demographic data, medical information, and all laboratory results of the included patients were gathered from the electronic database of our center, which is routinely used for patient follow-up. However, the technical details of the apheresis procedures were obtained from the patient files stored in the apheresis unit.

An approval letter was obtained from the Clinical Research Ethics Committee of Adana City Training and Research Hospital (date: 04.07.2022, number: 109/2037), and the Helsinki Declaration criteria were considered.

The records were searched for concomitant diabetes mellitus, hypertension, chronic renal failure, and other comorbid chronic diseases for all cases included in the analysis, and the presence of sepsis/septic shock, acute respiratory distress syndrome (ARDS), and multiple organ failure (MOF) was noted. For every patient, the type of hospital unit (any type of inpatient care service or intensive care unit) where the cases were being followed on the first day of the TPE procedure was noted. The existence of pneumonia and the presence of pneumonia the need for

oxygen and intubation was searched and the WHO criteria were used for the definition and grading of COVID-19 associated pneumonia. All the treatments other than TPE started for COVID-19 were also noted for all patients. Patients were categorized according to the results of laboratory tests including lymphocyte count, C-reactive protein (CRP), ferritin, and D-dimer. According to the guidelines published by the Turkish Ministry of Health, exceeding certain threshold values in these tests have been defined as poor prognostic indicators, and the thresholds were given as follows: the absolute lymphocyte count below $0.8 \times 10^3/\mu\text{L}$. CRP level equal to or above 40 mg/L, ferritin level equal to or above 500 ng/mL, and D-dimer level equal to or above 1000 ng/mL. For all patients included in this study, these critical tests were screened and included in the analysis, considering the results just before the TPE was started (on the day of the TPE procedure or 24 h before).

In addition to demographic, clinical, and laboratory data, the length of stay in the intensive care unit and hospital, and the survival status of the patients who underwent the procedure were noted for further analysis.

Statistical Analysis

SPSS v.25.0 (IBM Corp. Armonk, NY) software was used in the analysis of the data. The compatibility of the variables to the normal distribution was examined by histogram graphics and the Kolmogorov-Smirnov test. Descriptive analyzes were presented using mean and standard deviation, median and minimum-maximum values, numbers, and percentages. Categorical variables were compared with the Pearson chi-square and Fisher's exact tests. Following univariate analyzes, the variables which were found to be significant considering the mortality groups at the end of the 14th day (CRP, ferritin, ARDS, MOF, intubation) and 28th day (D-dimer, sepsis/septic shock, MOF, intubation) were analyzed by including in the multivariate logistic regression (enter method) model. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 80 patients who had TPE due to COVID-19 in our apheresis unit were included in the study. The median age of the cases was 60 (19-85) years. The ratio of male patients was 67.5% (n=54), whereas the ratio of female patients was 32.5% (n=26). Hypertension (37.5%, n=30) and diabetes mellitus (23.8%, n=19) were the most frequently noted comorbid diseases in patients who underwent TPE (Table 1). When the procedure was started, 98.8% (n=79) cases had severe pneumonia and 77.5% (n=62) were intubated. The ratio of patients followed in the intensive care unit was 93.8% (n=75), and ARDS was noted in most of them at 65.0% (n=52). The ratio of patients with sepsis/septic shock was 82.5% (n=66), and the ratio of patients with MOF was 38.8% (n=31).

All included patients were receiving anti-viral therapy (favipiravir and/or hydroxychloroquine) and prophylactic anticoagulant therapy in accordance with the algorithm recommended by the Turkish Ministry of Health, and TPE was started in addition to these treatments.

The results of laboratory tests performed just before the initiation of the TPE and are known to have prognostic

significance were summarized in Table 2. Accordingly, the presence of lymphopenia at levels exceeding the critical threshold was remarkable in 82.5 % (n=66) of all cases. Similarly, more than three-quarters of the patients had critically elevated CRP and D-dimer. Ferritin levels above 500 ng/mL, which is considered the critical threshold, were recorded in 41.0% (n=32) of the cases.

During the procedure, only fresh frozen plasma was used as the replacement fluid in half of the cases 50.0% (n=40), while fresh frozen plasma and albumin were used together in the other half 50.0% (n=40). The number of procedures applied to the patients ranged from 1 to 11. While only one session of TPE was performed in 28.8% (n=23) cases, 2 sessions were performed in 22.5% (n=18) and 3 sessions were performed in 27.5% (n=22). Only 1 case had 11 procedures performed, and the ratio of patients who had more than 3 procedures was 21.3% (n=17).

Considering the clinical outcomes of the patients, for a total of 75 patients followed in the intensive care unit, the median length of stay was 26 (5-124) days. When all patients were evaluated together, the mortality rate observed on the 14th day following the TPE procedure was 51.3% (n=41) and it was 75.0% (n=60) on the 28th day.

Patients with different clinical features were compared for mortality rates and the results of the univariate analysis were summarized in Table 3. Regarding the mortality on the 14th day of TPE, statistically significant results were obtained for critical elevations in CRP (p=0.039) and ferritin levels (p=0.021), the presence of ARDS (p=0.005), the presence of MOF (p<0.001), the presence of intubation (p<0.001), and hospitalization at the intensive care unit instead of inpatient care service (p=0.018). The mortality rate on the 28th day was worse according to elevated D-dimer levels (p=0.013), the presence of ARDS (p=0.001), the presence of MOF (p<0.001), the presence of sepsis/septic shock (p=0.002), the presence of intubation (p<0.001), and hospitalization at the intensive care unit (p<0.001). According to statistical analysis, mortality rates did not increase in the presence of concomitant diseases such as hypertension, diabetes mellitus, and chronic renal failure.

According to the results of univariate analyses, the variables of CRP, ferritin, ARDS, MOF, and intubation showed statistically significant differences between mortality groups at the end of the 14th day (Table 3). These variables that were found to be significant as a result of univariate analyzes were included in the multivariate logistic regression model. And, it was found that ferritin level >500 ng/mL (OR: 3.68, 95% CI: 1.03-13.10, p=0.044), presence of MOF (OR: 5.07 95% CI: 1.27-20.10, p=0.021) and intubation (OR: 12.39 95% CI: 1.13-135.24, p=0.039) increased the mortality risk at 14th day (Table 4).

Regarding mortality rate on the 28th day, the variables of D-dimer, sepsis/septic shock, MOF, and intubation showed statistically significant differences between mortality groups (Table 3). These variables that were found to be significant as a result of univariate analyzes were included in the multivariate logistic regression model and according to the results of the multivariate logistic regression model, it was determined that the presence of intubation during the procedure (OR: 8.45, 95% CI: 1.64-43.35, p=0.010) increased the risk of mortality (Table 4).

DISCUSSION

The patients who underwent TPE due to COVID-19 at our center were examined retrospectively, and the clinical features of these patients, their prognostic status according to some certain blood tests considered critical during COVID-19, and clinical outcomes such as the length of stay in the intensive care unit and mortality rate were displayed in our study.

It was observed for all cases that the TPE procedure was used as a supportive therapy in addition to the treatment algorithm recommended in the guidelines published by the Turkish Ministry of Health for COVID-19 patients. When the literature on the subject was examined, the predicted mechanism of action for TPE was found to be the reduction of auto- and allo-antibodies, inflammatory mediators, and some other proteins in the plasma, thereby reducing the destructive and uncontrolled cycle that progressed in a cascade (11,12). Although some articles have suggested that plasma exchange reduces viral load in patients with COVID-19, no published source has been found in the literature to date that TPE and SARS-CoV-2 load decreases.

During the application of the TPE procedure, there is a need for a replacement fluid and it has been hypothesized that the use of plasma instead of albumin may replace some of the protective factors preserving microcirculation, which are depleted during the disease (such as protein C and ADAMTS-13: a disintegrin and metalloproteinase with

Table 1. Clinical characteristics of patients undergoing therapeutic plasma exchange (n=80)

Comorbid diseases, n (%)	
Diabetes Mellitus	19 (23.8)
Hypertension	30 (37.5)
Chronic Renal Failure	4 (5.0)
Follow-Up in the Intensive Care Unit, n (%)	75 (93.8)
Intubation, n (%)	62 (77.5)
Acute Respiratory Distress Syndrome, n (%)	52 (65.0)
Sepsis/Septic Shock, n (%)	66 (82.5)
Multiple Organ Failure, n (%)	31 (38.8)

Table 2. Laboratory values of patients before therapeutic plasma exchange

Laboratory Test (n*)	Median (Min-Max)	Critical Threshold for Poor Prognosis**	Rate of Patients over the Critical Threshold
WBC , 10 ³ /μL (n=80)	13.7 (1.0-33.7)		
Lymphocyte , 10 ³ /μL (n=80)	0.4 (0.1-18.1)	<0.8	82.5% (n=66)
C-reactive protein , mg/L (n=79)	86 (2-17300)	>40	81.0% (n=64)
Ferritin , ng/mL (n=78)	424 (17-20880)	>500	41.0% (n=32)
D-dimer , ng/mL (n=74)	2320 (1-14340)	>1000	75.7% (n=56)

*: the number of patients who have been tested, **: poor prognosis criteria of the Turkish Ministry of Health

Table 3. Mortality rates according to the characteristics of patients on the 14th and 28th days of therapeutic plasma exchange

	Total number	14 th day mortality	p*	28 th day mortality	p*
Gender, n (%)					
Male	54 (67.5)	27 (50.0)	0.747 ^a	39 (72.2)	0.408 ^a
Female	26 (32.5)	14 (53.9)		21 (80.8)	
Lymphocyte, n (%)					
<0.8 x10 ³ /μL	66 (82.5)	36 (54.6)	0.200 ^a	52 (78.8)	0.089 ^a
>0.8 x10 ³ /μL	14 (17.5)	5 (35.7)		8 (57.1)	
D-dimer, n (%)					
<1000 ng/mL	18 (24.3)	8 (44.4)	0.420 ^a	10 (55.6)	0.013^a
>1000 ng/mL	56 (75.6)	31 (55.4)		47 (83.9)	
CRP, n (%)					
<40 mg/L	15 (18.9)	4 (26.7)	0.039^a	10 (66.7)	0.428 ^a
>40 mg/L	64 (81.0)	36 (56.3)		49 (76.6)	
Ferritin, n (%)					
<500 ng/mL	46 (58.9)	18 (39.1)	0.021^a	33 (71.7)	0.525 ^a
>500 ng/mL	32 (41.0)	21 (65.6)		25 (78.1)	
ARDS, n (%)					
No	28 (35.0)	8 (28.6)	0.005^a	14 (50.0)	0.001^b
Moderate	35 (43.7)	20 (57.1)		30 (85.7)	
Severe	17 (21.3)	13 (76.5)		16 (94.1)	
Multiple Organ Failure, n (%)					
No	49 (61.2)	16 (32.7)	<0.001^a	30 (61.2)	<0.001^b
Yes	31 (38.8)	25 (80.7)		30 (96.8)	
Sepsis/Septic Shock, n (%)					
No	14 (17.5)	4 (28.6)	0.062 ^a	6 (42.9)	0.002^a
Yes	66 (82.5)	37 (56.1)		54 (81.8)	
Intubation, n (%)					
No	18 (22.5)	2 (11.1)	<0.001^b	5 (27.8)	<0.001^a
Yes	62 (77.5)	39 (62.9)		55 (88.7)	
Follow-up Unit, n (%)					
Inpatient Care Service	5 (6.2)	0 (0.0)	0.018^b	0 (0.0)	<0.001^b
Intensive Care Unit	75 (93.7)	41 (54.7)		60 (80.0)	

a: Pearson chi-square test, b: Fisher's exact test

Table 4. Logistic regression analysis for mortality rates on the 14th and 28th day of therapeutic plasma exchange

14 th day Mortality	OR (95% CI)	p
CRP (>40 mg/L)	1.22 (0.25-5.83)	0.797
Ferritin (>500 ng/mL)	3.68 (1.03-13.10)	0.044
ARDS (No)		0.945
ARDS (Moderate)	1.24 (0.25-5.97)	0.786
ARDS (Severe)	1.02 (0.14-7.44)	0.984
MOF (Yes)	5.07 (1.27-20.10)	0.021
Intubation (Yes)	12.39 (1.13-135.24)	0.039
28 th day Mortality	OR (95% CI)	p
D-dimer (>1000 ng/mL)	3.39 (0.76-15.04)	0.107
Sepsis/septic shock (Yes)	1.01 (0.18-5.70)	0.985
MOF (Yes)	6.87 (0.71-66.01)	0.095
Intubation (Yes)	8.45 (1.64-43.35)	0.010

CRP: C-reactive protein, ARDS: acute respiratory distress syndrome, MOF: multiple organ failure, OR: odds ratio, CI: confidence interval

a thrombospondin type 1 motif, member 13) (9,13). In parallel with these suggestions, we found that fresh frozen plasma was used as the replacement fluid in half of our patients who underwent TPE, whereas albumin was used together with fresh frozen plasma in the other ones. Nearly all of the study population had severe COVID-19 related pneumonia, of which more than half developed

ARDS and we found that the presence of intubation at the start of TPE increased the mortality rates. Different sources have reported ARDS and cytokine storm syndrome as the main causes of death for patients with COVID-19 infection (14-16). However, approximately half of the patients presenting with cytokine storm syndrome develop ARDS (17). Therefore, early recognition and control of uncontrolled immune reactions are essential in these patients. Ferritin, CRP, and D-dimer levels are clues used to detect the severity of systemic inflammatory responses (18). In our study population, CRP levels in 81.0% (n=64), D-dimer in 75.7% (n=56), and ferritin levels in 41.0% (n=32) were found to be above the critical threshold values that predict poor prognosis and this suggests the presence of intensive cytokine storms during the TPE procedure in most of our population. Additionally, our statistical analysis revealed a relation between mortality rate and increased levels of ferritin, which is in parallel with the previously reported literature (19,20).

Unfortunately, we do not have a control group with whom the included cases could be compared, who were positive for COVID-19 and did not undergo TPE. However, the follow-up rate in the intensive care unit of our current study population is 93.8% (n=75), and the mortality rate reported in the literature for patients who need follow-up in the intensive care is around 50% (21,22). In a study published in Turkey, on the other hand, this rate was reported as 78% (23). The mortality rate observed for the

patients who underwent TPE was 75.0% (n=60) on the 28th day of the procedure in our study, and this rate is similar to the cases in which the procedure was not performed compared to the literature. As a matter of fact, it was also concluded in a similar observational study published in Iran that TPE could not reduce mortality rates (24). These findings contradict the literature reported on the effectiveness of TPE to date and needs to be confirmed by further observation and prospective studies (9,25,26).

In this study, most of the research population consisted of cases with poor prognostic features according to both clinical and laboratory characteristics. The median age of these cases is 60 years, and when considered from the perspective of the attending physician, there is a relatively young patient group who have exhausted all the treatment options. It appears that TPE is applied as a last-resort treatment in these cases. Current literature for recommendations on the selection of COVID-19 patients for TPE is scant, and it is unclear which patient will be selected at which stage of the disease. Therefore, studies involving large case series on the subject are urgently needed.

CONCLUSION

In this study, we found that elevated ferritin levels, intubation, and the presence of MOF increase the risk of mortality in TPE applied patients with COVID-19 and severe pneumonia. However, the mortality rates did not seem to be much different in our TPE treated patient population considering the literature on TPE naïve patients with COVID-19.

Although the development of vaccines and new treatments has come a long way in the pandemic process, for some patients, COVID-19 is still a deadly disease that progresses to the critical illness stage. Prospective studies in which the best treatment algorithms will be developed will primarily be led by a retrospective analysis of the existing data and it is thought that our study and findings will lead to larger-scale, multicenter prospective studies on the subject and that TPE will maintain its importance as a supportive treatment approach in critically ill patients.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Adana City Training and Research Hospital (04.07.2022, 109/2037).

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Evaluation of In Vitro Fertilization Outcomes in Women with Hypogonadotropic Hypogonadism

Hipogonadotropik Hipogonadizimli Kadınlarda İn Vitro Fertilizasyon Sonuçlarının Değerlendirilmesi


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ABSTRACT

Aim: Hypogonadotropic hypogonadism (HH) is a rare clinical condition resulting from gonadal insufficiency due to low pituitary gonadotropin levels. Since ovulation occurs rarely in these patients, the probability of spontaneous pregnancy is very low. The study aimed to evaluate the in vitro fertilization (IVF) treatment outcomes in patients with HH and to compare these results with that of patients with unexplained infertility (UI) who underwent IVF treatment.

Material and Methods: In this study, 36 cycles of 28 HH patients who underwent IVF treatment and 72 cycles of 68 patients who underwent IVF treatment for UI were included. Demographic data, ovarian hyperstimulation and cycle outcomes, clinical pregnancy rates, and predictive factors for clinical pregnancy were evaluated retrospectively, and the two groups were compared.

Results: In the HH group, clinical pregnancy, and live birth rates per cycle after IVF were significantly higher compared with the UI group (n=16, 44.4% vs. n=17, 23.6%, p=0.027; and n=14, 38.9% vs. n=14, 19.4%; p=0.030, respectively). Although the number of antral follicles (p=0.001) and retrieved oocytes (p=0.042) were significantly higher in the UI group, the number of mature oocytes and grade I-II embryos were similar in the HH and UI groups. The total gonadotropin dose used and duration of stimulation in the HH group were significantly higher than in the UI group (both p=0.001).

Conclusion: HH patients responded well to IVF treatment and had better IVF outcomes compared to women who underwent IVF for UI. No prognostic factor that affected pregnancy success in HH patients was detected.

Keywords: Hypogonadotropic hypogonadism; IVF outcomes; unexplained infertility.

ÖZ

Amaç: Hipogonadotropik hipogonadizm (HH), düşük hipofizer gonadotropin düzeylerine bağlı gonadal yetmezlikten kaynaklanan nadir bir klinik durumdur. Bu hastalarda ovulasyon nadiren gerçekleştiği için spontan gebelik olasılığı çok düşüktür. Bu çalışmanın amacı, HH hastalarında in vitro fertilizasyon (IVF) tedavi sonuçlarını değerlendirmek ve bu sonuçları açıklanamayan infertilitesi (Aİ) olan ve IVF tedavisi uygulanan hastalarla karşılaştırmaktır.

Gereç ve Yöntemler: Bu çalışmaya IVF tedavisi uygulanan 28 HH hastasının 36 siklusu ve Aİ nedeniyle IVF tedavisi uygulanan 68 hastanın 72 siklusu dahil edildi. Demografik veriler, ovaryan hiperstimülasyon ve siklus sonuçları, klinik gebelik oranları ve klinik gebelik için prediktif faktörler geriye dönük olarak değerlendirildi ve iki grup karşılaştırıldı.

Bulgular: IVF sonrası siklus başına klinik gebelik ve canlı doğum oranları Aİ grubu ile karşılaştırıldığında HH grubunda anlamlı derecede daha yüksekti (sırasıyla, n=16, %44,4'e karşı n=17, %23,6; p=0,027 ve n=14, %38,9'a karşı n=14, %19,4; p=0,030). Antral folikül sayısı (p=0,001) ve toplanan oosit sayısı (p=0,042) Aİ grubunda anlamlı olarak daha yüksek olmasına rağmen, matür oosit ve grade I-II embriyo sayısı HH ve Aİ gruplarında benzerdi. HH grubunda kullanılan toplam gonadotropin dozu ve stimülasyon süresi Aİ grubuna göre anlamlı derecede daha yüksekti (her iki p=0,001).

Sonuç: HH hastaları, IVF tedavisine iyi yanıt verdiler ve Aİ nedeniyle IVF uygulanan kadınlara oranla IVF sonuçları daha iyi oldu. HH hastalarında gebelik başarısını etkileyen herhangi bir prognostik faktör saptanmadı.

Anahtar kelimeler: Hipogonadotropik hipogonadizm; IVF sonuçları; açıklanamayan infertilite.

INTRODUCTION

Hypogonadotropic hypogonadism (HH) is a rare clinical syndrome caused by hypothalamic or pituitary defects that lead to gonadal insufficiency (1). HH is classified as group 1 ovulation disorders according to the World Health Organization (WHO) classification (2). Depending on the age of onset, patients experience delayed or arrested puberty, secondary amenorrhea, and infertility. Biochemically, it is characterized by low serum sex steroid hormone levels, low or normal luteinizing hormone (LH), and low follicle stimulating hormone (FSH) levels (3). Since ovulation occurs rarely in these patients, the probability of spontaneous pregnancy is very low. Therefore, fertility in these patients is achieved by assisted reproductive techniques (4).

As it is a rare disease, there are a limited number of studies evaluating the reproductive capacity and infertility treatment outcomes in women with HH. These patients appear to be definite candidates for ovulation induction with exogenous gonadotropins (5). Studies have found that the duration of ovarian stimulation is long and the total gonadotropin dose used is high in HH patients (5,6). In the literature, pregnancy outcomes in patients who underwent in vitro fertilization (IVF) for HH were compared with the IVF outcomes of different infertility groups (5,7,8), and the outcomes were reported to be comparable and even better in patients with the diagnosis of HH.

In this study, we aimed to evaluate the treatment results of patients with HH who underwent IVF treatment and to compare these results with the results of patients who underwent IVF for unexplained infertility (UI).

MATERIAL AND METHODS

In this study, the records of the patients who attended to the Etlik Zübeyde Hanım Women's Health Training and Research Hospital, Health Sciences University, Assisted Reproductive Technologies Clinic, Ankara, Turkey between September 2007 and July 2019 for IVF treatment were retrospectively analyzed. A total of 36 IVF cycles of 28 patients diagnosed as HH were examined as the study group. It was decided to recruit twice the number of study patients as the control group and 72 IVF cycles of 68 patients determined by randomization table among the patients with an International Statistical Classification of Diseases and Related Health Problems (ICD) code of N97.9 diagnosed as UI during the time span were evaluated. The study was approved by the institutional ethics committee (22/07/2020, 2020/100) and was conducted in accordance with the Declaration of Helsinki. At the beginning of the treatment, all patients were informed that the treatment process data could be used in scientific research and consent had been obtained.

In the study group, patients aged 18-35 years old who were diagnosed as HH with a basal serum FSH level <5 mIU/ml and LH level <5 mIU/ml, body mass index (BMI) below 30 kg/m^2 , and who received IVF treatment in our hospital were included. Patients over 35 years of age, patients with BMI $>30 \text{ kg/m}^2$, concomitant male factor, diminished ovarian reserve (DOR), and/or endometriosis or leiomyoma of the uterus were excluded. In the UI group, the inclusion criteria were being aged 18-35 years, having normal ovarian reserve tests (day 3 basal serum FSH level ≤ 10 mIU/mL and estradiol (E2) level ≤ 80 pg/ml, serum

anti-Müllerian hormone (AMH) level >1.1 ng/ml and antral follicle count >5), normal hysterosalpingography and normal spermogram values in accordance with the WHO criteria. Patients with endometriosis, endocrine pathology, leiomyoma were also excluded from the control group.

In the UI group, standard gonadotropin releasing hormone agonist (GnRH-a) or gonadotropin releasing hormone antagonist (GnRH-ant) protocols were applied after the patient's baseline evaluation. Ovarian hyperstimulation was started on the 2nd or 3rd day of menstruation using human menopausal gonadotropin (hMG, Menogon, Ferring, Turkey or Merional, IBSA, Turkey) in the HH patient group and recombinant FSH (Gonal F, Merck Serono, İstanbul, Turkey or Puregon, Organon, İstanbul, Turkey) and/or hMG was used in the UI patient group. In both groups, the dose of gonadotropins was personalized according to the patient's age, antral follicle count, and BMI, and necessary dose changes were made according to the ovarian response. Patients were monitored with serial transvaginal ultrasonography (TVUSG) for follicular development and serum E2, LH, and progesterone level measurements until the ovulation trigger. Recombinant human chorionic gonadotropin (hCG) was applied when at least 3 follicles reached a mean diameter of 18 mm. The oocyte retrieval (oocyte pick-up, OPU) procedure was performed 34-36 hours following hCG administration with TVUSG-guided aspiration.

All mature oocytes were inseminated using an intracytoplasmic sperm injection (ICSI) procedure. The presence of fertilization was confirmed by the appearance of two pronuclei 18-20 hours after ICSI. Day 3 embryos were classified in accordance with the embryo classification system 61-65 hours after ICSI using the number, size, and symmetry of the cells and the degree of fragmentation (9). At the blastocyst stage, embryo scoring was based on the equal-sized blastomere number and presence of adhesion, visible blastocyst cavity and inner cell mass, zona pellucida thickness, and trophectoderm with adequate cellular continuity (10).

In the presence of an available embryo(s), embryo transfer (ET) was performed under the guidance of transabdominal ultrasonography.

Luteal phase support was started for all patients following the OPU procedure with vaginal progesterone (Crinone 8% gel, Serono, İstanbul, Turkey) or vaginal progesterone plus 100 mg intramuscular progesterone (Progestan, Kocak, İstanbul, Turkey). The pregnancy test was performed on the 14th day after OPU and in case of a positive test, β hCG measurement was repeated 2-4 days later. Patients with sufficient elevation were called for a follow-up 14 days later and an ultrasonographic examination was performed. The presence of a fetal heartbeat was considered as clinical pregnancy. Luteal support was continued until 12 weeks of gestation.

The ET could not be performed when i) no follicular development was observed with ovarian stimulation, ii) no mature oocytes were retrieved, and iii) fertilization failure or embryo development arrest was encountered.

Demographic characteristics, ovarian hyperstimulation and OPU outcomes, embryo development, ET, and clinical pregnancy and live birth rates of the HH patients

undergoing IVF treatment were evaluated. The data obtained were compared with the data of the patients who underwent IVF with the diagnosis of UI.

Statistical Analysis

Data analysis was done with IBM SPSS v.22.0 package. Descriptive statistics were presented as mean±standard deviation and median (minimum-maximum) for continuous variables, and as numbers and percentages for categorical variables. Whether the distribution of continuous variables was normal was evaluated with the Shapiro-Wilk test. In cases where normal distribution was obtained, the groups were compared with Student's t-test, and in cases where normal distribution was not achieved with the Mann-Whitney U test. The Pearson chi-square or Fisher's exact test was used to compare the categorical variables. For $p < 0.05$, the results were considered statistically significant.

RESULTS

The study included 28 patients who underwent IVF treatment for HH and 68 patients who underwent IVF treatment for UI. The data from 36 cycles of 28 patients in the HH group and 72 cycles of 68 patients in the UI group were evaluated. The demographic characteristics of the patients were shown in Table 1.

The total gonadotropin dose used and the duration of ovarian hyperstimulation in the HH group were

significantly higher than in the UI patients ($p=0.001$). However, the number of oocytes retrieved ($p=0.042$) and serum progesterone level on the day of OPU ($p=0.020$) were higher in the UI group than in the HH group, and these differences were statistically significant. On the other hand, mature oocyte count, fertilized oocyte count, grade I-II embryo count, and ET rate were similar in both groups. The cycle characteristics of the patients were shown in Table 2.

There was no statistically significant difference between the number of patients who underwent ET in the HH group and in the UI group ($p=0.120$). Pregnancy occurred in 17 of 36 cycles (47.2%) in the HH group and the clinical pregnancy rate was 44.4% ($n=16$). In the UI group, 20 of 72 cycles (27.8%) resulted in pregnancy. In the HH group, the clinical pregnancy rate was 44.4% ($n=16/36$) per cycle and 57.1% ($n=16/28$) per patient whereas, in the UI group, the clinical pregnancy rate was 23.6% ($n=17/72$) per cycle and 25.0% ($n=17/68$) per patient. Clinical pregnancy rates per cycle and per patient were significantly higher in the HH group compared with the UI group ($p=0.027$, and $p=0.003$, respectively). The data on pregnancy outcomes were compared in Table 3.

The demographic characteristics and cycle outcomes of the HH patients with and without clinical pregnancy were compared and no significant difference was found apart from duration of infertility, serum E2 level on ET day, and the number of patients who underwent ET (Table 4). The duration of infertility was significantly longer and serum E2 level on ET day was significantly higher in patients who could not achieve clinical pregnancy ($p=0.019$, and $p=0.011$, respectively).

DISCUSSION

Hypogonadotropic hypogonadism is a rare disorder characterized with hypothalamic or pituitary defects that lead to gonadal insufficiency (1) with a spectrum of clinical symptoms depending on the age of onset and the degree of FSH and LH deficiency. Fertility is reduced in these patients as a result of anovulation, therefore assisted reproductive technologies are provided to achieve pregnancy (4). The infertility treatment protocol in HH

Table 1. The demographic characteristics of the patients

	HH (n=28)	UI (n=68)	p
Age (years)	30 (23-35)	32 (23-34)	0.858
Height (cm)	163.5 (146-178)	153.7 (144-179)	0.001
Weight (kg)	66 (47-80)	55 (43-94)	0.001
BMI (kg/m ²)	24.7 (18.4-30.0)	23.1 (18.9-31.6)	0.002
FSH (mIU/ml)	0.37 (0.0-4.6)	3.85 (0.7-9.8)	0.001
LH (mIU/ml)	0.20 (0.0-3.6)	4.82 (1.4-12.6)	0.001
E2 (pg/ml)	14.5 (3.0-54.0)	56.2 (11.8-181.0)	0.001
Duration* (months)	69 (18-168)	72 (24-228)	0.315

HH: hypogonadotropic hypogonadism, UI: unexplained infertility, BMI: body mass index, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, *: duration of infertility, data presented as median (minimum-maximum)

Table 2. Comparison of the in vitro fertilization cycle characteristics of the HH and UI groups

	HH (n=36)	UI (n=72)	p
Total gonadotropin dose (IU)	4045±1370	1950±936	0.001
Ovarian stimulation duration (days)	11.2±1.6	9.1±3.1	0.001
Antral follicle count	6.5 (0-30)	14 (2-44)	0.001
E2 level on OPU day (pg/ml)	1376.75±1015.43	1515.63±1197.44	0.552
Progesterone level on OPU day (ng/ml)	0.18±0.30	0.95±1.82	0.020
Endometrial thickness on OPU day (mm)	9.8±2.4	8.8±3.5	0.139
Number of oocytes retrieved	9 (0-35)	13 (2-43)	0.042
Number of mature oocytes	7 (1-26)	9 (0-30)	0.580
Number of fertilized oocytes	4 (0-21)	5 (0-25)	0.069
Number of good quality embryos	1 (0-3)	1 (0-3)	0.246
ET ratio, n (%)	29 (80.6)	66 (91.7)	0.120
Progesterone level on ET day (ng/ml)	61.57±34.42	93.59±64.69	0.077
E2 level on ET day (pg/ml)	1515.87±1287.22	1780.19±1217.49	0.154
Endometrial thickness on ET day (mm)	9.5±2.2	10.3±1.9	0.075

HH: hypogonadotropic hypogonadism, UI: unexplained infertility, OPU: oocyte pick-up, E2: estradiol, ET: embryo transfer, descriptive statistics of the variables were presented as mean±standard deviation or median (minimum-maximum)

Table 3. Comparison of the in vitro fertilization cycle outcomes of the patients with HH and UI

	HH (n=36)	UI (n=72)	p
Number of cycles with ET, n (%)	29 (80.6)	66 (91.7)	0.120
Implantation rate, n (%)	17 (47.2)	20 (27.8)	0.045
Clinical pregnancy rate per cycle, n (%)	16 (44.4)	17 (23.6)	0.027
Clinical pregnancy rate per patient, n (%)	16/28 (57.1)	17/68 (25.0)	0.003
Biochemical pregnancy, n (%)	1 (2.8)	3 (4.2)	0.999
Clinical pregnancy outcomes, n (%)			
Live birth rate per cycle	14 (38.9)	14 (19.4)	0.030
Live birth rate per patient	14/28 (50.0)	14/68 (20.6)	0.004
Miscarriage rate	2 (5.6)	3 (4.2)	0.999

HH: hypogonadotropic hypogonadism, UI: unexplained infertility, ET: embryo transfer, data presented as n (%)

Table 4. Comparison of demographic characteristics and IVF cycle data in patients with HH who achieved clinical pregnancy and who did not

	Clinical Pregnancy (n=16)	No Pregnancy (n=20)	p
Age (years)	29.1±4.1	29.8±3.22	0.970
BMI (kg/m ²)	25.1 (18.6-30.0)	23.9 (18.4-30.0)	0.626
Duration of infertility (months)	51 (18-132)	72 (36-168)	0.019
FSH (mIU/ml)	0.32 (0-4.59)	0.61 (0.01-3.70)	0.737
LH (mIU/ml)	0.10 (0-3.60)	0.25 (0.07-2.09)	0.680
E2 (pg/ml)	13.95 (3.00-54.00)	17.05 (5.00-43.72)	0.737
Total gonadotropin dose (IU)	3776±1134	4260±1528	0.300
Ovarian stimulation duration (days)	11.3±1.4	11.2±1.7	0.835
Antral follicle count	7 (3-18)	5 (0-30)	0.228
E2 level on OPU day (pg/ml)	1096±795	1600±1131	0.142
Number of oocytes retrieved	10 (4-35)	8.5 (0-27)	0.970
Number of mature oocytes	9 (3-26)	7 (1-17)	0.760
Number of good quality embryos	1 (1-3)	0.5 (0-3)	0.239
E2 level on ET day (pg/ml)	987±800	2166±1494	0.011
Endometrial thickness on ET day (mm)	9.5±2.5	9.5±1.8	0.966

BMI: body mass index; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol, OPU: oocyte pick-up; ET: embryo transfer, descriptive statistics of the variables were presented as mean±standard deviation or median (minimum-maximum)

patients is not clear yet. In HH patients, both FSH and LH are required for ovarian hyperstimulation to achieve full maturation of follicles and to obtain oocytes with fertilization capacity. In our study, the IVF outcomes in HH patients were investigated, and 16 clinical pregnancies occurred in these patients with 14 live births. The rate of achieving clinical pregnancy with IVF treatment in HH patients was found to be 44.4% per cycle and 57.1% per patient, and the live birth rate was 38.9% per cycle, 50% per patient. It has been concluded that successful results can be obtained with IVF in HH patients.

Previous studies have shown that the duration of ovarian hyperstimulation and total gonadotropin dose used in HH patients is higher than in patients with other infertility etiologies (5,6). Similarly, in our study, we found that the HH patient group required longer and higher doses of gonadotropin administration than the patients with UI. This can be explained by the presence of silent ovaries that need activation before a follicular response can be obtained. However, it has been shown that high gonadotropin doses may have adverse effects on the oocytes or embryos (11,12) and may impair fertilization. The results regarding the fertilization rate in HH patients

are conflicting. Gaffari et al. (13) concluded in their study that the fertilization rate was higher in patients with tubal infertility compared with the HH patient group whereas Ulug et al. (5) found no difference in terms of fertilization rate between patients who underwent IVF for tubal infertility and HH. Kumbak et al. (7) on the other hand reported a higher fertilization rate in HH patients than in UI patients. Despite the differences in fertilization rates in all these studies, the number of mature oocytes and good-quality embryos were reported to be similar (5,7,13). In our study, the number of mature oocytes, fertilization rate, and the number of good-quality embryos were found to be similar in the HH group and the control group and it was concluded that high-dose gonadotropins did not have a negative effect.

In previous studies, the IVF outcomes of HH patients were compared with the outcomes of patients who underwent IVF for different etiologies. In a study similar to the presented study performed by Kumbak et al. (7) the IVF outcomes of 27 patients with HH and 39 patients with UI were compared and the pregnancy rate was found to be 59% in HH patients and 46% in patients with UI. It was stated that there was no significant difference in pregnancy

rates between the two groups. Ulug et al. (5) compared the IVF results of 58 HH patients with the results of patients with tubal infertility. ET was performed in 53 of 58 (91.3%) HH patients. In the presented study, the ET ratio in the HH group was slightly lower than the ratio in the UI group (80.6% vs 91.7% respectively) but the difference was not statistically significant. Ulug et al. (5) reported that 30 patients (51.7%) achieved pregnancy, and the pregnancy rate per ET was 56.6% which was found to be similar with the pregnancy rate of women with tubal factor infertility. In a recent study performed by Zhang et al. (14), the live birth rate of the first fresh cycle was found to be higher in the control group which included patients with tubal or male factor infertility compared with the HH group but the difference was not statistically significant. In our study, the clinical pregnancy rate per cycle and per patient after IVF treatment in HH patients was found to be significantly higher, than that of women with UI (44.4% per cycle and 57.1% per patient versus 23.6% per cycle and 25.0% per patient). The reported pregnancy rates after IVF-ET treatment in women with UI vary between 20.7 to 45.8% in the literature (15-17). The reason for this wide range can be explained by the fact that the success of achieving pregnancy with IVF in UI patients is affected by other factors including age, BMI, duration of infertility, and basal gonadotropin levels.

In another study, Yılmaz et al. (2) compared the IVF outcomes of HH patients with couples who had IVF treatment for male factor infertility. While the pregnancy rate was 30% (10/33) in patients with HH, this rate was 31.4% in women who underwent IVF for male factor infertility in their study, and they stated that the IVF treatment results were similar in both groups. In a similar study, Yıldırım et al. (8) examined the IVF outcomes in 13 women with HH and compared the results with the outcomes of women with tubal factor infertility. In conclusion, they reported that 4 (30%) of 13 HH patients achieved pregnancy, and the pregnancy and live birth rates were similar with tubal factor infertility patients.

When the prognostic factors for achieving pregnancy in infertile patients other than the HH group basal serum FSH, LH, E2, and serum AMH levels are found to be associated with ovarian response to hyperstimulation and IVF outcomes. However, these factors cannot be used in women with HH as the basal gonadotropin levels are very low. In our study, it was shown that the basal FSH, LH, and E2 levels, albeit low, were not associated with IVF treatment results in HH patients. Eroglu et al. (18) reported that higher E2 level on hCG day, increased endometrial thickness, and a higher number of oocytes retrieved were the factors affecting the ongoing pregnancy rates in the HH group. We found that the number of oocytes retrieved and the endometrial thickness on ET day were similar in the HH patients who achieved clinical pregnancy and who did not, but higher E2 levels were measured on ET day in HH patients who did not get clinical pregnancy.

CONCLUSION

In conclusion, the IVF outcomes in HH patients are quite promising, and clinical pregnancy and live birth rates are higher compared with the patients undergoing IVF with the diagnosis of UI.

Ethics Committee Approval: The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Women's Health Training and Research Hospital (22.07.2020, 100).

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Fractal Dimension of Silhouette Magnetic Resonance Brain Images as a Measure of Age-Associated Changes in Cerebral Hemispheres

Serebral Hemisferdeki Yaşa Bağlı Değişikliklerin Bir Ölçüsü Olarak Silüet Manyetik Rezonans Beyin Görüntülerinin Fraktal Boyutu

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ABSTRACT

Aim: The aim of the present study was to characterize age-associated changes in the spatial configuration of cerebral hemispheres (including changes in spatial complexity and space-filling capacity) using fractal analysis of silhouette magnetic resonance brain images.

Material and Methods: Magnetic resonance brain images of 100 (44 male, 56 female) participants aged between 18-86 years were studied. Five magnetic resonance images were selected from the magnetic resonance imaging dataset of each brain, including four tomographic sections in the coronal plane and one in the axial plane. Fractal dimension values of the cerebral hemispheres silhouettes were measured using the two-dimensional box-counting algorithm. Morphometric parameters based on Euclidean geometry (perimeter, area, and their derivative values) were determined as well.

Results: The average fractal dimension value of the five studied tomographic sections was 1.878 ± 0.0009 , the average value of four coronal sections was 1.868 ± 0.0010 . It was shown that fractal dimension values of cerebral silhouettes for all studied tomographic sections and four coronal sections significantly decrease with age ($r = -0.512$, $p < 0.001$ and $r = -0.491$, $p < 0.001$, respectively). The difference in the character of age-related changes in males and females was not statistically significant. Based on the age and the fractal dimension values of the studied sample, the confidence intervals of the fractal dimension values of cerebral hemispheres silhouettes were determined, which can be used as norm criteria in clinical neuroimaging.

Conclusion: The fractal analysis and obtained data can be used in neuroimaging for assessing the degree of age-related cerebral atrophy and for differentiating between normal aging and neurodegenerative diseases.

Keywords: Fractal; cerebrum; aging; atrophy; neuroimaging.

ÖZ

Amaç: Bu çalışmanın amacı, manyetik rezonans beyin görüntülerinden elde edilen silüet görüntülerin fraktal analizini kullanarak serebral hemisferlerin uzaysal konfigürasyonundaki yaşa bağlı değişiklikleri (uzaysal karmaşıklık ve boşluk doldurma kapasitesindeki değişiklikler dahil) tanımlamaktır.

Gereç ve Yöntemler: Yaşları 18-86 yıl arasında olan 100 (44 erkek, 56 kadın) katılımcının manyetik rezonans beyin görüntüleri incelenmiştir. Her bir beyin manyetik rezonans görüntülemeye, koronal düzlemde dört tomografik kesit ve aksiyal düzlemde bir kesit dahil olmak üzere beş manyetik rezonans görüntüsü seçildi. Serebral hemisfer silüetlerinin fraktal boyut değerleri, iki boyutlu kutu sayma algoritması kullanılarak ölçüldü. Öklid geometrisine dayalı morfolometrik parametreler (çevre, alan ve bunlardan türetilen değerler) de belirlendi.

Bulgular: Çalışılan beş tomografik kesitin ortalama fraktal boyut değeri $1,878 \pm 0,0009$ ve dört koronal kesitin ortalama değeri ise $1,868 \pm 0,0010$ idi. İncelenen tüm tomografi kesitleri ve dört koronal kesit için serebral silüetlerin fraktal boyut değerlerinin yaş ile birlikte anlamlı olarak azaldığı gösterildi (sırasıyla $r = -0,512$, $p < 0,001$ ve $r = -0,491$, $p < 0,001$). Erkeklerde ve kadınlarda yaşa bağlı değişikliklerin karakterindeki fark istatistiksel olarak anlamlı değildi. Çalışılan örneğin yaş ve fraktal boyut değerleri esas alınarak, serebral hemisfer silüetlerinin fraktal boyut değerleri için klinik nörogörüntüleme norm kriteri olarak kullanılabilir güven aralıkları belirlendi.

Sonuç: Fraktal analiz ve elde edilen veriler, yaşa bağlı serebral atrofi derecesini değerlendirmek ve normal yaşlanma ile nörodejeneratif hastalıklar arasında ayırım yapmak için nörogörüntüleme kullanılabilir.

Anahtar kelimeler: Fraktal; beyin; yaşlanma; atrofi; nöro-görüntüleme.

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INTRODUCTION

Among the various structures and formations in the human body, the cerebrum has one of the most sophisticated shapes reflecting the complexity of its morphofunctional organization (1,2). During adulthood, morphological changes occur in different brain structures, reflecting its development and aging (3-5). Brain aging is accompanied by mostly atrophic changes, affecting the shape and dimensions of brain structures (3-5). Quantitative characterization of age-related changes in the brain shape is an important area of clinical neuroscience and neuro-morphology research (3-5). This is necessary both for determining the degree of atrophy and for differentiating changes in normal aging and in neurodegenerative diseases (3-7).

Numerous studies aimed to characterize and quantify brain structures using traditional morphometric methods, including volumetric studies, measurements of gray and white matter volumes (8-12), assessment of the cortical thickness (11-13), sulcal depth (12,13), and gyrification index, the ratio of the total brain surface to the superficially exposed brain surface (11,13-15). But the spatial configuration of the cerebral hemispheres is too irregular and difficult to be comprehensively assessed using morphometric methods based on Euclidean geometry (1,2,16).

In recent decades, the cerebral cortex and its configuration have been considered by scientists as a natural fractal (14-16). Therefore, for a comprehensive study of irregular structures, such as the cerebrum, various methods and modifications of fractal analysis were used (2,12-31). Fractal analysis is a method of mathematical analysis that quantitatively characterizes the structural complexity of geometric figures and their space-filling degree (1,2). The main parameter determined by fractal analysis is the fractal dimension (FD) which can be described as a measure of the space-filling capacity of the studied irregular geometric figures (1). In previous studies, various researchers used fractal analysis to study the cerebral cortical ribbon (14-20), the pial surface of the cerebral cortex (19-26), the cerebral white matter (20,27-31), including its outer surface, the linear boundary between cortical ribbon and white matter (20,30,31). However, despite the diversity of studies involving fractal analysis of brain structures, the whole silhouettes of cerebral hemispheres haven't been studied before.

In this study, we aimed to characterize age-associated changes in the spatial configuration of cerebral hemispheres (including changes in spatial complexity and space-filling capacity) using fractal analysis of silhouette magnetic resonance (MR) brain images.

MATERIAL AND METHODS

The present study was approved by the Commission on Ethics and Bioethics of Kharkiv National Medical University (No.10 of Nov 7, 2018).

MR brain images obtained from 100 (44 male, 56 female) participants with an 18-86 years age range were studied. Thirty-one (14 male, 17 female) of which were between 18-30 years, 29 (14 male, 15 female) were between 31-45 years, 24 (8 male, 16 female) were between 46-60 years, and 16 (8 male, 8 female) were between 61-86 years. The mean age was 41.72 ± 1.78 years. The mean age for male

participants was 41.43 ± 1.68 (range, 18-86) years and 41.95 ± 1.51 (range, 18-72) years for female participants. Patients of the studied sample underwent diagnostic magnetic resonance imaging (MRI) brain scanning and showed no apparent brain pathology upon examination. Their MRI data were considered as relatively normal. The exclusion criterion was the presence of pathological structural changes in the brain and surrounding structures.

Magnetic Resonance Imaging (MRI) Protocol

MRI was performed on a 1.5 T MRI machine (Siemens Magnetom Symphony). T2 and FLAIR sequences were chosen. MRI parameters were T2 sequence: TE (echo time) 130 ms, TR (repetition time) 4440 ms, and FLAIR sequence: TE (echo time) 114 ms, TR (repetition time) 9000 ms, TI (inversion time) 2500 ms. The slice (section) thickness for both MRI sequences was 5 mm. The resolution of digital MR brain images was 72 dpi.

Selection of Tomographic Sections

Five MR images were selected from the MRI dataset of each brain, including four tomographic sections in the coronal (frontal) plane and one in the axial (horizontal) plane. A total of 500 MR brain images were selected. Selection criteria were: location in different parts of the cerebral hemispheres, easy identification by anatomical landmarks, the inclusion of brain areas where pathological lesions are most often found in some neurodegenerative diseases, including Alzheimer's disease (15). The 1st coronal section (Coronal 1) was located at the level of the anterior points of the temporal lobes, the 2nd (Coronal 2) at the level of the mamillary bodies (corpus mamillare), the 3rd (Coronal 3) at the level of the quadrigeminal plate (lamina quadrigemina), and the 4th (Coronal 4) at the level of corpus callosum posterior pole (splenium corporis callosi). The axial section was selected as an uppermost horizontal tomographic section intercepting the thalamus (due to the section thickness of 5 mm this section was located at the upper part of the thalamus).

Image Pre-processing

Blank frame images were created using the Adobe Photoshop CS5 graphics editor. The size of the frame image was 512×400 pixels for coronal sections, and 512×800 pixels for axial. The absolute scale of the digital images was the following: 3 pixels (voxels) = 1 mm. The fragments of digital MR images were inserted into the blank frame images; the tomographic sections of cerebral hemispheres were completely placed in the frames and did not go beyond them (Figure 1, A). The structures surrounding the sections of cerebral hemispheres were initially removed from the images (Figure 1, B), and the pixels in these areas were colored white (pixel intensity value of 255) for the T2 sequence or black (pixel intensity value of 0) for the FLAIR sequence. Preliminary ("rough") segmentation was provided by image thresholding. The pixel intensity threshold value was 128 for the T2 sequence, and 65 for the FLAIR sequence. As a result of thresholding, grayscale MR images were converted into binary. Precise segmentation was performed to improve the anatomical accuracy of the obtained silhouette brain images (Figure 1, C). This was done using a manual correction by Adobe Photoshop CS5 tools. The preprocessing procedure was done for 500 selected images.

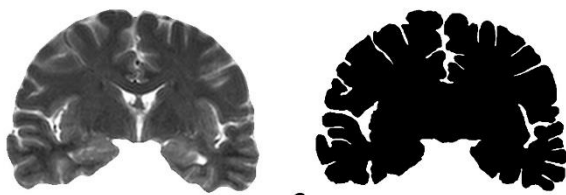
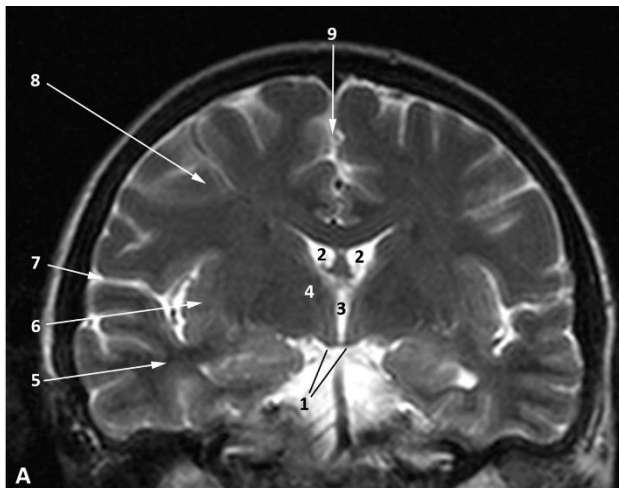


Figure 1. Pre-processing of magnetic resonance brain image (37 years old female). **A)** magnetic resonance image of cerebral hemispheres (Coronal 2, level of mamillary bodies), 1: mamillary bodies, 2: lateral ventricles, 3: 3rd ventricle, 4: thalamus, 5: temporal lobe, 6: insula, 7: lateral sulcus, 8: parietal lobe, 9: longitudinal cerebral fissure; **B)** non-segmented tomographic section after background removal; **C)** segmented silhouette image

Fractal Analysis

Fractal analysis was carried out using the 2D box-counting method tool of the Image J software (32). The FD values of the silhouette images of five different (Coronal 1 to 4,

and Axial) localizations were determined for each participant (Figure 2). The average FD value of all five sections and the average FD value of four coronal sections were also calculated.

Euclidean-Based Image Morphometry

Morphometric parameters based on Euclidean geometry were determined on two types of images. 1st type of images-non-segmented tomographic sections (Figure 1, B), the perimeter of which corresponds to the contour of the superficially exposed surface of cerebral hemispheres, and the area corresponds to the brain tissue as a whole, including the contents of the sulci. The following parameters were determined in these images: P₀ (perimeter), A₀ (area), P₀/A₀ (perimeter-to-area ratio), and SF₀ (shape factor). 2nd type of images-segmented silhouette images (Figure 1, C), the perimeter of which corresponds to the contour of the whole pial surface of the cerebral hemispheres (including the pial surface contour inside the sulci), and the area corresponds to the brain tissue as a whole (but not including the contents of the sulci). The following parameters were determined in these images: P_s (perimeter), A_s (area), P_s/A_s (perimeter-to-area ratio), and SF_s (shape factor). To determine the shape factor for both types of images we used the formula: “SF=(4π×A)/P²” (33). The ratios of the perimeter and the area of segmented silhouette images to the corresponding parameters of non-segmented images were also calculated (P_s/P₀ and A_s/A₀, respectively).

Statistical Analysis

Statistical data processing was done using Excel 2016 software. The mean, standard error of the mean, standard deviation, coefficient of variation (CV, relative standard deviation), median, 25th and 75th percentiles values, and minimum-maximum values were calculated. The normality of distribution was verified using the Shapiro-Wilk test. The statistical significance of the difference between FD values measured in different tomographic sections was

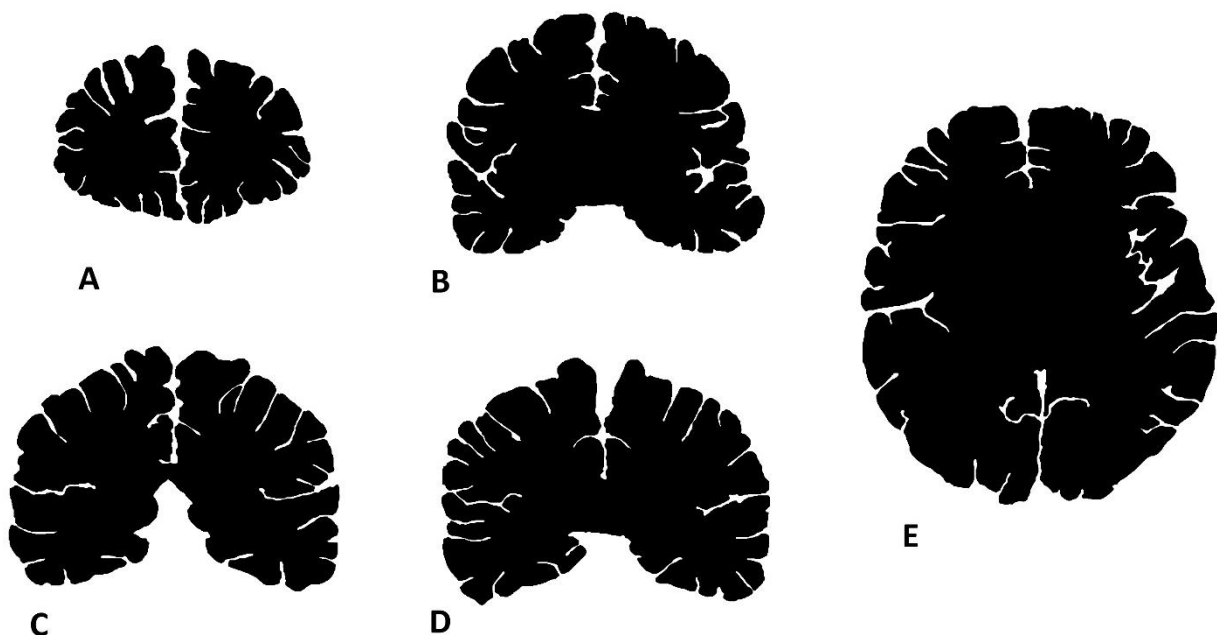


Figure 2. Silhouette images of cerebral hemispheres revealed after preprocessing of magnetic resonance images of different localization (36 years old female). **A)** Coronal 1, level of temporal lobes anterior pole; **B)** Coronal 2, level of mamillary bodies; **C)** Coronal 3, level of the quadrigeminal plate; **D)** Coronal 4, level of corpus callosum posterior pole (splenium corporis callosi); **E)** Axial, thalamus level

assessed using the Kruskal-Wallis H test and post-hoc Dunn's test with Bonferroni adjustment for multiple comparisons. The statistical significance of the difference between FD values and the age of sex groups were verified using the Mann-Whitney U test. The statistical significance of the difference between the linear regression equations was assessed using Fisher's F test. To characterize the correlation, Pearson's correlation coefficient was calculated, the significance of which was assessed using the Student's t-test. The significance level for all results was accepted as $p < 0.05$.

RESULTS

Fractal Dimension Values

The statistical descriptive parameters of FD values obtained in the present study were shown in Table 1. The FD values of the cerebral hemispheres had low values of the variation coefficient (0.39% to 1.01%), which indicates low variability of the cerebral FD.

The distributions of FD values were verified for normality and it was found that the distributions of FD values of the sections Coronal 1 and Coronal 2 did not differ significantly from the normal distribution ($p=0.310$, and $p=0.281$, respectively). The distribution of FD values of the sections Coronal 3 and Axial were significantly different from the normal distribution ($p=0.018$, and $p=0.036$, respectively). The difference between the distribution of FD values of the Coronal 4 section and the normal distribution was questionable ($p=0.059$). The distributions of the average FD values of the five sections and the average FD values of the four coronal

sections were significantly different from the normal distribution ($p=0.001$ for both values).

Since distributions of FD values of some tomographic sections were significantly different from the normal distribution, we chose the non-parametric Mann-Whitney U test to assess the statistical significance of the difference between FD values measured in male and female sex groups (Table 1). It indicated that mean ranks of FD values determined in female and male sex groups were not significantly different in all studied tomographic sections, Coronal 1 $p=0.168$, Coronal 2 $p=0.811$, Coronal 3 $p=0.177$, Coronal 4 $p=0.352$, Axial $p=0.361$, average FD values of the five sections $p=0.992$, average FD values of the four coronal sections $p=0.811$. We also compared the age of male and female sex groups using the Mann-Whitney U test, which indicated that mean ranks of age in male and female sex groups of the studied sample were not significantly different ($p=0.811$). Taking into account the absence of the difference between age and FD values of males and females, we used a non-divided sample for a few following analyses.

In the next stage of the study, we compared the FD values of five tomographic sections. The highest FD values were determined in the Axial section, and the lowest FD values were determined in the Coronal 1 section. There was a significant difference between the FD values of five sections ($p < 0.001$). The post-hoc comparison indicated that the mean ranks of the FD values of the following section pairs were significantly different, Coronal 1 vs Coronal 2, Coronal 1 vs Coronal 3, Coronal 1 vs Coronal 4, Coronal 1 vs Axial, Coronal 2 vs Axial, Coronal 3 vs Axial,

Table 1. Statistical parameters of fractal dimension values of cerebral hemispheres silhouette images

Section	Sex Group	Mean	SE	SD	CV (%)	Median	Q1-Q3	Min-Max
Coronal 1	Both	1.851	0.0016	0.016	0.84	1.852	1.841-1.862	1.809-1.885
	Male	1.848	0.0028	0.019	1.01	1.850	1.834-1.862	1.809-1.885
	Female	1.853	0.0017	0.012	0.67	1.854	1.846-1.862	1.825-1.880
Coronal 2	Both	1.874	0.0013	0.013	0.68	1.874	1.866-1.882	1.840-1.900
	Male	1.874	0.0018	0.012	0.65	1.874	1.868-1.884	1.840-1.896
	Female	1.873	0.0018	0.013	0.71	1.875	1.865-1.881	1.843-1.900
Coronal 3	Both	1.872	0.0014	0.014	0.75	1.874	1.864-1.880	1.834-1.905
	Male	1.873	0.0025	0.016	0.88	1.877	1.863-1.885	1.838-1.905
	Female	1.871	0.0016	0.012	0.64	1.873	1.865-1.878	1.834-1.898
Coronal 4	Both	1.876	0.0013	0.013	0.69	1.877	1.869-1.884	1.842-1.904
	Male	1.877	0.0022	0.015	0.79	1.880	1.868-1.885	1.842-1.904
	Female	1.875	0.0015	0.011	0.60	1.875	1.869-1.883	1.846-1.897
Axial	Both	1.918	0.0009	0.009	0.47	1.919	1.913-1.924	1.889-1.936
	Male	1.917	0.0013	0.009	0.44	1.919	1.913-1.922	1.893-1.933
	Female	1.919	0.0012	0.009	0.48	1.920	1.913-1.926	1.889-1.936
Average (All Sections)	Both	1.878	0.0009	0.009	0.48	1.880	1.873-1.884	1.852-1.894
	Male	1.878	0.0016	0.011	0.58	1.879	1.874-1.885	1.852-1.893
	Female	1.878	0.0010	0.007	0.39	1.880	1.873-1.883	1.859-1.894
Average (Coronal 1-4)	Both	1.868	0.0010	0.010	0.55	1.870	1.862-1.875	1.840-1.888
	Male	1.868	0.0019	0.012	0.66	1.870	1.863-1.877	1.840-1.887
	Female	1.868	0.0011	0.008	0.45	1.870	1.862-1.874	1.845-1.888

SE: standard error of the mean, SD: standard deviation, CV: coefficient of variation (relative standard deviation), Q1-Q3: 25th-75th percentile, min: minimum, max: maximum

and Coronal 4 vs Axial ($p < 0.001$ for all). It also indicated that the mean ranks of the FD values of the following section pairs were not significantly different, Coronal 2 vs Coronal 3 ($p = 0.570$), Coronal 2 vs Coronal 4 ($p = 0.378$), Coronal 3 vs Coronal 4 ($p = 0.147$).

Correlation Analysis

Significant positive correlations were found between the FD values of Coronal 1 and Coronal 2, Coronal 1 and Coronal 3, Coronal 1 and Coronal 4, Coronal 2 and Coronal 3, Coronal 2 and Coronal 4, Coronal 3 and Coronal 4, Coronal 3 and Axial, and Coronal 4 and Axial section pairs (Table 2). The highest correlation coefficient values were found between the FD values of adjacent coronal tomographic sections, Coronal 3 and Coronal 4, and Coronal 2 and Coronal 3. No statistically significant correlations were found between the FD values of the Coronal 1 and Axial, and Coronal 2 and Axial section pairs.

Correlations between the FD values and the morphometric parameters of non-segmented tomographic sections were analyzed (Table 3). Significant positive correlations were found between the FD values and values of area (A_0) and shape factor (SF_0) in coronal sections. The correlations between FD values and perimeter values (P_0) were significant only in the sections Coronal 3 and Coronal 4. Significant negative correlations were found between the FD values and perimeter-to-area ratio (P_0/A_0) in coronal sections.

We also analyzed correlations between the FD values and the morphometric parameters of segmented silhouette images (Table 3). Significant positive correlations were found between the FD values and values of area (A_s) and shape factor (SF_s) in all studied sections. Significant negative correlations were found between the FD values and perimeter-to-area ratio (P_s/A_s) in all sections. The perimeter values (P_s) didn't have a significant correlation with FD values in coronal sections, but there was a

significant negative correlation between these values in the Axial section. Significant positive correlations between the FD values and the two-dimensional gyrification index (ratio of perimeters, P_s/P_0) were found only in the Axial section. Also, significant correlations were found between the FD values and the ratio of the areas (A_s/A_0) in all coronal sections.

To study changes in the spatial configuration of the cerebral hemispheres, we conducted a correlation analysis between the FD values and age. We found negative significant correlations between those parameters. It indicates a tendency for FD values to decrease with age. Figure 3 illustrates FD values distribution throughout adulthood. The strongest correlations between age and FD were found in the sections Coronal 2 ($r = -0.511$, $p < 0.001$) and Coronal 3 ($r = -0.429$, $p < 0.001$). Weak correlations were found in the sections Coronal 1 ($r = -0.232$, $p < 0.05$), Coronal 4 ($r = -0.312$, $p < 0.001$), Axial ($r = -0.313$, $p < 0.001$). Also, we found moderate negative significant correlations between the age and the average FD values of all five tomographic sections ($r = -0.512$, $p < 0.001$) and the average FD values of four coronal sections ($r = -0.491$, $p < 0.001$).

Table 2. Correlation of the cerebral silhouettes fractal dimension values measured in different tomographic sections

Section	Coronal 1	Coronal 2	Coronal 3	Coronal 4
Coronal 2	r	0.249		
	p	0.012		
Coronal 3	r	0.333	0.440	
	p	<0.001	<0.001	
Coronal 4	r	0.367	0.351	0.701
	p	<0.001	<0.001	<0.001
Axial	r	0.104	0.155	0.425
	p	0.303	0.124	<0.001

Table 3. Correlation of the cerebral silhouettes fractal dimension values and values of Euclidean geometry-based morphometric parameters of cerebral hemispheres

Type of Image	Morphometric Parameter	Tomographic Section of Cerebral Hemispheres					
		Coronal 1	Coronal 2	Coronal 3	Coronal 4	Axial	
Non-segmented Tomographic Sections	Perimeter (P_0)	r	0.079	0.129	0.228	0.209	0.166
		p	0.435	0.201	0.023	0.036	0.099
	Area (A_0)	r	0.242	0.230	0.434	0.389	0.094
		p	0.015	0.021	<0.001	<0.001	0.352
	Perimeter-to-area ratio (P_0/A_0)	r	-0.319	-0.275	-0.478	-0.477	0.113
		p	0.001	0.006	<0.001	<0.001	0.263
	Shape Factor (SF_0)	r	0.376	0.133	0.258	0.316	-0.035
		p	<0.001	0.187	0.010	0.001	0.730
Segmented Silhouette Images	Perimeter (P_s)	r	-0.029	0.009	0.072	0.039	-0.285
		p	0.775	0.929	0.477	0.700	0.004
	Area (A_s)	r	0.446	0.463	0.694	0.600	0.388
		p	<0.001	<0.001	<0.001	<0.001	<0.001
	Perimeter-to-area ratio (P_s/A_s)	r	-0.519	-0.410	-0.509	-0.503	-0.610
		p	<0.001	<0.001	<0.001	<0.001	<0.001
	Shape Factor (SF_s)	r	0.339	0.231	0.246	0.276	0.467
		p	<0.001	0.021	0.014	0.005	<0.001
Both Types of Images	Ratio of perimeters (P_s/P_0) (2D gyrification index)	r	-0.082	-0.072	-0.050	-0.088	-0.426
		p	0.417	0.477	0.621	0.384	<0.001
	Ratio of areas (A_s/A_0)	r	0.573	0.628	0.605	0.590	0.156
		p	<0.001	<0.001	<0.001	<0.001	0.121

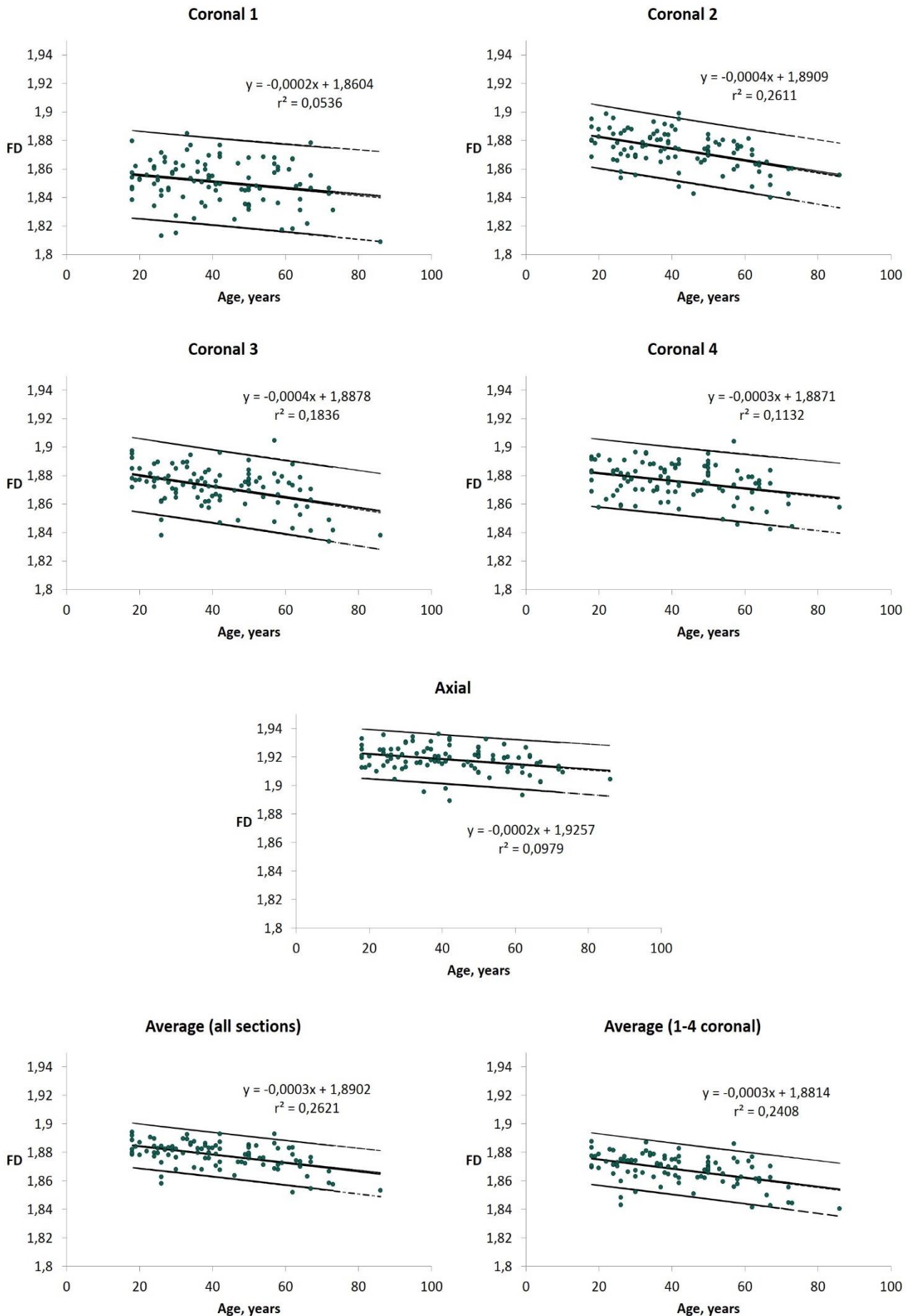


Figure 3. Fractal dimension values of cerebral silhouettes, changes throughout adulthood, confidence intervals, and linear regression equations in different tomographic sections

Figure 4 shows two silhouette images of the cerebral hemispheres of young and old persons (23 and 72 years old, respectively). We can notice the age-associated atrophic changes in the silhouette brain image of the older person, the widening and deepening of the sulci, and the atrophic changes in the gyri shape. The FD values of these images were significantly different, the FD value of the young person was 1.88, and the FD value of the old person was 1.85.

As we have noticed earlier, FD values determined in the male and female groups of the studied sample were not significantly different as well as age. Additionally, we analyzed the relationships of FD values with age in the male and female groups (Figure 5). Negative correlations were found between FD values and age in both sex groups in all studied tomographic sections: $r=-0.292$, $p=0.054$ for males, and $r=-0.174$, $p=0.120$ for females in the Coronal 1 section; $r=-0.573$, $p<0.001$ for males, and $r=-0.465$, $p<0.001$ for females in the Coronal 2 section; $r=-0.431$, $p=0.003$ for males, and $r=-0.432$, $p<0.001$ for females in the Coronal 3 section; $r=-0.317$, $p=0.036$ for males, and $r=-0.307$, $p=0.021$ for females in the Coronal 4 section; $r=-0.502$, $p<0.001$ for males, and $r=-0.174$, $p=0.120$ for females in the Axial section; $r=-0.526$, $p<0.001$ for males, and $r=-0.508$, $p<0.001$ for females for the average FD values of all five tomographic sections; $r=-0.489$, $p<0.001$ for males, and $r=-0.505$, $p<0.001$ for females for the average FD values of four coronal sections. The character of correlations in female and male groups was close to that determined in the undivided sample. The correlation coefficients determined in male and female groups were close to each other almost in all studied sections (except the Axial section, females showed no significant correlations between FD value and age). However, in addition to the strength and character of correlations, it is important to compare the dynamics of age-related changes in FD in the studied groups. We calculated linear regression equations characterizing age dynamics of FD values (FD value was the response variable, and age was the predictor variable) and compared obtained equations in female and male groups (Figure 5). The analysis showed that linear regression equations were not significantly different in males and females in all studied tomographic sections ($p\approx 1$ for all sections).

DISCUSSION

In this study, we have described features of age-associated changes in cerebral hemispheres using fractal analysis of MR silhouette brain images. We have found that the FD values of cerebral silhouettes significantly decrease with age. Our findings are consistent with data from some previous studies, which have shown that FD values of different brain structures had significant negative correlations with age (12,21,29,30).

Some studies regarding age-related changes in fractal measurements involved the assessment of the cortical ribbon or the cortical surface (12,21,22).

The study of Podgórski et al. (12) included the comparative analysis of brain aging using methods of fractal and Euclidean geometry-based morphometries. The brain MR images of healthy volunteers were studied. The following parameters were measured to assess the aging changes: volumes of total gray matter, white matter and

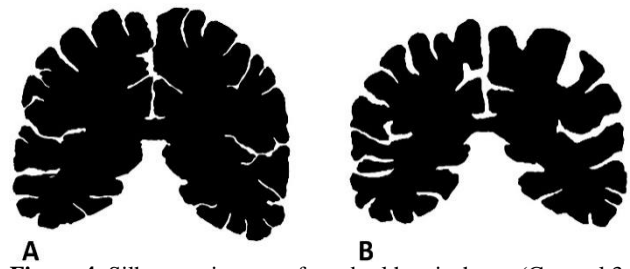


Figure 4. Silhouette images of cerebral hemispheres (Coronal 3, level of quadrigeminal plate) of young and old persons. **A)** 23 years old male with a fractal dimension value of 1.88, **B)** 72 years old male with a fractal dimension value of 1.85

cerebrospinal fluid, cortical thickness, sulcal depth, gyrification index, and FD. The authors described a decrease in gray and white matter volumes and an increase in cerebrospinal fluid volume. Cortical thickness and gyrification index decreased with age, meanwhile, the sulcal depth increased. The authors also investigated cortical maps of the age-related distribution of FD and found that FD was changed during aging.

The study of Madan and Kensinger (21) aimed to examine differences in cortical complexity across the adult lifespan. The authors used a three-dimensional box-counting algorithm for the fractal analysis of the MR brain images. The study revealed that the FD of the cortical ribbon significantly decreased with age ($r=-0.732$) as well as the FD of the cortical surface ($r=-0.719$), mean cortical thickness ($r=-0.603$), and gyrification index ($r=-0.494$). The authors also estimated correlation relationships and found that values of the correlation index between FD of the cortical ribbon and cortical thickness, FD, and gyrification index were $r=0.863$ and $r=0.626$, respectively. In the study of Kalmanti and Maris (22) two-dimensional fractal analysis of the cerebral cortex was provided. Parasagittal tomographic sections were used for the measurements. The studied sample included 93 persons aged from 3 months to 78 years (mainly children and adolescents). The authors demonstrated differences in two-dimensional FD between ages, possibly related to brain development.

The study of Im et al. (25) involved fractal analysis of the three-dimensional cortical surface, using the box-counting method. The authors showed significant correlation relationships of the FD of the cortical surface with cortical thickness, sulcal depth, and folding area. Also, an interesting finding of this study was the correlation of FD with intelligence quotient and the number of education years; the results showed that the FD of the cortical surface had a significant relationship with intelligence and education.

Cortex and cortical ribbon were studied in the studies regarding the atrophic changes in neurodegenerative diseases. In the studies of King et al. (15,19) cerebral cortical ribbons in Alzheimer's disease were studied by two-dimensional and three-dimensional box-counting algorithms of fractal analysis. The authors quantified atrophic changes in cerebral hemispheres and described the significant difference between FDs of the cortical ribbon of healthy persons and patients with Alzheimer's disease. Fractal analysis of the cortex and cortical ribbon

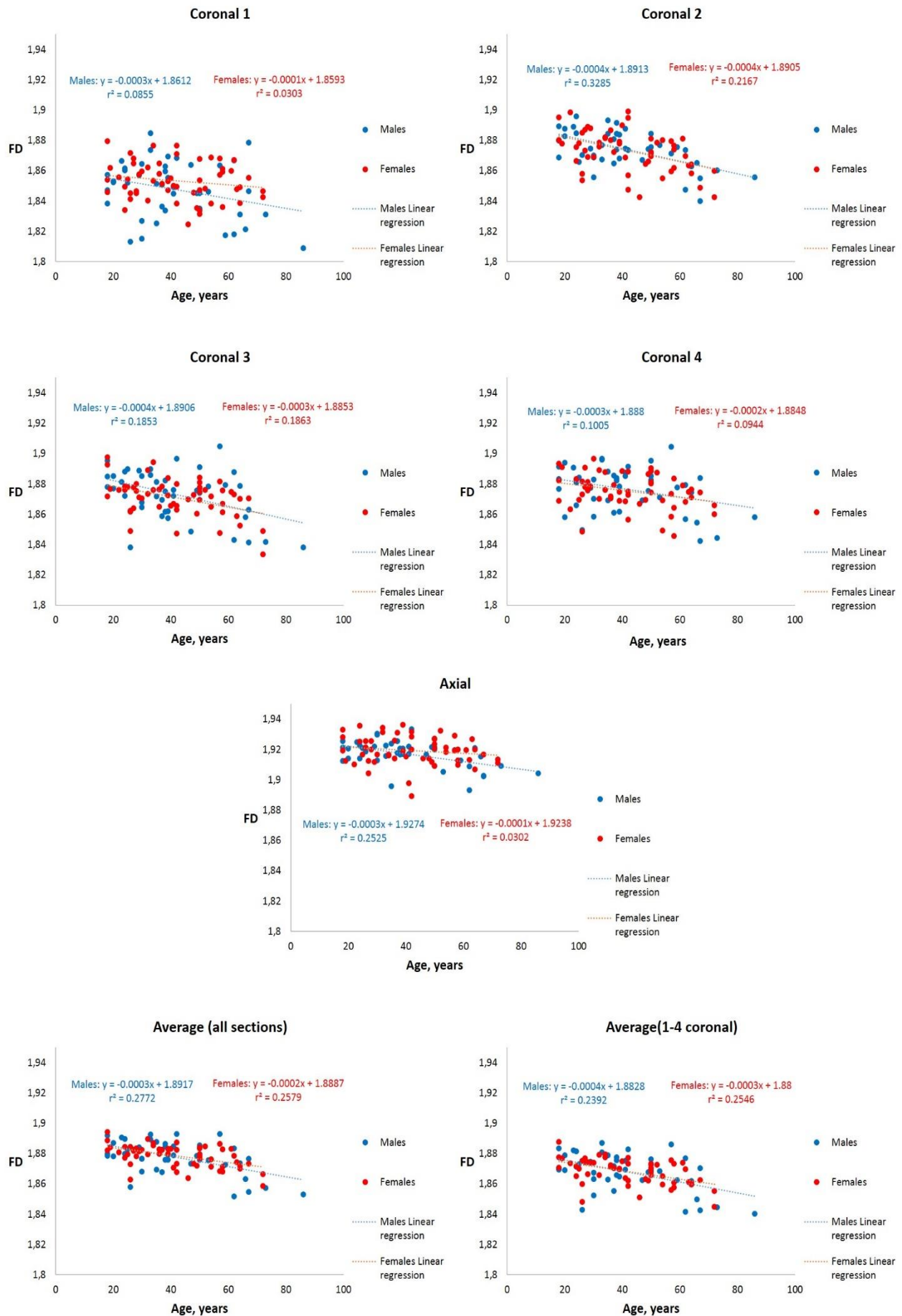


Figure 5. Fractal dimension values of cerebral silhouettes in female and male sex groups, changes throughout adulthood, and linear regression equations in different tomographic sections

also revealed significant changes in patients with multiple sclerosis (17,18), schizophrenia and obsessive-compulsive disorder (23), and auditory verbal hallucinations (24).

Other studies on age-dependent changes of the FD were focused on the brain white matter (29,30). In the study of Zhang et al. (29), a three-dimensional modification of the box-counting method was applied for the fractal analysis of MR brain images. The authors measured the FD of white matter volume (whole white matter), surface (white matter/gray matter boundary), and skeletonized white matter images. There were significant differences between FD values of white matter volume, surface, and skeleton between young and old persons.

In the study of Farahibozorg et al. (30) cerebral white matter was studied. The authors used a three-dimensional box-counting method to provide fractal analysis of brain MR images. In each brain, three FD values were measured, general (volume, whole white matter), skeleton, and boundaries (surface, white matter/gray matter boundary). FDs of white matter volume and skeleton showed inverse U-shape patterns with aging ($r^2=0.116$ for general FD, $r^2=0.202$ for skeleton FD), and FD of white matter surface was less affected by age ($r^2=0.040$). The authors found positive correlations between FD and white matter volume, $r=0.480$ for general FD, $r=0.558$ for skeleton FD, and $r=0.385$ for boundary FD.

Fractal analysis of cerebral white matter was also provided in some diseases and pathological conditions, significant changes were found in patients with multiple sclerosis (27) and traumatic brain injury (28).

Some studies involved the assessment of both white and gray matter. The study of Goñi et al. (20) aimed to quantify the reliability of fractal measures within the human brain. Authors in their study used MR brain images and measured the FDs of the pial cortical surface, the cortical ribbon volume, the white matter volume, and the gray matter/white matter boundary. The three-dimensional box-counting method was used for the fractal analysis. The authors estimated three different FDs (the Kolmogorov capacity dimension, the information dimension, and the correlation dimension) and concluded that the highest reproducibility was achieved with the correlation dimension.

The three-dimensional box-counting algorithm is the most commonly used fractal analysis method in recent studies. It has its undeniable advantages, as it determines a single FD value of the whole cortical ribbon or whole cerebral white matter or whole surface of cerebral hemispheres (19-21,25,29-31). Most three-dimensional fractal analysis algorithms require the construction of three-dimensional brain models complicating the research algorithm (19-21,25,30). For the present study, we used a two-dimensional box-counting algorithm which includes fractal analysis of two-dimensional tomographic image sections (15,22). This method has its own advantages. We chose this method to evaluate the informativeness of a simpler and more accessible fractal analysis algorithm that does not require the construction of three-dimensional brain models. In some cases, three-dimensional brain model construction is difficult or impossible (due to too thick tomographic sections or poor MR image quality). In addition, sometimes it is necessary to assess only a certain brain area (for example, one particular tomographic

section) and compare the obtained FD value with values measured in adjacent areas to reveal and assess the changes in the region of interest. In such cases, it is advisable to use a two-dimensional box-counting algorithm. Kalmanti and Maris (22) in their study said that “interpretation of two-dimensional pictures is common in everyday medical practice and measurement of two-dimensional FD can easily be used since it does not need complicated volumetric methods and can indicate a threshold between normal as well as pathological changes”. In our opinion, a two-dimensional box-counting algorithm is more accessible and convenient for routine medical practice than a three-dimensional one.

As the object of the present study, we chose cerebral silhouettes. The quantitative assessment of the silhouette images makes it possible to simplify morphometric studies of cerebral hemispheres since it does not require the segmentation of brain tissue into constituent components (white and gray matter) and does not require the construction of three-dimensional brain models. The segmentation of brain tissue into white and gray matter usually requires special software and high-quality MR images. In some cases, the quality of routine MRI used in clinical practice is not good enough for this type of segmentation. Silhouettes obtained in our study weren't divided into components and included both cerebral tissue components, gray and white matter. The segmentation used in our study is simpler and more accessible for medical practitioners and it does not require high-quality MR images. Thus, the morphometric method used by us is more convenient for use in cases where segmentation of white and gray matter is not possible, or the thickness of the tomographic sections is not small enough to construct and quantify three-dimensional brain models.

Silhouettes obtained as a result of the segmentation of MR brain images make it possible to assess the spatial location of the brain tissues, its spatial configuration, and the space-filling degree. Age-related atrophic changes lead to a decrease in the volumes of white and gray matter, which is manifested in the shape changes of the cerebral hemispheres; the widening and deepening of the sulci, the reduction and simplification of the gyri may be found in the aged brain (3-5,21). These changes lead to a decrease in the intracranial volume filled with brain structures. At the same time, the percentage of intracranial space corresponding to the space inside the widened and deepened sulci (cerebrospinal fluid-filled space) increases (21). Thus, aging changes are associated with a decrease in the space-filling capacity of the cerebrum. In addition, atrophic changes lead to a simplification of the shape of the brain as a whole, which is reflected in the decrease in the complexity of the brain spatial configuration. FD is a parameter of fractal geometry that quantitatively characterizes the degree of space-filling and the complexity of the spatial configuration of the studied structures. Therefore, the FD of silhouette brain images is the appropriate morphometric parameter, which provides a specific and objective assessment of age-related changes in the shape of cerebral hemispheres.

Fractal analysis of the cerebral silhouettes of young and old persons gave us significantly different FD values. Thus, the FD of silhouette images proved to be a

sufficiently sensitive indicator to quantitatively distinguish two images, without atrophy and with age-related atrophic changes and to quantify the degree of atrophic changes. Based on the age and the FD values of the studied sample, we have calculated confidence intervals of the FD values of cerebral hemispheres silhouette images, which can be used as norm criteria in clinical neuroimaging.

The study of Podgórski et al (12) focused on the comparative analysis of brain aging in males and females. The authors found that FD, volumes of total gray matter, white matter, cerebrospinal fluid, cortical thickness, sulcal depth, gyrification index, and FD were changed during aging in both males and females. The study results showed that cortical FDs were changed during aging only in a small number of locations in males (2.0%), while that number was significantly higher in females (2.7%). Authors stated that male and female brains start aging at a similar age of 45, but compared to males, in females, the cortex is affected earlier and in a more complex pattern. Sex differences in brain age-related FD changes were also evaluated in the study of Farahibozorg et al. (30). This study focused on cerebral white matter. Researchers revealed that the interaction of age- and sex-dependent differences were significant mainly in the hemispheric analysis, the age-related changes were sharper in males. The authors noticed that after adjusting for the volume effect, age-related results remained approximately the same, but females had higher values, specifically in the left hemisphere and boundaries.

Our study also has revealed a decrease in FD values during life in males and females, but there are some differences between the present study and mentioned studies (12,30). The fractal analysis algorithms were quite different. In the study of Podgórski et al. (12) cortical FD maps were obtained, the study of Farahibozorg et al. (30) included fractal analysis of three-dimensional models of cerebral white matter, and the present study included analysis of two-dimensional cerebral silhouettes. Our study has shown no significant differences in the character of male and female brain aging. We revealed a continuous and gradual type of FD decrease during aging in both males and females. The character and strength of correlations between FD and age were similar in male and female groups. Some differences in correlation coefficients (between age and FD in male and female groups) can be explained by data heterogeneity and a relatively small sample size. Taking into account the absence of a significant difference between linear regression equations characterizing FD dynamics throughout life in males and females, we can conclude that there are no significant sex differences in the character of age-related brain changes estimated by fractal analysis of cerebral silhouettes.

CONCLUSION

Fractal analysis of silhouette MR images of the cerebral hemispheres has revealed and quantified age-associated changes in the brain spatial complexity and space-filling degree that characterize atrophic changes in normal aging. The obtained data can be used as normal criteria for assessing the degree of age-related cerebral atrophy and for differentiating between normal aging and neurodegenerative diseases.

Ethics Committee Approval: The study was approved by the Ethics Committee of Kharkiv National Medical University (07.11.2018, 10).

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Evaluation of the Correlation Between Vitamin D Level and Insulin Resistance in Children with Overweight and Obesity

Fazla Kilolu ve Obeziteli Çocuklarda D Vitamini Düzeyi ile İnsülin Direnci Arasındaki Korelasyonunun Değerlendirilmesi


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ABSTRACT

Aim: This study aimed to evaluate the relationship between vitamin D levels and insulin resistance parameters in children with overweight and obesity.

Material and Methods: A total of 174 children, 64.4% (n=112) female, and 35.6% (n=62) male, aged between 6-17 years were included in the study. The participants were divided into three groups as normal (29.9%, n=52), overweight (23.6%, n=41), and obesity (46.5%, n=81) based on the criteria of the World Health Organization body mass index (BMI) classification. The insulin resistance status of the participants was evaluated by homeostatic model assessment of insulin resistance (HOMA-IR), fasting glucose to insulin ratio (FGIR), and quantitative insulin sensitivity check index (QUICKI).

Results: HOMA-IR was found higher in the obesity group (3.2±2.1) compared to the overweight (2.2±1.0) and the normal weight (1.5±1.0) groups (p<0.001). It was observed that QUICKI values of the overweight (0.34±0.03) and the obesity (0.33±0.03) groups were lower than the normal weight (0.37±0.03) group (p<0.001). FGIR was noticed as higher in the normal weight (16.8±10.4) group compared to the overweight (10.6±6.0) and the obesity (8.5±5.5) groups (p<0.001). The mean serum 25-hydroxyvitamin D [25(OH)D] levels of the children and adolescents were 19.6±10.7 ng/mL, and no statistically significant difference was found between the groups (p=0.153). A significant weak negative correlation between serum 25(OH)D and HOMA-IR values was found (r=-0.170, p=0.025). Additionally, a weak positive statistically significant correlation was found between serum 25(OH)D level and QUICKI and FGIR values (r=0.173, p=0.022, and r=0.173, p=0.023, respectively).

Conclusion: Vitamin D levels can affect insulin resistance parameters.

Keywords: Insulin resistance; pediatric obesity; vitamin D.

ÖZ

Amaç: Bu çalışmada fazla kilo ve obezitesi olan çocuklarda D vitamini düzeyleri ile insülin direnci parametreleri arasındaki ilişkinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya 6-17 yaş aralığında, %64,4 (n=112) kız ve %35,6 (n=62) erkek, toplam 174 çocuk dahil edilmiştir. Katılımcılar, Dünya Sağlık Örgütü beden kitle indeksi (BKİ) sınıflaması kriterlerine göre normal (%29,9; n=52), fazla kilolu (%23,6; n=41) ve obezite (%46,5; n=81) olmak üzere üç gruba ayrılmıştır. Katılımcıların insülin direnci durumları, insülin direncinin homeostatik model değerlendirilmesi (homeostatic model assessment of insulin resistance, HOMA-IR), açlık glikozunun insüline oranı (fasting glucose to insulin ratio, FGIR) ve kantitatif insülin duyarlılığı kontrol indeksi (quantitative insulin sensitivity check index, QUICKI) ile değerlendirilmiştir.

Bulgular: HOMA-IR, fazla kilolu (2,2±1,0) ve normal kilolu (1,5±1,0) gruplara göre obezite grubunda (3,2±2,1) yüksek bulunmuştur (p<0,001). Fazla kilolu (0,34±0,03) ve obezite (0,33±0,03) gruplarının QUICKI değerlerinin normal kilolu (0,37±0,03) gruptan daha düşük olduğu görülmüştür (p<0,001). FGIR, normal kilolu grupta (16,8±10,4), fazla kilolu (10,6±6,0) ve obezite (8,5±5,5) gruplarına göre daha yüksek saptanmıştır (p<0,001). Çocuk ve adölesanların ortalama serum 25-hidroksivitamin D [25(OH)D] düzeyi 19,6±10,7 ng/mL olup, gruplar arasında istatistiksel olarak anlamlı bir farklılık bulunmamıştır (p=0,153). Serum 25(OH)D ile HOMA-IR değerleri arasında anlamlı, zayıf negatif (r=-0,170; p=0,025) bir korelasyon bulunmuştur. Buna ek olarak, serum 25(OH)D düzeyi ile QUICKI ve FGIR değerleri arasında zayıf pozitif istatistiksel olarak anlamlı (sırasıyla, r=0,173; p=0,022 ve r=0,173, p=0,023) bir korelasyon bulunmuştur.

Sonuç: D vitamini düzeyleri insülin direnci parametrelerini etkileyebilir.

Anahtar kelimeler: İnsülin direnci; pediatrik obezite; D vitamini.

INTRODUCTION

Insulin resistance, one of the complications of increasing body weight, can be seen widely in childhood, parallel with the increase in obesity frequency (1). Insulin resistance is a disorder that affects many organ systems and causes significant metabolic damage (2). Multiple factors, including various genetic, environmental, and biochemical reasons, influence the development of insulin resistance. Additionally, dietary patterns (especially diets with a high saturated fatty acid, sucrose, and energy content) increase hepatic insulin resistance and free oxygen species formation. The risk of developing insulin resistance reduces with preventive measures and therapeutic interventions, so it is crucial to define environmental factors. In this context, the most critical risk factors are low physical activity level, weight gain, obesity, and unhealthy nutrition habits. These factors can impair or alter insulin sensitivity (3,4). Besides, vitamin D, which involves in the function of insulin-sensitive tissues, including the liver and skeletal muscle, and has potential effects on the regulation of insulin secretion and the survival of pancreatic beta cells, may play a role in the pathogenesis of insulin resistance (5-7). However, the contradictory results of the studies (8-10) necessitate the evaluation of the relationship between vitamin D levels and insulin resistance in childhood. Based on the study results, a more explicit demonstration of the association between vitamin D deficiency and insufficiency and the risk of developing insulin resistance may be an effective strategy to fight against insulin resistance (7,11).

This study aimed to evaluate the relationship between insulin resistance and vitamin D levels in children and adolescents with overweight and obesity aged 6-17.

MATERIAL AND METHODS

This cross-sectional study was conducted with 174 children and adolescents (ages 6-17) who were admitted to the Gülhane Training and Research Hospital Pediatric Endocrine Department between August to December 2019. Those who regularly used any nutritional supplements (vitamin D, iron, fish oil, prebiotics, probiotics, etc.) in the last three months, those with perception and communication problems, those with thinness, chronic diseases, and growth retardation, were not included in this study.

To conduct the study, Ethics Committee Approval dated 18 July 2019 and Decision No: İ2-32-19 from Ankara University Ethics Committee and Research Permission dated 28 August 2019 from the Gülhane Training and Research Hospital, where the data will be collected, were obtained. Before the data started to be collected, the participants and their parents were informed about the research, and the purpose was explained. Those who agreed to participate and those with parental consent were included in the study. Also, each participant signed the informed volunteer consent form.

The dieticians measured height and body weight, and the body mass index (BMI) of the participants was calculated with the formula, $\text{weight (kg)}/\text{height}^2 (\text{m}^2)$. BMI values were evaluated according to the growth standards determined by the WHO for 5-19 years old. BMI was categorized as follows: $-1\text{SD} \geq \text{BMI} \leq +1\text{SD}$ indicating normal weight, $+1\text{SD} > \text{BMI} \leq 2\text{SD}$ indicating overweight,

and $\text{BMI} > 2\text{SD}$ indicating obesity (12). WHO Antroplus program was used to determine the BMI z-scores of the participants (13). Some biochemical parameters, fasting blood glucose, serum 25-hydroxyvitamin D [25(OH)D], and fasting insulin checked in the last three months were obtained from the patient folders and evaluated by the pediatric endocrinologists.

The status of insulin resistance in participants was determined using homeostatic model assessment of insulin resistance (HOMA-IR) calculated by the formula $\text{fasting insulin (uIU/mL)} \times \text{fasting blood glucose (mg/dL)} / 405$, fasting glucose to insulin ratio (FGIR), and quantitative insulin sensitivity check index (QUICKI) (14). There is no standard value for HOMA-IR to define insulin resistance in children and adolescents, and different cut-off values are referenced in different populations (15). In this study, HOMA-IR reference values for Turkish children and adolescents (5-18 years) were used. The HOMA-IR reference values in the prepubertal period, ≥ 2.67 in males and ≥ 2.22 in females; and in the pubertal period, ≥ 5.22 in males and ≥ 3.82 in females, were defined as insulin resistance (16). FGIR value < 7 was considered insulin resistance (17). QUICKI was calculated with the formula: $1 / [\log (\text{fasting insulin (uIU/mL)}) + \log (\text{fasting glucose (mg/dL)})]$ (14). QUICKI value < 0.34 was defined as insulin resistance (18). Serum 25(OH)D level was categorized as follows, deficiency: < 12 ng/mL, insufficiency: 12-20 ng/mL, and normal: > 20 ng/mL (19).

Statistical Analysis

The data were evaluated with IBM SPSS v.26.0 for Windows. Basic descriptive statistics (frequency, percentage, mean, standard deviation, median, interquartile range, minimum, and maximum) were used. The chi-square test was used to compare categorical variables. Normality was examined using the Kolmogorov-Smirnov test. While comparing more than two groups, the one-way analysis of variance (ANOVA) was used under normal distribution assumptions, and the Kruskal-Wallis test was used when not. Bonferroni test was used to determine from which group the difference originated for ANOVA and in case the Kruskal-Wallis test, the Bonferroni corrected Mann-Whitney U test was applied as a post hoc test. Pearson or Spearman correlation analysis was used to evaluate the correlation between the quantitative variables according to the normality assumptions. A p value of < 0.05 was considered as statistical significance.

RESULTS

The study was conducted with 174 children (35.6% (n=62) male, and 64.4% (n=112) female) with a mean age of 11.6 ± 3.17 years. In accordance with BMI z-scores, 46.5% (n=81) of the children were with obesity, while 23.6% (n=41) were overweight, and 29.9% (n=52) were normal weight. There was no statistically significant difference between the BMI groups in regard to general characteristics (Table 1).

The participants' mean fasting blood glucose level was 88.2 ± 7.79 mg/dL, and no significant difference was found between the BMI groups (p=0.974). Participants with normal weight had lower mean fasting insulin levels compared to overweight and obesity groups (p<0.001).

HOMA-IR value was found to be higher in the obesity group compared to the overweight and the normal weight groups ($p<0.001$). The QUICKI value was higher in participants with normal weight compared to the overweight and the obesity groups ($p<0.001$). Similarly, the FGIR value was higher in children with normal weight compared to the overweight and obesity groups ($p<0.001$). The participants' mean serum 25(OH)D level was 19.6 ± 10.7 ng/mL. Additionally, no significant difference was found between the BMI groups ($p=0.153$, Table 2). According to the HOMA-IR classification, 5.8% ($n=3$) of the participants with normal weight, 9.8% ($n=4$) of the participants with overweight, and 37.0% ($n=30$) of the participants with obesity had insulin resistance. Compared

to the children with normal weight, insulin resistance prevalence was higher in children with overweight and obesity ($p<0.001$, Table 3).

Vitamin D was deficient in 22.4% ($n=39$) and was insufficient in 39.1% ($n=68$) of children and adolescents participating in the study. These values were 28.8% ($n=15$) and 34.6% ($n=18$), respectively, in children with normal weight. While the ratios of those who were deficient and insufficient in terms of vitamin D levels in the overweight group were 9.8% ($n=4$), and 48.8% ($n=20$), these ratios were 24.7% ($n=20$) and 37.0% ($n=30$), respectively, among the obesity group. No statistically significant difference was found between the BMI groups in terms of the distribution of 25(OH)D levels ($p=0.234$, Table 3).

Table 1. Demographic characteristics of participants according to the BMI groups

	Normal (n=52)	Overweight (n=41)	Obesity (n=81)	p	Total (n=174)
Gender, n (%)					
Male	14 (26.9)	12 (29.3)	36 (44.4)	0.750	62 (35.6)
Female	38 (73.1)	29 (70.7)	45 (55.6)		112 (64.4)
Age groups, n (%)					
6-9 years	19 (36.5)	11 (26.8)	19 (23.5)	0.256	49 (28.2)
10-17 years	33 (63.5)	30 (73.2)	62 (76.5)		125 (71.8)
Age (year), mean\pmSD	11.2 \pm 3.2	12.4 \pm 3.4	11.6 \pm 3.0		11.6 \pm 3.2
median (IQR) [min-max]	11 (5.8) [6-17]	12 (7) [6-17]	11 (4) [6-17]	0.192	11 (5) [6-17]

BMI: body mass index, SD: standard deviation, IQR: interquartile range

Table 2. Comparison of biochemical findings, insulin resistance, and serum 25(OH)D levels

	Normal (n=52)	Overweight (n=41)	Obesity (n=81)	p	Total (n=174)
Glucose (mg/dL)	88.0 \pm 8.4 87.5 (9.0) [63-111]	88.3 \pm 8.0 87.0 (7.5) [63-115]	88.2 \pm 7.4 87.0 (11.0) [73-106]	0.974	88.2 \pm 7.8 87.0 (9.0) [63-115]
Insulin (uIU/mL)	7.0 \pm 4.2 ^a 6.4 (4.8) [1.3-26.6]	10.2 \pm 4.2 ^b 10.1 (6.3) [2.2-18.6]	14.4 \pm 8.8 ^c 12.2 (9.6) [2.9-48.0]	<0.001	11.2 \pm 7.5 9.5 (8.5) [1.3-48.0]
HOMA-IR	1.5 \pm 1.0 ^a 1.4 (1.1) [0.3-6.1]	2.2 \pm 1.0 ^b 2.1 (1.5) [0.4-4.9]	3.2 \pm 2.1 ^c 2.7 (2.4) [0.6-11.3]	<0.001	2.5 \pm 1.8 2.1 (1.9) [0.3-11.3]
QUICKI	0.37 \pm 0.03 ^a 0.36 (0.05) [0.29-0.50]	0.34 \pm 0.03 ^b 0.34 (0.04) [0.30-0.44]	0.33 \pm 0.03 ^b 0.32 (0.04) [0.27-0.42]	<0.001	0.34 \pm 0.04 0.34 (0.04) [0.27-0.50]
FGIR	16.8 \pm 10.4 ^a 13.6 (9.9) [3.5-58.2]	10.6 \pm 6.0 ^b 8.9 (4.9) [4.6-36.9]	8.5 \pm 5.5 ^b 6.9 (4.9) [1.9-29.3]	<0.001	11.5 \pm 8.2 9.2 (7.4) [1.9-58.2]
25(OH)D (ng/mL)	19.8 \pm 13.4 16.8 (15.4) [4.9-64.6]	21.8 \pm 10.3 18.9 (10.5) [5.9-52.2]	18.3 \pm 8.8 17.1 (10.7) [4.9-40.9]	0.153	19.6 \pm 10.7 17.6 (11.7) [4.9-64.6]

25(OH)D: 25-hydroxyvitamin D, HOMA-IR: homeostatic model assessment of insulin resistance, QUICKI: quantitative insulin sensitivity check index, FGIR: fasting glucose to insulin ratio, ^{a,b,c}: each different superscript letter denotes significant differences between groups, mean \pm standard deviation and median (interquartile range) [min-max]

Table 3. Distribution of participants according to the insulin resistance parameters and 25(OH)D levels

	Normal (n=52)	Overweight (n=41)	Obesity (n=81)	p	Total (n=174)
HOMA-IR, n (%)					
Suboptimal	3 (5.8) ^a	4 (9.8) ^a	30 (37.0) ^b	<0.001	37 (21.3)
Optimal	49 (94.2) ^a	37 (90.2) ^a	51 (63.0) ^b		137 (78.7)
QUICKI, n (%)					
<0.34	7 (13.5) ^a	19 (46.3) ^b	51 (63.0) ^b	<0.001	77 (44.3)
\geq 0.34	45 (86.5) ^a	22 (53.7) ^b	30 (37.0) ^b		97 (55.7)
FGIR, n (%)					
<7	3 (5.8) ^a	12 (29.3) ^b	42 (51.9) ^b	<0.001	57 (32.8)
\geq 7	49 (94.2) ^a	29 (70.7) ^b	39 (48.1) ^b		117 (67.2)
25(OH)D, n (%)					
Deficient	15 (28.8)	4 (9.8)	20 (24.7)	0.234	39 (22.4)
Insufficient	18 (34.6)	20 (48.8)	30 (37.0)		68 (39.1)
Normal	19 (36.6)	17 (41.4)	31 (38.3)		67 (38.5)

25(OH)D: 25-hydroxyvitamin D, HOMA-IR: homeostatic model assessment of insulin resistance, QUICKI: quantitative insulin sensitivity check index, FGIR: fasting glucose to insulin ratio, ^{a,b}: each different superscript letter denotes significant differences between groups

Of the participants with insulin resistance according to the HOMA-IR value, 21.6% (n=8) were vitamin D deficient, 37.8% (n=14) were insufficient, and 40.6% (n=15) were normal. No significant difference was determined between the distribution of 25(OH)D levels of the participants according to insulin resistance status (p=0.960, Table 4).

A weak negative correlation was determined between HOMA-IR and 25(OH)D levels (r=-0.170, p=0.025). A weak positive correlation was determined between serum 25(OH)D and QUICKI (r=0.173, p=0.022), and between serum 25(OH)D and FGIR (r=0.173, p=0.023, Table 5).

DISCUSSION

Obesity is the most crucial risk factor for insulin resistance and type 2 diabetes. Nevertheless, diabetes may not develop in every individual with obesity and insulin resistance. In the absence of functional impairment in the beta cells of the pancreas, insulin resistance in individuals with obesity without diabetes can be compensated by increasing insulin levels. Increased insulin levels in patients with obesity compared to normal-weight individuals may compensate for the decreased insulin effect, resulting in glucose levels similar to normal-weight individuals (20). In this study, the similarity in blood glucose levels of children with normal weight, overweight, and obesity can be explained by the fact that they did not have type 2 diabetes, and those with insulin resistance were in the asymptomatic stages.

In this study, the mean fasting insulin level of participants with normal weight was lower compared to the overweight and obesity groups. In Düzce, a study with similar results was also found (21). A study conducted in Denmark found that as body weight increases, fasting insulin level also increases (22). Various mechanisms in obesity may cause the development of hyperinsulinemia, such as dysregulation of lipid and glucose metabolism, hormone imbalance, and inflammation (23). These reasons may explain the higher insulin levels in individuals with obesity.

According to the results of this study, insulin resistance was more common in children with obesity (37.0%) compared to those with overweight (9.8%) and with

normal weight (5.8%), and this result is consistent with the literature (24,25). In other words, the HOMA-IR value was higher in the obesity group (3.2±2.1) compared to the overweight group (2.2±1.0) and normal weight group (1.5±1.0). QUICKI value was higher in participants with normal weight (0.37±0.03) compared to those with overweight (0.34±0.03) and with obesity (0.33±0.03). Similarly, the FGIR value was higher in the normal weight group (16.8±10.4) compared to the overweight (10.6±6.0) and obesity groups (8.5±5.5). In a study conducted in Ankara, the mean HOMA-IR value of 164 children with overweight and obesity between the ages of 9-13 was determined to be 4.7±2.7, and insulin resistance was found in 62.2% of the participants (26). In another study conducted with children with obesity in Ankara, the mean HOMA-IR value was found 3.3±2.8, and the frequency of insulin resistance was 44.4% (27). Changes in adipose tissue secretions such as increased free fatty acids and leptin levels and decreased adiponectin levels in obesity cause insulin resistance development (20). Also, increased secretion of pro-inflammatory cytokines (IL-6, TNF-α) plays a role in developing insulin resistance (28). For these reasons, the connection between obesity and insulin resistance is not surprising. However, it should be noted that standard methods are needed to evaluate the prevalence of insulin resistance in children (29). According to countries, ages, and gender, different parameters and cut-off points can be used to diagnose insulin resistance. Even in the same region, various cut-off points are used in several studies (15). In this study, too, insulin resistance was evaluated based on the HOMA-IR, FGIR, and QUICKI index, and different results were obtained. Therefore, it is difficult to get exact data on the prevalence of insulin resistance in childhood and compare it based on age, gender, body weight, and country.

The mean serum 25(OH)D levels of children and adolescents were 19.6±10.7 ng/mL and were insufficient. In general, vitamin D level was deficient in 22.4% of the participants, insufficient in 39.1%, and normal in 38.5%. No significant difference was found between normal weight, overweight, and obesity groups in terms of serum

Table 4. Distribution of vitamin D levels of participants by the status of insulin resistance

	HOMA-IR		P	QUICKI		P	FGIR		P
	IR+ (n=37)	IR- (n=137)		IR+ (n=77)	IR- (n=97)		IR+ (n=57)	IR- (n=117)	
25(OH)D, n (%)									
Deficient	8 (21.6)	31 (22.6)	0.960	21 (27.3)	18 (18.6)	0.165	15 (26.3)	24 (20.5)	0.400
Insufficient	14 (37.8)	54 (39.4)		32 (41.5)	36 (37.1)		24 (42.1)	44 (37.6)	
Normal	15 (40.6)	52 (38.0)		24 (31.2)	43 (44.3)		18 (31.6)	49 (41.9)	

25(OH)D: 25-hydroxyvitamin D, HOMA-IR: homeostatic model assessment of insulin resistance, QUICKI: quantitative insulin sensitivity check index, FGIR: fasting glucose to insulin ratio, IR: insulin resistance

Table 5. Correlation of serum 25(OH)D level and insulin resistance parameters

	Normal (n=52)		Overweight (n=41)		Obesity (n=81)		Total (n=174)	
	r _s	p	r	p	r	p	r _s	p
HOMA-IR	-0.178	0.206	-0.024	0.881	-0.249	0.025	-0.170	0.025
QUICKI	0.179	0.203	-0.009	0.954	0.272	0.014	0.173	0.022
FGIR	0.223	0.112	0.039	0.806	0.266	0.017	0.173	0.023

25(OH)D: 25-hydroxyvitamin D, HOMA-IR: homeostatic model assessment of insulin resistance, QUICKI: quantitative insulin sensitivity check index, FGIR: fasting glucose to insulin ratio, r: Pearson correlation coefficient, r_s: Spearman's rho

25(OH)D level distribution. A study conducted with a large population (14473 participants) between the ages of 6-17 years in China found that the serum vitamin D levels did not differ significantly according to the BMI classification (30). A study in Istanbul found that 9.0% of the children were in vitamin D deficiency, and 22.9% in vitamin D insufficiency (31). In another study conducted with 640 children in the 6-9 age group in Istanbul, the frequency of vitamin D deficiency was 5.62%, and the insufficiency was 18.6% (32). The studies conducted in different countries also show that vitamin D deficiency is common in children and adolescents (30,33-36). There are a variety of reasons why vitamin D deficiency is common. A limited number of foods such as salmon, mackerel, eggs, and liver naturally contain vitamin D. Inadequate consumption of foods containing vitamin D may play a role in its deficiency. Inadequate sun exposure, reduced outdoor activities, clothing style, intensive use of sunscreens, air pollution, and physiological factors such as dark skin pigmentation, malabsorption syndromes, and hepatic/renal failure may also cause vitamin D deficiency (37). The common prevalence of vitamin D deficiency and insufficiency in this study may be due to the study being conducted in a wide season range, including autumn and winter. Because the weather is cold and overcast, the children reduced their time outdoors, which caused them to not benefit from sunlight, which is the primary vitamin D synthesis source.

This study did not show a significant difference between the distribution of 25(OH)D levels according to the insulin resistance status. However, the decrease in vitamin D levels was significantly associated with an increase in HOMA-IR values and decreased QUICKI and FGIR values. The studies conducted with children and adolescents in different countries also support this result (38,39). Various mechanisms play a role in the relationship between vitamin D and insulin metabolism. Since pancreatic β -cells express the vitamin D receptor, vitamin D seems to be required for proper insulin secretion. Increasing insulin sensitivity, inhibiting inflammatory factors, and reducing the chronic inflammation process of the pancreas are all possible outcomes of combining calcitriol with the vitamin D receptor on islet β cells. This ultimately leads to an improvement in the function of islet β cells. Additionally, vitamin D may reduce hyperactivity of the renin-angiotensin system and improve the function of β -cells. In addition to this, vitamin D may alter insulin release mediated by calcium channel opening and closing (40,41). This study had potential limitations. Vitamin D insufficiency and deficiency were frequent in children in the study may be due to the wide season period, which includes cold and rainy months. In future studies, it is recommended to limit the season period and evaluate the sun exposure of the participants (such as outdoor time, playing, and outdoor sports activities). This study did not assess vitamin D intake via the food of participants because of the lack of products enriched with vitamin D in Turkey and insufficient vitamin D intake through diet (8). Also, Tanner's stage of puberty and sex hormones were not assessed in the participants. In future studies, it is recommended to consider this situation, and it will be useful to make plans according to countries.

CONCLUSION

In this study, the insufficiency or deficiency of serum vitamin D levels in most children and adolescents indicates that outdoor activities should be increased, especially in school health programs. Additionally, finding a relation between serum vitamin D levels and insulin resistance parameters suggests that vitamin D may have a role in the pathogenesis of insulin resistance. In this context, to better understand the role of vitamin D on insulin resistance, it is recommended to conduct comprehensive studies that take into account the other factors that may affect vitamin D function, for instance, the physical (season, clothing style), and the biochemical parameters (calcium, parathyroid hormone).

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Effect of Paternal Age on Miscarriage Rates in Couples Undergoing In Vitro Fertilization/Intracytoplasmic Sperm Injection

İn Vitro Fertilizasyon/İntrasitoplazmik Sperm Enjeksiyonu Uygulanan Çiftlerde Paternal Yaşın Düşük Oranlarına Etkisi

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ABSTRACT

Aim: The aim of this study was to determine the effect of paternal age on miscarriage rates in couples undergoing in vitro fertilization/intracytoplasmic sperm injection treatment cycles.

Material and Methods: Patients were classified into two groups. The study group consisted of the patients whose pregnancy resulted in a miscarriage (n=73) and a control group in which the patients had a live singleton birth (n=256). Demographic characteristics, treatment indications, duration of infertility, menstruation day 3 follicle stimulating hormone, estradiol, luteinizing hormone, total antral follicle count, anti-Mullerian hormone levels and controlled ovarian stimulation parameters, day of trigger, estradiol and progesterone levels on the day of trigger, the total number of oocytes retrieved, the number of mature oocytes, the number and quality of the embryo, endometrial thickness on the day of trigger, oocyte pick up and embryo transfer, the distance of embryo-fundus and the day of embryo transfer were recorded.

Results: Totally 329 women were included in the present study. The number of patients with the diagnosis of unexplained infertility was statistically significantly higher in the study group than in the control group (p=0.020). Maternal age was found statistically significantly higher in the study group than in the control group (p=0.025). When maternal age increased by 1 unit, the risk of miscarriage increased by 8.7% and those with unexplained infertility had a 75.6% higher risk of miscarriage than those without unexplained infertility.

Conclusion: Paternal age was not associated with miscarriage whereas maternal age and unexplained infertility had a positive correlation with miscarriage rate.

Keywords: Paternal age; miscarriage rate; unexplained infertility; maternal age; IVF/ICSI.

ÖZ

Amaç: Bu çalışmanın amacı in vitro fertilizasyon/intrasitoplazmik sperm enjeksiyonu tedavi siklusları uygulanan çiftlerde paternal yaşın düşük oranları üzerindeki etkisini belirlemektir.

Gereç ve Yöntemler: Hastalar iki gruba ayrıldı. Gebeliği düşükle sonuçlanan hastalar (n=73) çalışma grubunu ve canlı tekil doğum yapan hastalar (n=256) ise kontrol grubunu oluşturdu. Demografik özellikler, tedavi endikasyonları, infertilite süresi, adet 3. günü folikül uyarıcı hormon, östradiol, lüteinize edici hormon, toplam antral folikül sayısı, anti-Müllerian hormon seviyeleri ve kontrollü ovaryan stimülasyon parametreleri, tetikleme günü, tetikleme gününde östradiol ve progesteron seviyeleri, toplanan toplam oosit sayısı, matur oosit sayısı, embriyo sayısı ve kalitesi, tetikleme, oosit pick-up ve embriyo transfer gününde endometrial kalınlık, embriyo-fundus mesafesi ve embriyo transfer günü kaydedildi.

Bulgular: Bu çalışmaya toplam 329 kadın dahil edildi. Açıklanamayan infertilite tanısı alan hasta sayısı çalışma grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede daha yüksek idi (p=0,020). Anne yaşının, çalışma grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede daha yüksek olduğu bulundu (p=0,025). Anne yaşı 1 birim arttığında, düşük yapma riskinin de %8,7 oranında arttığı ve açıklanamayan infertilitesi olanların açıklanamayan infertilitesi olmayanlara göre ise %75,6 oranında daha yüksek oranda düşük yapma riskine sahip olduğu görüldü.

Sonuç: Paternal yaş düşük yapma ile ilişkili olarak bulunmazken, anne yaşı ve açıklanamayan infertilite ile düşük oranı arasında pozitif bir korelasyon olduğu görüldü.

Anahtar kelimeler: Paternal yaş; düşük oranı; açıklanamayan infertilite; anne yaşı; IVF/ICSI.

INTRODUCTION

Pursuing higher education, increasing life expectancy and women having an active role in business management delayed childbearing in developed countries (1). The detrimental effects of advanced maternal age on reproductive outcomes have been thoroughly researched (2-4). In contrast, there was limited information about the effects of paternal age on reproductive outcomes and unfortunately, the results were conflicting. Some studies have shown that the embryo quality, implantation, and live birth rate declined with advanced paternal age (APA), and also reported that abnormal semen parameters, sperm DNA fragmentations, and sperm aneuploidy rates were higher in APA (5-7). On the other hand, other studies did not find any correlation between implantation, miscarriage, and live birth rates with APA (8-10).

This study aimed to determine the effect of paternal age on miscarriage rates in couples undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment cycles.

MATERIAL AND METHODS

This retrospective study was performed at the center of assisted reproduction of Etlik Zübeyde Hanım Women's Health Training and Research Hospital in Ankara, Türkiye, and a total of 329 women were included. The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Women's Health Training and Research Hospital (21.09.2022, 2022/139). Patients were classified into two groups. The study group consisted of the patients whose pregnancy resulted in a miscarriage (n=73) and a control group in which the patients had a live singleton birth (n=256).

Exclusion criteria were as follows: the history of chronic disease, multiple embryo transfer cycles, multiple pregnancies, natural or mild stimulation cycles, severe male factor infertility (azoospermia or total progressive motile sperm count was less than 1 million), and preimplantation genetic diagnosis cycles.

Demographic characteristics (paternal and maternal age, body mass index (BMI), gravidity, abortion, and live birth), treatment indications (male factor, unexplained infertility, and others), duration of infertility, menstruation day 3 (D3) follicle stimulating hormone (FSH), D3 estradiol (E2), D3 luteinizing hormone (LH), D3 total antral follicle count, anti-Müllerian hormone (AMH) levels, and controlled ovarian stimulation parameters (stimulation protocol, total gonadotropin dose (recombinant FSH, Gonal-F® Merck, Germany, human menopausal gonadotropin (hMG), Menopur®, Ferring Pharmaceuticals,

Germany), day of trigger, E2 and progesterone levels on the day of trigger, the total number of oocytes retrieved, the number of mature oocytes, the number and quality of embryo (11), endometrial thickness on the day of trigger, oocyte pick up (OPU) and embryo transfer (ET), the distance of embryo-fundus and the day of ET were recorded. The same luteal phase support was used in both groups.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics v.26. Frequency tables and descriptive statistics were referred to interpret the findings. Non-parametric methods were employed for the values that are not normally distributed. In accordance with non-parametric methods, two independent groups were compared through the Mann-Whitney U test method. Pearson chi-square test was resorted to in examining the relationships between two qualitative variables. Binary logistic regression (Backward LR model) was deployed whilst identifying the factors affecting the abortion status. Receiver operating characteristics (ROC) curve analysis was used to detect the performance of maternal age in predicting the risk of miscarriage. A p value of <0.05 was considered to be statistically significant

RESULTS

Totally 329 women were included in the present study. No significant difference was found in the male factor, tubal factor, diminished ovarian reserve, and ovulation induction protocol between the two groups. The number of patients with the diagnosis of unexplained infertility was significantly higher in the study group than in the control group (p=0.020, Table 1).

There was no significant difference in gravidity, abortion, live birth, BMI, paternal age, D3 E2-FSH-LH, total antral follicle count, AMH, the number of cycles, and duration of infertility between the two groups. Maternal age was significantly higher in the study group than in the control group (p=0.025, Table 2).

There was no significant difference in controlled ovarian stimulation parameters between the two groups (Table 3). As a result of the Backward: LR logistic regression analysis according to the risk of miscarriage, the optimal model is given in Table 4 using all the predictive parameters that could have a significant effect in the univariate analysis. In the current model, it has been determined that maternal age and unexplained infertility are important parameters impacting the occurrence of miscarriage (p=0.032, and p=0.038, respectively). When

Table 1. Comparison of clinical indications and ovulation induction protocols

	Miscarriage (n=73)	Live Singleton Birth (n=256)	p
Male factor , n (%)	18 (24.7)	75 (29.3)	0.437
Tubal factor , n (%)	5 (6.8)	20 (7.8)	0.784
Unexplained infertility , n (%)	42 (57.5)	108 (42.2)	0.020
Diminished ovarian reserve , n (%)	12 (16.4)	52 (20.3)	0.461
Ovulation induction protocol , n (%)	(n=66)	(n=238)	
Long luteal protocol	24 (36.4)	97 (40.8)	
Antagonist protocol	37 (56.1)	130 (54.6)	0.569
Luteal Estradiol + Antagonist protocol	5 (7.5)	11 (4.6)	

Table 2. Comparison of demographic and obstetric characteristics

	Miscarriage (n=73)		Live Singleton Birth (n=256)		p
	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	
Gravidity	0.37±0.76	0 [0-4]	0.50±0.89	0 [0-5]	0.331
Abortion	0.23±0.51	0 [0-2]	0.27±0.65	0 [0-5]	0.912
Live birth	0.04±0.20	0 [0-1]	0.11±0.41	0 [0-3]	0.195
Maternal age (year)	29.78±3.40	30 [21-35]	28.77±3.52	28 [20-38]	0.025
Paternal age (year)	32.25±4.31	32 [25-45]	31.81±3.24	32 [25-41]	0.774
BMI (kg/m ²)	26.68±5.93	25.6 [16.6-50.0]	25.83±4.91	25.2 [15.8-42.7]	0.320
D3 FSH (mIU/mL)	7.42±3.68	6.8 [1.0-25.4]	7.45±3.42	7.0 [0.0-33.6]	0.634
D3 LH (mIU/mL)	5.48±2.67	4.9 [0.5-11.7]	5.28±3.54	4.6 [0.1-25.0]	0.171
D3 E2 (pg/mL)	47.23±23.03	44.5 [5.0-126.0]	44.77±21.60	41.0 [3.0-134.0]	0.360
Total antral follicle count	15.62±9.38	13 [2-36]	14.51±8.17	13 [0-33]	0.622
AMH (ng/ml)	4.53±4.56	3.1 [0.1-15.0]	3.17±3.70	2.1 [0.1-18.1]	0.096
Cycle number	1.77±1.09	1 [1-6]	1.61±0.91	1 [1-6]	0.374
Duration of infertility (month)	69.82±44.10	60 [7-180]	59.98±36.39	48 [2-204]	0.374

BMI: body mass index, D3: day 3, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, AMH: anti-Müllerian hormone, SD: standard deviation

Table 3. Comparison of controlled ovarian stimulation parameters

	Miscarriage (n=73)		Live Singleton Birth (n=256)		p
	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	
Total doses of gonadotropins (IU)	2322.95±989.74	2100 [925-5300]	2145.67±824.09	2025 [600-4725]	0.400
Day of trigger progesterone (ng/mL)	1.05±0.66	0.9 [0.2-2.6]	0.92±1.33	0.7 [0.1-14.6]	0.127
Day of trigger E2 (pg/mL)	3061.66±2721.96	2525.0 [653.0-19372.0]	2529.29±1611.89	2184.0 [248.9-11041.7]	0.146
Day of trigger	10.52±1.92	10 [7-16]	10.44±1.73	10 [7-18]	0.794
Day of OPU E2 (pg/mL)	1984.46±1739.88	1524.3 [16.8-11681.0]	1647.54±1075.96	1299.6 [149.0-6112.9]	0.247
Day of OPU Progesterone (ng/mL)	8.23±5.95	7.1 [0.6-29.2]	7.33±4.89	6.1 [0.5-36.5]	0.320
Day of ET E2 (pg/mL)	1796.11±1476.91	1469.0 [178.8-9317.0]	1607.47±1173.42	1325.7 [183.8-6663.5]	0.432
Day of ET Progesterone (ng/mL)	73.70±47.26	60.0 [3.1-205.0]	77.48±54.71	60.0 [3.2-387.3]	0.620
Follicle number (15-17 mm)	4.74±4.18	4 [0-25]	4.00±2.83	4 [0-16]	0.462
Follicle number (≥17 mm)	4.03±3.50	3 [0-23]	3.53±2.56	3 [0-18]	0.507
Total oocyte count	13.16±7.58	11 [1-33]	12.26±6.33	11 [2-34]	0.689
Mature oocyte count	9.89±5.85	9 [1-26]	9.46±5.04	9 [1-30]	0.867
Grade 1 embryo	0.93±0.89	1 [0-3]	0.71±0.60	1 [0-3]	0.133
Grade 2 embryo	0.49±0.69	0 [0-3]	0.34±0.52	0 [0-2]	0.137
Grade 3 embryo	0.18±0.45	0 [0-2]	0.09±0.31	0 [0-2]	0.058
Endometrial thickness day of trigger (mm)	9.97±2.27	9.5 [6.5-20.0]	10.24±1.82	10.2 [6.5-16.0]	0.143
Endometrial thickness day of OPU (mm)	9.69±2.48	9.6 [3.2-17.0]	10.04±2.29	9.9 [3.6-18.0]	0.362
Endometrial thickness day of ET (mm)	10.13±2.14	9.8 [5.0-17.0]	10.54±2.31	10.1 [4.6-19.3]	0.150
Distance of embryo-fundus	9.39±4.83	9.7 [0.5-24.9]	8.90±3.81	8.4 [0.1-20.6]	0.456
Day of embryo transfer	3.99±1.02	3 [3-6]	3.88±1.00	3 [2-5]	0.439

E2: estradiol, OPU: oocyte pick up, ET: embryo transfer, SD: standard deviation

maternal age (year) increased by 1 unit, the risk of miscarriage increased by 8.7% and those with unexplained infertility had a 75.6% higher risk of miscarriage than those without unexplained infertility.

Maternal age in relation to the risk of miscarriage was determined as 28.5 year with a sensitivity of 67.1% and a specificity of 50.4% (area under the curve (AUC): 0.586; SE=0.037; 95% CI: 0.513-0.658; p=0.026, Figure 1).

DISCUSSION

In the current study, we observed that paternal age had no major effect on the miscarriage in couples undergoing IVF/ICSI while maternal age and the diagnosis of unexplained infertility were associated with increased miscarriage rate.

It was demonstrated that one of the most common causes of miscarriage was aneuploidies (12). Vagnini et al. (13) reported that sperm DNA damage increased with paternal age, and Garcia-Ferreya et al. (14) also revealed supporting the previous study that sperm DNA damage increased with APA and genetic screening was necessary for patients whose paternal age is >50 years of age. The study by Tiegs et al. (15) investigated that APA did not affect IVF pregnancy outcomes when the euploid embryo was transferred. In addition, APA is associated with increased sperm DNA fragmentation that leads to impaired implantation and increases the miscarriage rate (16). APA predisposes to the formation of de novo mutations by

increasing the replication errors in the germ line with an increase of reactive oxygen species and decreasing in antioxidant capacity. This situation is aggravated by the deterioration of DNA repair mechanisms with age (17). As opposed to the mentioned studies, a fair number of other studies showed that there was no correlation between miscarriage rates and APA as in agreement with the present study (18-20).

In one study by Stone et al. (21) the sperm concentration, the rate of normal morphology, and motility declined as paternal age progressed. In accordance with this, ICSI is gaining popularity particularly in preventing the detrimental effect of APA on pregnancy outcomes by improving the fertilization rate (9). In the present study, ICSI was used in all cycles hence, the detrimental effect of sperm abnormalities on the pregnancy outcomes was eliminated.

When the literature was reviewed, it appeared that IVF failure can be caused by many factors, but clearly, the fetal loss was greatly affected by advanced maternal age and the rate of age-related aneuploidy boosted with increasing maternal age (22,23). In the present study, the cut-off value of maternal age for risk of miscarriage was found as 28.5 years.

Unexplained infertility is indeed a “gordian knot” in IVF that forms nearly a third of infertility patients (24), and the lack of understanding of its pathogenesis has made it a mysterious phenomenon.

In accordance with the present study, Fuchinoue et al. (25) reported that miscarriage and unexplained infertility had a relation with each other and determined that natural killer 22 (NK22) cells were elevated in the peripheral blood and endometrium of women with unexplained infertility and women suffering from recurrent miscarriage. In addition, Ran et al. (26) revealed that the core gene of the unexplained infertility and recurrent miscarriage had similar functions such as regulation of immune cells and cellular secretion.

Table 4. Logistic regression analysis of factors for the prediction of a miscarriage

	OR	95% CI	p
Maternal age (year)	1.087	1.007 - 1.172	0.032
Unexplained infertility	1.756	1.032 - 2.988	0.038

OR: odds ratio, CI: confidence interval, CCR=77.8%, $\chi^2_{(8)}=10.330$; p=0.243

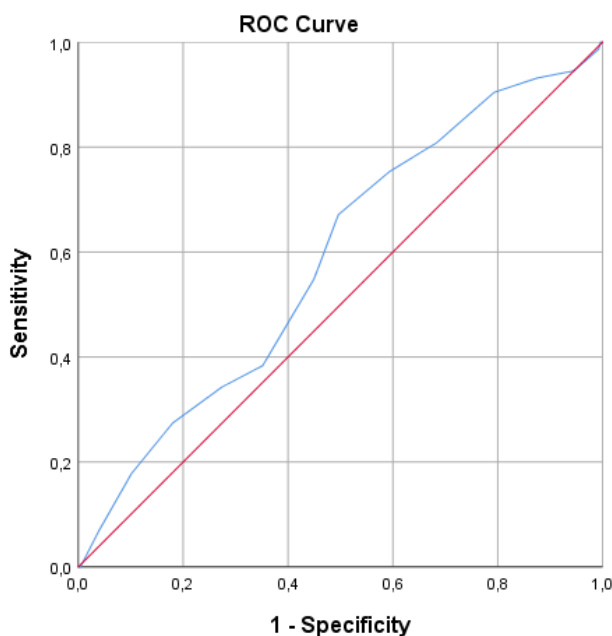


Figure 1. Receiver operating characteristics curve of maternal age for the risk of miscarriage

CONCLUSION

In conclusion, paternal age was not associated with miscarriage whereas maternal age and unexplained infertility had a positive correlation with miscarriage rate. Larger studies are needed to confirm the results of this study.

Ethics Committee Approval: The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Women’s Health Training and Research Hospital (21.09.2022, 139).

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Outcomes and Pathological Features of Total Thyroidectomy in Patients with Multifocal Papillary Thyroid Carcinoma

Multifokal Papiller Tiroid Karsinomlu Hastalarda Total Tiroidektominin Sonuçları ve Patolojik Özellikleri


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ABSTRACT

Aim: The incidence of thyroid cancer has increased dramatically in recent decades. Multifocality is considered a poor prognostic factor for papillary thyroid carcinoma (PTC). Patients with multifocal PTC (MPTC) are at high risk for local recurrence, as well as lymphatic and distal metastases. This study examined the features and outcomes of MPTC.

Material and Methods: This retrospective study was conducted on 300 patients with PTC. Patients were classified into a multifocal group and a unifocal group. The pathological features of the PTC and the patients' outcomes were analyzed and compared.

Results: The multifocal group included 146 patients (48.7%), while the unifocal group included 154 patients (51.3%). The occurrence of multifocality was higher in females than in males (Odds ratio, OR: 2.37, 95% confidence interval, CI: 1.20-4.67, p=0.015). Tumor size of >1 cm in the multifocal group was larger than in the unifocal group (2.5 and 2.2 cm, respectively, p=0.021). Moreover, in multifocal group higher moderate risk of recurrence was detected than in the unifocal group (OR: 1.63, 95% CI: 1.01-2.60, p=0.044). At follow-up after treatment, MPTC patients had higher lymph node metastasis (OR: 2.89, 95% CI: 1.23-6.80, p=0.014). In addition, significantly higher thyroglobulin plasma levels (p=0.026) and disease recurrence (OR: 2.41, 95% CI: 1.05-5.52, p=0.037) were found in the multifocal group compared to the unifocal group.

Conclusion: Patients with MPTC had a higher risk of disease recurrence, and multifocality was concluded to be an independent prognostic factor for overall disease recurrence.

Keywords: Papillary thyroid carcinoma; thyroidectomy; multifocality; recurrence.

ÖZ

Amaç: Tiroid kanseri insidansı son yıllarda önemli ölçüde artmıştır. Multifokalite, papiller tiroid karsinomu (papillary thyroid carcinoma, PTC) için kötü bir prognostik faktör olarak kabul edilir. Multifokal PTC (MPTC)li hastalar, lenfatik ve distal metastazların yanı sıra lokal nüks açısından yüksek risk altındadır. Bu çalışmada MPTC özellikleri ve sonuçları incelendi.

Gereç ve Yöntemler: Bu retrospektif çalışma, 300 PTC'li hasta üzerinde yapıldı. Hastalar multifokal grup ve unifokal grup olmak üzere sınıflandırıldı. PTC'nin patolojik özellikleri ve hastaların sonuçları analiz edildi ve karşılaştırıldı.

Bulgular: Multifokal grupta 146 hasta (%48,7), unifokal grupta ise 154 hasta (%51,3) vardı. Multifokalite oluşumu kadınlarda erkeklerden daha yüksekti (Odds ratio, OR: 2,37, %95 güven aralığı, GA: 1,20-4,67; p=0,015). Multifokal gruptaki >1 cm olan tümör boyutu, unifokal gruptakinden daha büyüktü (sırasıyla 2,5 ve 2,2 cm; p=0,021). Ayrıca, multifokal gruptaki orta düzeyde nüks riskinin unifokal gruba göre daha yüksek olduğu saptandı (OR: 1,63, %95 GA: 1,01-2,60; p=0,044). Tedavi sonrası takipte, MPTC hastalarında daha yüksek lenf nodu metastazı vardı (OR: 2,89, %95 CI: 1,23-6,80; p=0,014). Ek olarak, tiroglobulin plazma seviyeleri (p=0,026) ve hastalık nüksü (OR: 2,41, %95 CI: 1,05-5,52; p=0,037), multifokal grupta unifokal gruba göre anlamlı olarak daha yüksek bulundu.

Sonuç: MPTC'li hastalarda hastalık nüksü riski daha yüksekti ve multifokalitenin genel hastalık nüksü için bağımsız bir prognostik faktör olduğu sonucuna ulaşıldı.

Anahtar kelimeler: Papiller tiroid karsinomu; tiroidektomi; multifokalite; nüks.

INTRODUCTION

The incidence of papillary thyroid carcinoma (PTC) has increased over the past years. That's about 5% in the last decade in the United States (1,2). These tumors comprise 80 to 85% of all thyroid cancers among people living in iodine-rich areas (3).

Multifocality is considered a poor prognostic factor for PTC (4). The prevalence of multifocal PTC (MPTC) has risen over the past decade (5,6). Patients with MPTC are at high risk for local recurrence, as well as lymphatic and distal metastases. In addition, patients with bilateral MPTC have a worse prognosis than patients with unilateral PTC (7). While patients with intrathyroidal MPTC are less likely to have a recurrence (8,9), patients with extrathyroidal MPTC have an increased risk of lymphatic metastases (10). MPTC may present as a microcarcinoma (≤ 10 mm) or as large tumors in two or more separate locations in the thyroid gland. Multifocal or bilateral malignant nodules are often detected after surgery in surgical specimens (11).

Fine needle aspiration biopsy (FNAB) of thyroid nodules is commonly used to detect malignancies and has a 99% accuracy (12,13). A preoperative diagnosis of MPTC may help surgeons decide on the best surgical treatment. A thyroidectomy is the standard approach to treating patients with PTC, followed by treatment with radioactive iodine (RAI; I-131) and levothyroxine therapy. Choosing an appropriate treatment can significantly reduce disease recurrence and mortality from PTC (9).

This study aimed to evaluate outcomes and pathological features of total thyroidectomy in patients with MPTC.

MATERIAL AND METHODS

Study Design and Patients

This retrospective cohort study involved patients with PTC who underwent total thyroidectomy in the thoracic ward of Imam Reza Hospital, Tabriz University of Medical Sciences, Iran. Patients were treated from January 2014 to July 2019. Patients with pathologically confirmed PTC were divided into two groups, unifocal PTC group and multifocal PTC group. The ethics committee of Tabriz Azad University of Medical Sciences approved the study proposal on 29.06.2021 with the ethics code IR.IAU.TABRIZ.REC.1400.042

Inclusion Criteria and Exclusion Criteria

Patients who had been confirmed with PTC by a pathological examination after total thyroidectomy were included in this study. Patients who had not been diagnosed with PTC and patients with different pathologic types other than classical PTC (CPTC) were excluded from this study. In addition, pregnant women and patients who underwent a hemithyroidectomy were also excluded.

Clinicopathological Variables and Follow-up

Demographic and medical data, including age, gender, underlying diseases, type of surgery, radiation exposure, family history of PTC, ultrasound (US) findings, FNAB reports, and RAI therapy, were collected for each patient. In addition, pathological parameters, including tumor size, vascular invasion, capsular invasion, extrathyroidal extension (ETE), and lymph node metastasis (LNM) were recorded according to the tumor node metastasis (TNM) staging system (9). The disease persistence at last visit and

disease recurrence were evaluated according to the finding of histopathology, imaging, and thyroglobulin (Tg) levels. Negative finding of pathological evaluation and Tg level indicated the disease-free condition. Determining the response to primary treatment, disease outcome 12 months after initial treatment, and disease recurrence during follow-up was considered according to the 2015 American Thyroid Association (ATA) management guidelines (9).

Patients with an excellent response defined as no clinical, biochemical, or structural evidences of disease. Abnormal Tg values or rising anti-Tg antibodies in the absence of localizable disease was considered as incomplete biochemical response to therapy. Incomplete structural response was considered when a newly identified locoregional or distant metastases were observed. An indeterminate response was indicated by non-specific biochemical and/or structural findings that were not reliable enough to differentiate between benign or malignant responses (9).

Statistical Analysis

All demographic information and studied data were analyzed using IBM® SPSS® Statistics v.21.0 software. The mean, standard deviation, and 95% confidence interval (CI) were calculated with the quantitative variables for descriptive purposes. The chi-squared and Fisher's exact test were used for the categorical variables, and the independent t-test was considered for continuous variables. Qualitative variables were expressed as numbers and percentages. In addition, continuous variables were evaluated as mean and standard deviation or as median and interquartile ranges. An odds ratio (OR) was used to indicate the strength of the association between the risk factors and clinical outcomes. A p-value ≤ 0.05 was considered to be statistically significant.

RESULTS

Patient Characteristics

The study involved 300 patients with pathologically confirmed PTC who underwent a total thyroidectomy: 45 patients (15%) were male, and 255 patients (85%) were female. The demographics and characteristics of PTC patients were shown in Table 1. The mean age of the patients was 43.3 ± 13.8 years. A family history of PTC was detected in 22 patients (7.3%). LNM was found in 136 patients (45.3%) and not found in 164 patients (54.7%); central LNM (CLNM) and lateral LNM (LLNM) were observed in 88 patients (29.3%) and 48 patients (16%), respectively. The FNAB examination results were consistent with the results of the pathological examination in 288 patients (96%). In addition, the US examination reports showed the same results as the pathological findings in 271 patients (90.3%). Before surgery, the evaluation of thyroid function showed that 291 patients (97%) were euthyroid. A total thyroidectomy was performed on 166 patients (55.3%). A dissection of the lymph nodes to remove lymph nodes in the neck was performed in 133 patients (44.3%). A total of 291 patients (97.0%) received a cumulative dose of I-131 (≤ 30 mCi) postoperatively. The median follow-up was 38.5 months. The Tg level of 54 patients was not measurable. However, in 246 patients Tg level measured,

the median Tg level was 0.2 (range, 0.16-1.2) IU/mL. After treatment, LNM and disease recurrent were detected in 28 patients (9.3%).

Disease Outcome 12 Months after Primary Treatment

One year after primary treatment, 281 patients (93.7%) showed an excellent response. However, 19 (6.3%) patients, including 15 females (78.9%) and 4 males (21.1%), had non-excellent responses according to the 2015 ATA management guidelines (incomplete biochemical response, incomplete structural response, and indeterminate response). Variables related to the non-excellent response after 12 months of primary treatment were

shown in Table 2. These variables included the focality of disease (multifocal and unifocal), LNM, vascular invasion, ETE, distant metastasis, and tumor size.

Comparison of the Unifocal and Multifocal Groups

The comparison of the multifocal and the unifocal groups were shown in Table 3. The multifocal group included 146 patients (48.7%), while the unifocal group included 154 patients (51.3%). The mean ages in the multifocal and unifocal groups were 43.4±13.9 and 43.3±13.7 years, respectively (p=0.971). Multifocality occurred more often in females than males (OR: 2.37, 95% CI: 1.20-4.67, p=0.015). In the tumor size >1 cm, the multifocal group had a larger

Table 1. Patients' characteristics

Age (years), mean±SD	43.3±13.8
Gender (female), n (%)	255 (85%)
Gender (male), n (%)	45 (15%)
History of radiation, n (%)	2 (0.7%)
Family history, n (%)	22 (7.3%)
Diabetes, n (%)	42 (14.0%)
Hypothyroidism (follow-up), n (%)	8 (2.7%)
Hyperthyroidism (follow-up), n (%)	3 (1.0%)
Normothyroidism (follow-up), n (%)	291 (97.0%)
Ultrasound findings, n (%)	271 (90.3%)
FNAB, n (%)	288 (96.0%)
Tumor size (cm), median (IQR) [min-max]	2 (1.5-3.0) [0.3-9.0]
Tumor size (<1 cm), n (%)	39 (13.0%)
Tumor size (≥1 cm), n (%)	261 (87.0%)
LNM (CLNM and LLNM) at diagnosis, n (%)	136 (45.3%)
Multifocal disease, n (%)	146 (48.7%)
Unifocal disease, n (%)	154 (51.3%)
Capsule invasion, n (%)	164 (54.7%)
Vascular invasion, n (%)	160 (53.3%)
Extrathyroidal extension, n (%)	34 (11.3%)
Distant metastasis, n (%)	5 (1.7%)
Cervical LN dissection, n (%)	133 (44.3%)
TT surgery, n (%)	166 (55.3%)
TT + LND surgery, n (%)	14 (4.7%)
TT + CND surgery, n (%)	40 (13.3%)
TT + LND + CND surgery, n (%)	79 (26.3%)
TNM stage I, n (%)	265 (88.3%)
TNM stage II, n (%)	22 (7.3%)
TNM stage III, n (%)	9 (3.0%)
TNM stage IV, n (%)	4 (1.3%)
Treatment with I-131 (ATA), n (%)	291 (97.0%)
Transient hypocalcemia, n (%)	16 (5.3%)
Vitamin D deficiency hypocalcemia, n (%)	16 (5.3%)
Persistent hypocalcemia, n (%)	1 (0.3%)
Tg level at follow-up (IU/ml), median (IQR) [min-max]	0.2 (0.18-0.23) [0.16-1.20]
Low recurrence risk (ATA), n (%)	110 (36.7%)
Medium recurrence risk (ATA), n (%)	188 (62.7%)
High recurrence risk (ATA), n (%)	6 (2.0%)
LNM (CLNM and LLNM) at follow-up, n (%)	28 (9.3%)
Recurrence of the disease, n (%)	28 (9.3%)

SD: standard deviation, IQR: interquartile range, FNAB: fine needle aspiration biopsy, LNM: lymph node metastasis, CLNM: central lymph node metastasis, LLNM: lateral lymph node metastasis, LN: lymph node, TT: total thyroidectomy, LND: lateral node dissection, CND: central node dissection, TNM: tumor node metastasis, ATA: American thyroid association, Tg: thyroglobulin

Table 2. Comparison of variables between excellent response group and non-excellent response group of patients with PTC

	Excellent Response (n=281)	Non-Excellent Response (n=19)	p
Gender (female), n (%)	240 (85.4)	15 (78.9)	0.502
Family history, n (%)	20 (7.1)	2 (10.5)	0.639
Multifocal disease, n (%)	132 (47.0)	14 (73.7)	0.032
Lymph node metastasis, n (%)	73 (26.0)	13 (68.4)	<0.001
Vascular invasion, n (%)	149 (53.0)	11 (57.9)	0.813
Extrathyroidal extension, n (%)	30 (10.7)	4 (21.1)	0.249
Distant metastasis, n (%)	4 (1.4)	1 (5.3)	0.281
Tumor size (cm), median (IQR) [min-max]	2 [0.3-9.0]	3.65 [1.5-6.0]	<0.001

PTC: papillary thyroid carcinoma, IQR: interquartile range, excellent response: no clinical, biochemical, or structural evidence of disease, non-excellent response: composed of incomplete biochemical, structural, and indeterminate response, definition criteria was according to the 2015 American Thyroid Association thyroid cancer guidelines

Table 3. Comparison of variables between multifocal group and unifocal group of patients with PTC

	Multifocal (n=146)	Unifocal (n=154)	p
Gender (female), n (%)	132 (90.4)	123 (79.9)	0.015
Age (years), mean±SD	43.4±13.9	43.3±13.7	0.971
Family history, n (%)	13 (8.9)	9 (5.8)	0.378
Diabetes, n (%)	20 (13.7)	22 (14.3)	>0.999
Hypothyroidism, n (%)	6 (4.1)	2 (1.3)	0.164
Hyperthyroidism, n (%)	1 (0.7)	2 (1.3)	>0.999
Normothyroidism, n (%)	141 (96.6)	150 (97.4)	0.744
FNAB, n (%)	139 (95.2)	149 (96.8)	0.565
Tumor size >1cm (cm), median (IQR) [min-max]	2.5 (1.8-3.9) [1.1-9]	2.2 (1.5-3.0) [1.1-6.5]	0.021
Tumor size ≤1 cm (cm), median (IQR) [min-max]	0.8 (0.6-1) [0.3-1.0]	1 (0.93-1) [0.5-1.0]	0.003
Tumor size (<1 cm), n (%)	19 (13.0)	20 (13.0)	>0.999
Tumor size (≥1 cm), n (%)	127 (87.0)	134 (87.0)	0.994
LNM at diagnosis, n (%)	82 (56.2)	54 (35.1)	<0.001
CLNM, n (%)	53 (36.3)	35 (22.7)	0.011
LLNM, n (%)	29 (19.9)	19 (12.3)	0.084
Capsule invasion, n (%)	87 (59.6)	77 (50.0)	0.095
Vascular invasion, n (%)	82 (56.2)	78 (50.6)	0.338
Extra thyroidal extension, n (%)	17 (11.6)	17 (11.0)	0.868
Distant metastasis, n (%)	4 (2.7)	1 (0.7)	0.193
Cervical LN dissection, n (%)	80 (54.8)	53 (34.4)	<0.001
TT surgery, n (%)	66 (45.2)	100 (64.9)	<0.001
TT + LND surgery, n (%)	10 (6.8)	4 (2.6)	0.102
TT + CND surgery, n (%)	25 (17.1)	15 (9.7)	0.064
TT + LND + CND surgery, n (%)	45 (30.8)	34 (22.1)	0.090
TNM I, n (%)	126 (86.3)	139 (90.3)	0.369
TNM II, n (%)	11 (7.5)	11 (7.1)	>0.999
TNM III, n (%)	6 (4.1)	3 (1.9)	0.325
TNM IV, n (%)	3 (2.1)	1 (0.7)	0.359
Treatment with I131 (ATA), n (%)	137 (45.7)	154 (51.3)	-
Transient hypocalcemia, n (%)	11 (7.5)	5 (3.2)	0.125
Vitamin D deficiency hypocalcemia, n (%)	8 (5.5)	8 (5.2)	>0.999
Persistent hypocalcemia, n (%)	1 (0.7)	0 (0.0)	-
Tg level at follow-up (IU/ml), median (IQR) [min-max]	0.2 (0.18-0.24) [0.16-0.86]	0.19 (0.18-0.2) [0.17-1.2]	0.026
Low recurrence risk (ATA), n (%)	44 (30.1)	66 (42.9)	0.023
Medium recurrence risk (ATA), n (%)	100 (68.5)	88 (57.1)	0.044
High recurrence risk (ATA), n (%)	4 (2.7)	2 (1.3)	0.383
LNM (follow-up), n (%)	20 (13.7)	8 (5.2)	0.014
CLNM, n (%)	9 (6.2)	1 (0.7)	0.007
LLNM, n (%)	11 (7.5)	7 (4.5)	0.334
Recurrence of the disease, n (%)	19 (13.0)	9 (5.8)	0.037

PTC: papillary thyroid carcinoma, SD: standard deviation, IQR: interquartile range, FNAB: fine needle aspiration biopsy, LNM: lymph node metastasis, CLNM: central lymph node metastasis, LLNM: lateral lymph node metastasis, LN: lymph node, TT: total thyroidectomy, LND: lateral node dissection, CND: central node dissection, TNM: tumor node metastasis, ATA: American thyroid association, Tg: thyroglobulin

mean tumor size than the unifocal group (2.5 and 2.2 cm, respectively, $p=0.021$). While in tumor size ≤ 1 cm, the unifocal group had a larger mean tumor size than the multifocal group (1 and 0.8 cm, respectively, $p=0.003$). According to the TNM scoring system, 126 patients (86.3%) in the multifocal group and 139 patients (90.3%) in the unifocal group had stage I PTC ($p=0.369$). In addition, patients in the multifocal group had a more advanced stage of disease (i.e., stage III/IV) than patients in the unifocal group. According to the 2015 ATA guidelines, the moderate risk of recurrence was higher for the patients in the multifocal group than for the patients in the unifocal group (OR: 1.63, 95% CI: 1.01-2.60, $p=0.044$). More patients in the multifocal group had distant metastases than in the unifocal group (2.7% vs. 1.3%, $p=0.383$). The capsular invasion was detected in 87 patients (59.6%) of multifocal and 77 patients (50.0%) of unifocal groups ($p=0.095$). The vascular invasion was observed in 82 patients (56.2%) and 78 patients (50.6%) in the multifocal group and unifocal group, respectively ($p=0.338$). In comparing the primary treatments between groups, patients in the multifocal group underwent more cervical LN dissections than in the unifocal group (54.8% and 34.4%, respectively, OR: 2.30, 95% CI: 1.45-3.67, $p<0.001$). After treatment, LNM, particularly CLNM, occurred more frequently in patients in the multifocal group than in the unifocal group (OR: 2.89, 95% CI: 1.23-6.80, $p=0.014$ for LLNM, and OR: 10.05, 95% CI: 1.25-80.36, $p=0.007$ for CLNM). The median level of plasma Tg in the multifocal group was meaningfully elevated compared to the unifocal group (0.2 vs. 0.19, $p=0.026$). Recurrence of the disease in the multifocal group was significantly higher than in the unifocal group (OR: 2.41, 95% CI: 1.05-5.52, $p=0.037$).

DISCUSSION

PTC is the most common type of well-differentiated thyroid cancer. Multifocality is related to an increased risk of recurrence of PTC. However, its prognostic value in clinical outcomes is controversial. Choi et al. (14) performed a retrospective study on 2,390 patients with pathologically confirmed PTC. They found that multifocal tumors were an independent risk factor for the recurrence of PTC after total thyroidectomy. Geron et al. (15) studied the data of patients with PTC from tertiary medical centers. They analyzed the main characteristics of PTC as well as patient outcomes in order to evaluate prognostic implications in MPTC patients. In their study, the median follow-up of patients was 10.1 years. MPTC was detected more frequently than unifocal PTC, and often presented in older patients and more frequently in males. MPTC was also associated with higher LNM involvement, more advanced stages (III/IV), a higher risk of recurrence according to the ATA, more recurrence during follow-up, and higher overall mortality rates. However, the researchers concluded that multifocality was not an independent prognostic factor for long-term outcomes. In addition, 78.3% of all patients showed excellent response, 1 year after treatment. Patients in the unifocal group had a higher percentage of excellent response compared to patients in the multifocal group. After 12 months of initial treatment, worse response to the treatment was associated with multifocal disease, the male gender, a large tumor, advanced TNM stage, a high risk of recurrence, ETE,

LNM, and distant metastases. In a retrospective review on patients with PTC who underwent thyroid surgery (10), patients with MPTC were older, had a smaller average tumor size, presented an advanced TNM stage and had a higher rate of non-remission and a lower survival rate than patients with unifocal PTC. Within a mean follow-up of 7.1 ± 5.3 years, 25 patients with MPTC (4%) died.

In our study, a total thyroidectomy was performed in all patients and the median follow-up was 38.5 months. MPTC affected more females than males. The age of most of the patients was 43.3 ± 13.8 years old. In addition, patients in the multifocal group had an advanced level of disease (stage III/IV), a higher rate of distant metastasis, and a higher moderated recurrence risk than patients in the unifocal group. Furthermore, patients with a large tumor, multifocal disease, LNM, vascular invasion, and ETE did not have an excellent response after treatment.

The approaches for diagnosing and treating recurrent PTC are different from the management strategies for primary thyroid tumors. Tg is a tumor marker that is used for the follow-up of thyroid cancers (16). Patients with tumor recurrence after surgery are expected to have high levels of Tg (16,17). During the follow-up of PTC patients after surgery, we found that patients with MPTC had higher plasma levels of Tg than patients with unifocal PTC.

Kuo et al. (6) investigated the clinical features and therapeutic outcomes of multifocal papillary thyroid microcarcinoma (PTMC) in patients with PTC who had undergone a thyroidectomy at one medical center. More patients in the multifocal PTC group had stage I than in the non-PTMC group: 63.1% versus 56.8%. In addition, patients with multifocal PTMC had higher postoperative Tg levels. Patients in the non-PTMC group had larger tumors (2.6 ± 0.1 cm) than in the MPTC group (2.2 ± 0.1 cm) and showed a higher rate of postoperative disease progression than patients with PTMC. There was no statistical difference in disease-specific mortality and total mortality between patients with PTMC and non-PTMC.

Gur et al. (18) reviewed the documents of patients with PTC who underwent total or near-total thyroidectomies. Patients were classified into multifocal or unifocal groups. The researchers found that age, gender, the sizes of the tumors, ETE, and capsular invasion were statistically similar in both groups. LNM was also more common in the multifocal group than unifocal. Our data showed significantly high rates of LNM in patients in the multifocal group. We also found higher rates of capsular invasion and vascular invasion in the multifocal group than in the unifocal group. However, differences in capsular invasion and vascular invasion were not statistically significant. Moreover, we detected a significant disease recurrence in patients in the multifocal group than in the unifocal group. In addition, the median size of the tumor (>1 cm) in patients in the multifocal group was larger than patients in the unifocal group.

Limitations

In this retrospective study, we had some limitations. The first is that some patients did not meet the treatment criteria, for example, a few patients did not receive a postoperative dose of I-131 for residual tumor ablation. The second, approaches to the treatment of patients with PTC is a dynamic process, and the classification of the

disease stage, follow-up methods, and definitions of the persistent or recurrent disease can be changed over time. Furthermore, the mortality rate is low in PTC patients; consequently, we need a more population-based study to examine the relationship between multifocality and mortality.

CONCLUSION

MPTC affected more females than males and most patients with MPTC were young to middle-aged. Patients with MPTC had larger tumors and higher rates of LNM, capsular invasion, and vascular invasion than patients with unifocal PTC. Patients in the multifocal group were more likely to have recurrent disease and not to have excellent responses to treatment. Total thyroidectomy with lymph node dissection may be effective in reducing the rate of postoperative disease progression in patients with MPTC.

Ethics Committee Approval: The study was approved by the Research Ethics Committee of Tabriz Azad University of Medical Sciences (29.06.2021, 1400.042).

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Does Mesh Application by Using Inside-Out and Outside-In Techniques during Transobturator Tape Surgery Change the Success?

Transobturator Bant Cerrahisinde İçten Dışa ve Dıştan İçte Teknik Kullanılarak Meş Uygulaması Başarıyı Değiştirir mi?


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ABSTRACT

Aim: This study aimed to evaluate the effect of transobturator tape (TOT) surgery, which is performed in the treatment of stress urinary incontinence (SUI) in women, using the inside-out or outside-in technique on the success of the surgery.

Material and Methods: Sixty-five women diagnosed with SUI and for whom it was decided to perform TOT surgery were included in this prospective randomized study. Participants were divided into two groups, 32 women underwent TOT surgery using the inside-out technique and 33 women underwent TOT surgery using the outside-in technique. The surgical data of patients, the 3-month incidence of complications, pre-, and postoperative scores of the incontinence impact questionnaire (IIQ-7), and the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF) were recorded. Objective, subjective, and inadequate treatment rates were determined for the evaluation of the success of the treatment.

Results: When the two groups were compared, there was no statistically significant difference in terms of age, body mass index, parity, menopause status, duration of incontinence, and preoperative IIQ-7 and ICIQ-SF scores. There were no statistically significant differences between surgical durations, length of hospital stay, early surgical complications, and postoperative 3rd month IIQ-7 and ICIQ-SF scores. There were no statistically significant differences between the two groups in terms of objective, subjective, and inadequate treatment rates.

Conclusion: TOT surgery is successful with both techniques in patients with SUI. Choosing the technique with which the surgeon is experienced and feels most confident will increase the success of TOT surgery regardless of the technique.

Keywords: Transobturator tape; suburethral slings; urinary incontinence; stress.

ÖZ

Amaç: Bu çalışmanın amacı kadınlarda stres üriner inkontinans (SÜİ) tedavisinde uygulanan transobturator bant (TOT) cerrahisinin içten dışa veya dıştan içte tekniği kullanılarak yapılmasının cerrahinin başarısına etkisini değerlendirmektir.

Gereç ve Yöntemler: Bu ileriye yönelik randomize çalışmaya SÜİ tanısı alan ve TOT ameliyatı yapılmasına karar verilen 65 kadın dahil edildi. Katılımcılar iki gruba ayrıldı, 32 kadına içten dışa teknik kullanılarak TOT ameliyatı, 33 kadına ise dıştan içte teknik kullanılarak TOT ameliyatı yapıldı. Hastaların cerrahi verileri, 3 aylık komplikasyon insidansları, ameliyat öncesi ve sonrası idrar kaçırmanın etki anketleri (incontinence impact questionnaire, IIQ-7) ve Uluslararası İnkontinans Değerlendirmesi Anketi-Kısa Formu (International Consultation on Incontinence Questionnaire-Short Form, ICIQ-SF) skorları kaydedildi. Tedavinin başarısının değerlendirilmesinde objektif, subjektif ve yetersiz tedavi oranları belirlendi.

Bulgular: İki grup karşılaştırıldığında yaş, vücut kitle indeksi, parite, menopoz durumu, inkontinans süresi, ameliyat öncesi IIQ-7 skorları ve ICIQ-SF skorları açısından istatistiksel olarak anlamlı fark yoktu. Ameliyat süreleri, hastanede kalış süreleri, erken dönem cerrahi komplikasyonlar ve ameliyat sonrası 3. ay IIQ-7 ve ICIQ-SF skorları arasında istatistiksel olarak anlamlı fark saptanmadı. Objektif, subjektif ve yetersiz tedavi oranları açısından iki grup arasında istatistiksel olarak anlamlı fark yoktu.

Sonuç: TOT cerrahisi SÜİ olan hastalarda her iki teknikle de oldukça başarılıdır. Cerrahin deneyimli olduğu ve kendini en güvende hissettiği tekniği seçmesi, teknik ne olursa olsun TOT ameliyatının başarısını artıracaktır.

Anahtar kelimeler: Transobturator teyp; subüretal slingler; üriner inkontinans; stres.

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INTRODUCTION

Urinary incontinence is an important problem that reduces a woman's quality of life considerably and can cause them to withdraw from social life when it progresses (1,2). The incidence of incontinence and prolapse is increasing with the increase in the average life expectancy and the serious increase in obesity. With the widespread use of the internet and social media, more and more women have learned that treatment is possible. The fact that society is more informed about treatment options has increased the rate of hospital admissions, making stress urinary incontinence (SUI) a problem that we have started to see more often in our clinic (3,4).

The gold standard in the treatment of SUI is mid-urethral sling surgery, and when the long-term results are considered, it has a cure rate of 70-90% (5). As an alternative to this, to reduce bladder damage and provide wider support to the mid urethra, transobturator tape (TOT) surgery was described for the first time by Delorme (6) in 2001 by placing an external-to-inside tape. In 2003, a new technique was defined by de Leval (7), with tape application from the inside to the outside. Both techniques have been successfully applied in urogynecology and urology clinics for years. The bladder, obturator nerves, and blood vessels may be injured during the passage through the obturator foramen during TOT surgery. Similarly, passage through the adductor tendons and into the skin is thought to cause postoperative groin and thigh pain (8).

In surgical procedures, success depends on the surgeon's compliance with the technique and the perfect application of the technique, as much as the surgical technique itself. For this reason, surgeons should be in search of techniques that will make them feel safer in terms of complications and will not have difficulty in performing them. Most of the time, the complications that may occur are not caused by the technique itself, but by mistakes made in the application of the technique (9,10). Therefore, it is important to develop surgical techniques that we know to be successful in a way that will provide ease of application to surgeons.

Although studies have compared these two techniques, the results are limited. In our study, we aimed to compare these two techniques by comparing the postoperative objective and subjective treatment success and complication incidences of women with SUI.

MATERIAL AND METHODS

Our study was a prospective randomized double-blind case-control study. Before starting the study, approval was obtained from the ethics committee of Gaziosmanpaşa Training and Research Hospital (Date: 23/11/2022, Number: 121). All participants were included in the study by invitation and written informed consent was obtained from each if they agreed to participate in the study.

Sample Size

In the power analysis made before the study, it was found appropriate to include a minimum of 16 women in each group, a total of 32 women, to be able to observe a difference with an effect size of 0.5 (high degree) and 80% power and 5% type I error. The calculation was made using the Chi-square test in G*Power 3.1.9.7 program. A total of 65 women were included in the study who were applied to

the urogynecology outpatient clinic of Gaziosmanpaşa Training and Research Hospital.

Participants who were diagnosed as having SUI and for whom it was decided to perform TOT surgery were included. Detailed physical and urogynecological examinations of all participants were performed. Complete urinalysis, urine culture, voiding diary, Q test, and cough test results were recorded. Before the surgery, the patients were asked to rate their incontinence perceptions from 1 to 10, and the scores were recorded. All participants were asked to complete the Incontinence Impact Questionnaire (IIQ-7) (10) and the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF) (11), which were validated in Turkish.

Patients diagnosed as having true SUI, confirmed by history, physical examination, and tests, and patients without a cystocele greater than stage 1 were included in the study. Women who had undergone incontinence surgery before, those with neurologic diseases that might affect bladder functions, women with mixed-type or urge incontinence, patients who would undergo hysterectomy for gynecologic reasons, women with urinary system anomalies, those who were planning a pregnancy, patients who need additional surgery in addition to an incontinence surgeon, those with indications for surgery, and patients with cystocele and rectocele were excluded from the study. All patients were undergoing their first procedure.

The patients were divided into two groups according to the surgical technique to be used, but the patients were blind to which technique they would receive. Patients were randomized to the groups by the evaluating urogynecologist in the outpatient clinic; all patients had surgery performed by the same urogynecologist. After the surgery, follow-ups and evaluations were made by another gynecologist who was blind to the surgical technique in the early and late postoperative period.

Surgical Procedure

All surgeries were performed under spinal or epidural anesthesia in the dorsal lithotomy position. Thirty minutes before the surgery, prophylaxis was performed with 2 g of cefazolin. In accordance with the surgical technique, the anterior vaginal wall 1.5-2 cm sagittal was incised 1.5 cm below the urethra. The tunnel reaching the ischiopubic bone was opened using sharp and blunt dissection in the paraurethral areas. The skin was incised 1 cm lateral to the ischiopubic ramus on the line passing through the clitoris. In the first group, with the outside-in technique, a technique defined by Delorme (6) in 2001, and in the other group with the inside-out technique defined by de Leval (7) in 2003, the mesh was placed under the urethra with a specially curved trocar, close to the medial part of the obturator foramen. After the tension was adjusted, the excess mesh pieces from the skin were cut. Vaginal mucosa was sutured. The same mesh brand and material were used in both groups (Düzey SVT Helical Set, 10x450 mm thickness).

Urethral tubes were removed on the 1st postoperative day. The residual urine was measured after the first micturition. Measurement of postvoid residual urine volume (PVR), the amount of residual urine in the bladder after a voluntary void, is another noninvasive screening test for evaluating voiding dysfunction. Like uroflowmetry, PVR

measurement helps to identify patients in need of further evaluation and to evaluate treatment effect during the follow-up. Threshold values delineating what constitutes an abnormal PVR are poorly defined. However, most urologists agree that volumes of 50 mL to 100 mL constitute the lower threshold defining abnormal residual urine volume (12,13). We also accepted the residual volume of 100 ml as the limit value in our study. Patients with >100 cc and those who developed a bladder globe were re-catheterized. Patients whose residual urine was <100 cc and who did not develop early complications were discharged. All patients were called for follow-up in the 1st week, 6th week, and 12th week after the surgery. Objective treatment, subjective treatment, and unsuccessful treatment criteria recorded in the follow-ups of the patients were used to evaluate the effectiveness of the procedure. Objective treatment was defined as a negative cough stress pad test (CSPT) and complete resolution of symptoms, and subjective treatment was defined as CSPT negative on examination but persisting intermittent symptoms. The continuation of incontinence symptoms was considered an unsuccessful treatment. The IIQ-7 and ICIQ-SF questionnaires were completed again at the postoperative 12th-week follow-ups. Patients were again asked to rate their perceptions of incontinence from 1 to 10.

All authors declare that the study was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects.

Statistical Analysis

The IBM SPSS v.21 package program was used for the statistical analysis of data. The assessment of the normality of continuous variable distribution was performed using the Shapiro-Wilk test. Non-parametric analysis methods were used because the variables did not conform to normal distribution. The Mann-Whitney U test was used to compare the differences between each group. The difference between the first measurement and the second measurement was evaluated using the Wilcoxon test. Categorical data were analyzed using the Chi-square and

Fisher's exact test. A p value of <0.05 was considered a statistical significance level.

RESULTS

Of the 65 women who underwent sling procedures, 32 women received the inside-out technique, and 33 women had the outside-in technique. The demographic data of the patients were shown in Table 1.

When the two groups were compared, they matched each other in terms of age, parity, body mass index (BMI), menopausal status, smoking, duration of incontinence, and IIQ-7 and ICIQ-SF scores, and no statistically significant differences were found. Patients in all groups had at least one vaginal delivery. The preoperative cough stress test was performed on all patients before surgery and all were positive. There was no prolapse greater than grade 1 in any patient.

All patients were prepared for the surgery with the same procedure. The perioperative and early postoperative results of the patients were shown in Table 2.

The patients were called for follow-up in the 1st week, 6th week, and 12th week after the surgery. The effectiveness of the surgery was evaluated through examinations of the patients at the 3rd-month follow-up. There was a significant improvement in all scores and patient symptoms after surgery independent of the technique. When the two techniques were compared in this improvement, it was observed that there was no significant difference between the two groups in terms of IIQ-7 and ICIS-SF scores, incontinence perception scores (Table 3), objective treatment, subjective treatment, and inadequate treatment rates (Table 4).

Both the preoperative incontinence perception values and the postoperative incontinence perception values did not differ significantly according to the groups ($p=0.849$, and $p=0.325$, respectively). Preoperative and postoperative incontinence perception values were different in the inside-out TOT group ($p<0.001$). Preoperative and postoperative incontinence perception values in the outside-in TOT group were also statistically significantly different ($p<0.001$).

Table 1. Demographic data of the patients

	TOT Inside-Out (n=32)	TOT Outside-In (n=33)	p
Age (years), mean±SD, median (Q ₁ -Q ₃) [min-max]	62.28±8.07 64 (57-66) [47-78]	61.09±8.17 61 (55-67) [46-78]	0.557 ^a
BMI (kg/m ²), mean±SD, median (Q ₁ -Q ₃) [min-max]	30.64±5.85 30 (26-34) [21.87-44.1]	29.66±5.74 29 (24-30) [17.72-42.31]	0.442 ^a
Parity , mean±SD, median (Q ₁ -Q ₃) [min-max]	2.38±1.07 2 (2-3) [1-5]	2.48±1.00 3 (2-3) [1-5]	0.514 ^b
Incontinence duration (years), mean±SD, median (Q ₁ -Q ₃) [min-max]	2.5±1.69 2 (1-3) [1-7]	2.21±1.56 2 (1-3) [1-6]	0.367 ^b
Number of vaginal birth , mean±SD, median (Q ₁ -Q ₃) [min-max]	1.09±2.30 1 (1-2) [0-3]	1.12±2.33 1 (1-2) [0-3]	0.723 ^a
Vaginal birth , n (%)	26 (81.3)	28 (84.8)	0.699 ^c
Cesarean , n (%)	6 (18.8)	5 (15.2)	0.740 ^c
Menopause , n (%)	4 (12.5)	3 (9.1)	0.708 ^d
Smoker , n (%)	2 (6.3)	3 (9.1)	0.514 ^d

TOT: transobturator tape, SD: standard deviation, Q₁-Q₃: 25th-75th percentile, ^a: Student's t-test, ^b: Mann-Whitney U test, ^c: Chi-square test, ^d: Fisher's exact test

The change in the preoperative and postoperative incontinence perception values did not differ according to the groups ($p=0.696$).

Objective, subjective, and inadequate treatment rates were shown in Table 4, and no significant difference was detected between the groups.

DISCUSSION

In the current study, the effect of using the inside-out and outside-in TOT techniques, which are two different techniques of the same surgery, on the success of the surgery was evaluated in patients with SUI. When the data of our study were evaluated, no superiority of either method over the other could be determined statistically.

Although many surgical techniques have been described in the treatment of SUI, TOT surgery stands out because of its minimal invasiveness, low cost, low complication rates, and high success rates (14). There are many studies in the

literature comparing the inside-out and outside-in techniques with each other. In a study by Liapis et al. (15), although there was no statistically significant difference in treatment success, they found the outside-in technique disadvantageous in terms of vaginal mucosa perforation and bladder damage, and showed that the inside-out technique was disadvantageous in terms of vascular injury and nerve damage. In our study, vaginal sulcus perforation developed in patients during surgery, and the mesh was corrected by retracting and repositioning during the procedure. However, there was no significant difference between the two techniques in terms of sulcus perforations. This difference may be due to the difference in the number of cases.

In a systematic review and meta-analysis examining prospective studies conducted by Madhuvrata et al. (16) between 1966 and 2011, these two methods were compared and no significant difference was found in

Table 2. The per-operative and early postoperative results of the patients

	TOT Inside-Out (n=32)	TOT Outside-In (n=33)	p
Surgical time (minutes), mean±SD, median (Q ₁ -Q ₃) [min-max]	22.69±4.38 20 (20-25) [16-32]	22.21±4.04 20 (20-25) [16-32]	0.801 ^a
Hospital stay (day), mean±SD, median (Q ₁ -Q ₃) [min-max]	1.09±0.30 1 (1-2) [1-2]	1.12±0.33 1 (1-2) [1-2]	0.723 ^a
Vaginal mucosa perforation , n (%)	6 (18.8)	3 (9.1)	0.303 ^b
Groin pain , n (%)	1 (3.1)	0 (0.0)	0.492 ^b
Mesh erosion , n (%)	3 (9.4)	3 (9.1)	0.999 ^b
Urinary catheter (>24 hours) , n (%)	3 (9.4)	6 (18.2)	0.475 ^b
De novo urge incontinence , n (%)	2 (6.3)	2 (6.1)	0.999 ^b
Transient residual urine elevation , n (%)	6 (18.8)	7 (21.2)	0.804 ^c

TOT: transobturator tape, SD: standard deviation, Q₁-Q₃: 25th-75th percentile, ^a: Mann-Whitney U test, ^b: Fisher's exact test, ^c: Chi-square test

Table 3. Comparison of scores before and after surgery

	TOT Inside-Out (n=32)	TOT Outside-In (n=33)	p _{group}	p _{general}
Preop incontinence perception, mean±SD, median (Q ₁ -Q ₃) [min-max]	7.0±1.19 7 (6-8) [5-9]	7.06±1.06 7 (6-8) [5-9]	0.849	0.696
Postop incontinence perception, mean±SD, median (Q ₁ -Q ₃) [min-max]	1.22±0.75 1 (1-1) [0-4]	1.42±0.90 1 (1-1) [0-4]	0.325	
	p_{time}	<0.001	<0.001	
Preop IIQ-7 score, mean±SD, median (Q ₁ -Q ₃) [min-max]	15.66±1.10 16 (15-16) [14-18]	15.48±1.18 15 (14-16) [14-18]	0.509	0.763
Postop IIQ-7 score, mean±SD, median (Q ₁ -Q ₃) [min-max]	0.91±1.01 1 (0-1) [0-3]	0.94±0.97 1 (0-1) [0-3]	0.654	
	p_{time}	<0.001	<0.001	
Preop ICIQ-SF score, mean±SD, median (Q ₁ -Q ₃) [min-max]	13.00±1.19 13 (12-14) [11-15]	13.03±1.31 13 (12-14) [11-15]	0.925	0.393
Postop ICIQ-SF score, mean±SD, median (Q ₁ -Q ₃) [min-max]	1.66±1.49 1 (0.25-2.75) [0-5]	2.12±1.60 2 (1-3) [0-5]	0.216	
	p_{time}	<0.001	<0.001	

p_{group}: Mann-Whitney U test, p_{time}: Wilcoxon test, p_{general}: Repeated Measures ANOVA (time-group interaction)

Table 4. Objective, subjective, and inadequate treatment rates

	TOT Inside-Out (n=32)	TOT Outside-In (n=33)	p
Objective treatment , n (%)	31 (96.9)	31 (93.9)	0.999 ^a
Subjective treatment , n (%)	1 (3.1)	1 (3.0)	0.999 ^a
Inadequate treatment , n (%)	0 (0.0)	1 (3.0)	0.492 ^a

TOT: transobturator tape, ^a: Fisher's exact test

terms of treatment success and recurrence in 12-month follow-up. Groin and thigh pain was nonsignificantly more common with the inside-out route. Only vaginal angle injuries were significantly more common with the outside-in route.

A prospective study of 100 women in France in 2006 showed that, in agreement with the literature, the two transobturator access routes are equally safe and do not require perioperative cystoscopic examinations (17).

Nerve damage is one of the most feared complications. Spinosa et al. (18) argued that the inside-out technique might damage the posterior terminal branch of the obturator nerve, and even the dorsal nerve of the clitoris could be affected by possible lesions but did not reveal any anatomic evidence. In the continuation, Ahtari et al. (19) performed each technique in a study on a cadaver and placed a tape. In their examinations, although the distance to the nerve was the same in both methods, the inside-out technique also passed the tape closer to the obturator canal. The outside-in technique was found to be safer in terms of nerve damage.

All follow-ups in this literature included one-year results. The 9-year follow-up by Karmakar et al. (20) included the longest follow-up period in the literature. In their study, it was revealed that the success rate showed a significant decrease compared with the 1-year results (71.6% vs. 80%). However, this was a clinically insignificant reduction compared with the 3-year results (71.6% vs. 73.1%). This means that the failure of the surgery is higher in the first years and tends to decrease as time progresses. The authors emphasized that the success rate was almost constant after 3 years.

A prospective single-blind study of 341 women conducted by Abdel-Fattah et al. (21) in 2010 also evaluated the independent risk factors for 1-year failure. In the study, no statistical difference was found between the two methods in terms of success and failure. In terms of quality of life and sexual life, the difference before and after surgery was not different for the two methods.

The fact that the anatomic space is entered with a blunt needle, working very close to important anatomic areas, and the accuracy of the placement of the tape cannot be visualized during surgery is perhaps the most distressing process for surgeons in terms of surgical technique. To illuminate this process, studies were conducted on cadavers, and the band position was tried to be confirmed by placing radiopaque bands. One of these studies was a study conducted by Hinoul et al. (22) on 10 freshly thawed cadavers. After the propylene bands were made radiopaque, they were placed by an experienced surgeon using two different techniques, then scanned using computed tomography and the route of placement was evaluated. As a result, the inner angle of the tape applied from the inside to the outside was significantly narrower compared with the other method. This meant that the two techniques did not place the tape in the same location, despite using the same anatomic space and the same material.

In a study conducted by Cordeiro et al. (23), there was no statistical difference between the technical successes of the two methods, while the rates of de novo urge incontinence and mesh erosion were the same in the two groups when different materials were used during the technical application. However, there was more erosion in

microporous bands than in macroporous bands. When the same study looked at surgical times, the inside-out technique was associated with significantly shorter surgical time. However, this may be due to their increased experience with these methods because they perform more inside-out techniques in their practice. In another study conducted by Abdel-Fattah et al. (24), 238 of 341 women were followed for 3 years and the success of both methods was found to be the same, in line with the literature.

10-year long-term results were shared in 2018 in the study of Serdinšek et al. (25), which is one of the newest studies. They did not find a significant difference in terms of objective and subjective improvement rates, and satisfaction rates.

In the most recent study to date by Kovalev et al. (26) in 2021, the two techniques were compared in many ways. They defined the outside-in technique as the method performed by taking the anatomical soft tissues as a guide, and the inside-out technique as the method in which the bone tissue is the guide. The author, who stated that the perineal pain may be due to the damage of the obturator nerve branches, determined that the branch damage was seen at 70% in the outside-in technique, while 30% was seen in the inside-out technique. In their evaluation to detect band placement with tomography, they also found the inside-out technique to be disadvantageous, which may affect the clinical results. As a result, they argued that bone tissue is a more reliable guide than soft tissue.

CONCLUSION

Successful results are obtained with TOT procedures performed in the treatment of SUI. The perfect application of the technique is as important as the choice of technique in the success of the surgery. We believe that the choice of surgical procedure and technique should be decided after discussing the risks related to the patient in addition to the experience of the surgeon.

Ethics Committee Approval: The study was approved by the Ethics Committee of Gaziosmanpaşa Training and Research Hospital (23.11.2022, 121).

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The Effects of Bioimpedance Analysis Results and Upper Extremity Anthropometric Measurements on Grip Strength in Young Adults

Genç Erişkinlerde Biyoempedans Analiz Sonuçlarının ve Üst Ekstremitte Antropometrik Ölçümlerinin Kavrama Kuvvetine Etkisi


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ABSTRACT

Aim: Among the hand functions, grasping is an important function for the continuity of daily living activities. The assessment of grip strength is crucial for identifying athletic prowess, neuromuscular illnesses, assessing potential side effects following hand surgery, and treatment plans. It was aimed to examine whether bioimpedance analysis (BIA) and upper extremity anthropometric measurements are effected grip strength in young adults.

Material and Methods: The study was conducted on 110 young adults, 55 males and 55 females, aged 18-25 years. Individuals with a history of trauma, musculoskeletal problems, and cardiac, respiratory, metabolic, or systemic diseases were not included in the study. Non-flexible tape measure, Harpenden anthropometric set, digital caliper, hand dynamometer, and skinfold caliper were used for anthropometric measurements. Body fat percentage and lean body mass, muscle mass, basal metabolic rate, and total body fluid parameters were investigated by bioelectrical impedance measurement. Three measurements were taken to reduce the error rate, and the average of these measurements was considered.

Results: Excluding arm circumference, forearm length, and wrist circumference from anthropometric measurements, and mineral from BIA parameters; anthropometric measurements and BIA results were found to be statistically significantly correlated with grip strength.

Conclusion: Upper extremity anthropometric measurements and BIA were associated with grip strength and varied according to gender. Significant correlations were found between grip strength with most of the anthropometric parameters and BIA.

Keywords: Anthropometry; bioimpedance analysis; grip strength; upper extremity; young adults.

ÖZ

Amaç: El fonksiyonları arasında kavrama, günlük yaşam aktivitelerinin devamlılığı için önemli bir fonksiyondur. Kavrama gücünün değerlendirilmesi, atletik beceriyi belirlemek, nöromusküler hastalıklar, el cerrahisini takiben olası yan etkileri değerlendirmek ve tedavi planları için çok önemlidir. Genç erişkinlerde biyoempedans analizi (BIA) ve üst ekstremitte antropometrik ölçümlerinin kavrama gücünü etkileyip etkilemediğinin incelenmesi amaçlandı.

Gereç ve Yöntemler: Çalışma, yaşları 18-25 yıl arasında olan 55 erkek ve 55 kadın olmak üzere 110 genç yetişkin üzerinde gerçekleştirildi. Travma öyküsü, kas-iskelet sistemi sorunu ve kalp, solunum, metabolik veya sistemik hastalığı olan kişiler çalışmaya alınmadı. Antropometrik ölçümler için esnek olmayan mezura, Harpenden antropometrik seti, dijital kumpas, el dinamometresi ve deri kıvrım kalınlığı ölçüm cihazı kullanıldı. Biyoelektrik empedans ölçümü ile vücut yağ yüzdesi ve yağsız vücut kütlesi, kas kütlesi, bazal metabolizma hızı ve toplam vücut sıvısı parametreleri araştırıldı. Hata oranını düşürmek için 3 kez ölçüm alındı ve bu ölçümlerin ortalaması dikkate alındı.

Bulgular: Antropometrik ölçümlerden kol çevresi, ön kol uzunluğu ve bilek çevresi, BIA parametrelerinden ise mineral hariç olmak üzere; antropometrik ölçümler ve BIA sonuçlarının kavrama gücü ile istatistiksel olarak anlamlı bir şekilde ilişkili olduğu bulundu.

Sonuç: Üst ekstremitte antropometrik ölçümleri ve BIA kavrama kuvveti ile ilişkilidir ve cinsiyete göre farklılık göstermektedir. Kavrama kuvveti ile antropometrik parametrelerin çoğu arasında ve BIA ile anlamlı bir ilişki bulunmuştur.

Anahtar kelimeler: Antropometri; biyoempedans analizi; kavrama kuvveti; üst ekstremitte; genç erişkinler.

INTRODUCTION

Various body composition analysis methods have been developed in clinical and athletic fields to determine optimal body composition and to identify and classify diseases. Bioimpedance analysis (BIA) is a common approach used in body composition measurement and health assessment systems. BIA is a very easy-to-use and non-invasive method (1). Varied methods are used to interpret the data obtained by bioimpedance measurements. There are a wide variety of bioimpedance applications in healthcare, such as disease prognosis and monitoring of body vitality (2).

Anthropometry is a systematic technique-based science that measures the physical characteristics of the human body using measurement principles. Anthropometric measurements are made dynamically and statically. Measurements such as height, length, circumference, and skin thickness are static measurements. Dynamic data is obtained by measuring the limits of bending, elongation, and spinning/turning movements (3). Anthropometry is a widely used, portable, cheap, simple, and useable technique consisting of various body measurements (4).

Grip strength is an indicator of disability, morbidity, mortality, increased dependence on activities of daily living, and cognitive decline in adults (5). It is important for the auxiliary diagnosis of diseases in many clinical branches such as internal medicine, orthopedics, and physiotherapy. Studies on this subject have shown that grip strength is not only related to general body muscle strength but also to upper extremity muscle strength (6). Low grip strength is also correlated with hypertension, coronary artery disease, peripheral artery disease, heart failure, stroke, or chronic obstructive pulmonary disease. Furthermore, grip strength is negatively associated with physical frailty even after accounting for the effects of body mass index (BMI) and arm muscle circumference (7). Evaluation of grip strength helps to identify the risk of micro-trauma as well as to determine both the rehabilitation process of overuse injury and the performance level of athletes (8,9).

We aimed to benefit these various clinical and social areas to examine whether upper extremity anthropometric measurements and BIA results are effected grip strength in young adults. The present study's results contribute to determining the risk of future disease, with grip strength in the young population with less health problems. The correlation between grip strength and BIA and

anthropometric measurements is effective in determining health problems such as obesity and metabolic syndrome.

MATERIAL AND METHODS

This study was approved by the Non-Invasive Clinical Research Ethics Committee of Kırşehir Ahi Evran University Faculty of Medicine (Date: 10.05.2022, Ethical number: 2022-09/99). Volunteers were informed about the measurements and signed an informed consent form. The sample size of the study was determined by a priori power analysis. When the correlation was taken as $\rho H_1=0.3$, Power $(1-\beta)=0.85$, the minimum sample size to be studied was 110. The study was conducted on a total of 110 young adults (55 male and 55 female) between the ages of 18-25 years, collected cross-sectionally from Kırşehir Ahi Evran University Faculty of Medicine. Individuals without any history of trauma, musculoskeletal problems, or cardiac, respiratory, metabolic, or systemic diseases were included in the study. For anthropometric measurements, an inflexible tape measure, Harpenden anthropometric set, digital caliper, stadiometer, hand dynamometer, pinch meter, and skinfold caliper were used. To reduce the error rate, the measurements were made by the researcher, three times, and the average was taken. TANITA MC-780 is used for BIA. Height was measured with bare feet by a stadiometer in an anatomical position. Weight was measured with an electronic scale while the participants were hungry. BMI was calculated using the weight kg/m^2 method.

Triceps skinfold thickness, biceps skinfold thickness, arm circumference, forearm circumference, wrist circumference, upper extremity length, arm length, forearm length, hand length, palm length, third finger length, hand width, wrist medio-lateral diameter, wrist dorso-volar diameter, grip strength, tip pinch, key pinch, palmar pinch, weight (kg), height (cm) were measured. As the BIA measurements were taken such as BMI (kg/m^2), lean mass (%), muscle (%), fat mass (%), total body fluid (lt), intracellular fluid (kg), extracellular fluid (kg), protein (%), mineral (%), basal metabolic rate (kcal), obesity degree (%), waist/hip ratio, waist/height ratio, phase angle (provides information about cellular health and integrity).

Triceps and biceps skinfold thickness were measured by skinfold caliper from the dominant upper extremities of individuals (Figure 1A and Figure 1B). Grip strength was measured by hand dynamometer (Figure 1C).



Figure 1. A) Triceps skinfold thickness, B) Biceps skinfold thickness, C) Grip strength

Arm circumference (the criterion point is taken as the medial epicondyle of the humerus and is made by taking 10-15 cm above this point or the most swollen part of the muscle), forearm circumference (the distance between the olecranon and styloid process of radius) and wrist circumference were measured by inflexible tape measure. Upper extremity length (distance between the acromion and the most distal part of the third finger), arm length, and forearm length were measured by the Harpenden anthropometric set (Figure 2).

Hand length, palm length and third finger length, hand width, wrist medio-lateral diameter (wrist line level, from the medial and lateral points), and wrist dorso-volar diameter (anterior-posterior thickness was measured at the wrist crease line level) were measured by a digital caliper (Figure 3).

Tip pinch, key pinch, and palmar pinch were measured by pinch meter (Figure 4).

Weight, lean mass, muscle mass, fat mass, total body fluid, intracellular fluid, extracellular fluid, protein, mineral, basal metabolic rate, obesity degree, waist/hip ratio, waist/height ratio, and phase angle were measured by BIA device. Participants were asked to stand barefoot on the electrode panel and hold the electrodes in their anatomical position by contacting the fingers and palms of both hands.

Statistical Analysis

The conformity of the data to the normal distribution was evaluated with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Independent t-test and Mann-Whitney U tests were used for univariate analyzes according to the availability of assumptions. The correlation between variables was calculated by Pearson correlation analysis. Data analysis was performed using Statistical Package for Social Sciences for windows (IBM SPSS version 28.0, Armonk, NY, USA). The significance level was accepted as $p < 0.05$.

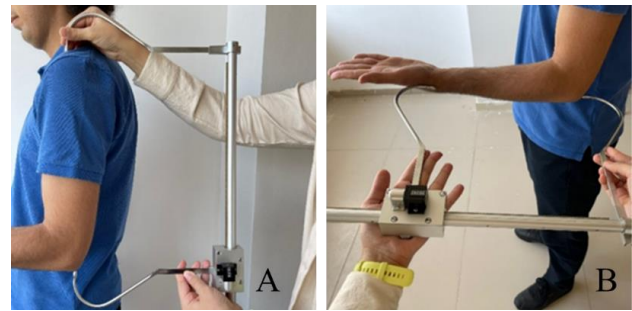


Figure 2. A) Arm length, B) Forearm length

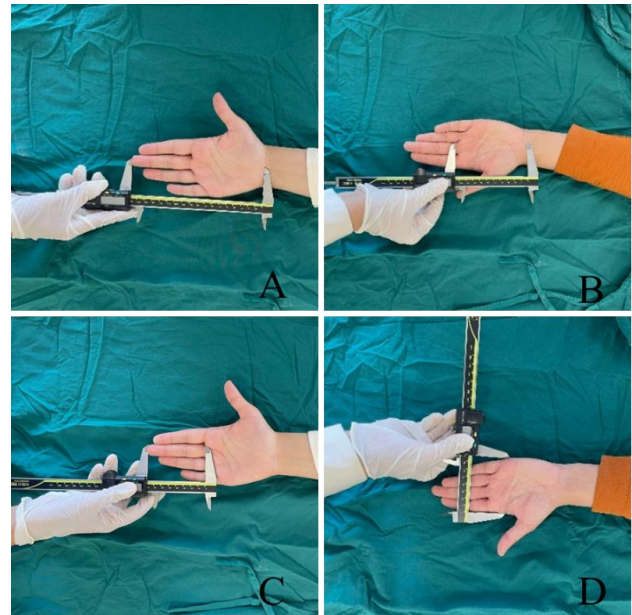


Figure 3. A) Hand length, B) Palm length, C) Third finger length, D) Hand width

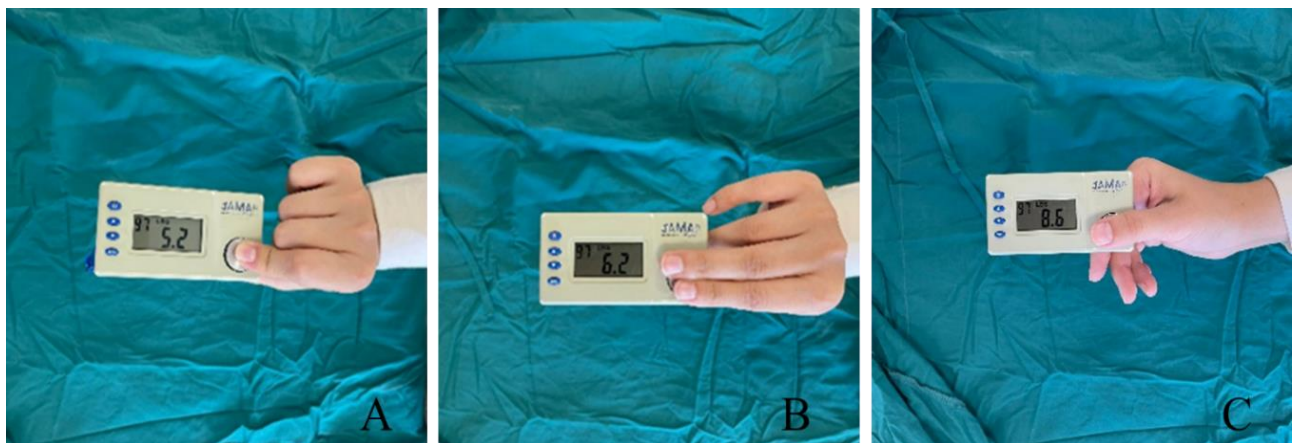


Figure 4. A) Tip pinch, B) Key pinch, C) Palmar pinch

RESULTS

Descriptive statistics and group comparisons of the variables that are the subject of the study were given in Table 1. The mean age of individuals was calculated as 19.07 ± 1.16 years. Height and weight were calculated to be higher in males than in females. A statistically significant difference was found between the genders in terms of all

variables ($p < 0.001$) except arm circumference and forearm length. Arm circumference, forearm circumference, wrist circumference, upper extremity length, arm length, forearm length, hand length, third finger length, palm length, hand width, wrist medio-lateral diameter, wrist dorso-volar diameter, tip pinch, key pinch, palmar pinch,

weight, height, BMI, lean mass, muscle mass, total body fluid, intracellular fluid, extracellular fluid, basal metabolic rate, protein, mineral, obesity degree, waist/height ratio, waist/hip ratio, phase angle, grip strength of males was higher than females. Even though arm circumference and forearm length are higher in males, these were found to be statistically insignificant ($p=0.070$, and $p=0.501$, respectively). Triceps skinfold thickness, biceps skinfold thickness, and fat mass (%) were found higher in females ($p<0.001$).

Arm length, hand length, palm length, hand width, wrist medio-lateral diameter, wrist dorso-volar diameter, BMI, lean mass, muscle mass, total body fluid, basal metabolic rate, waist/hip ratio, forearm circumference, upper extremity length, third finger length, tip pinch, key pinch, palmar pinch, weight, height, intracellular fluid, extracellular fluid, protein, obesity degree, waist/height ratio were found to be statistically significantly positively correlated with grip strength. There is a negative significant correlation with biceps skinfold thickness. Triceps skinfold thickness, fat mass, and phase angle were

found to be statistically significantly negatively correlated with grip strength (Table 2). It was determined that the correlation between grip strength and arm circumference, forearm length, and wrist circumference with mineral was not statistically significant.

DISCUSSION

Grip strength is very important for performing many activities of daily living. Studies in the literature have reported the effects of factors such as anthropometric parameters (forearm length, forearm circumference measurement), gender, hand dominance, height, BMI, and age on hand grip and finger grip strength (10-12). In a study by Stegink et al. (12), hand grip strength and finger grip strength, and anthropometric measurements were found to be positively related to the arm-forearm circumference measurements.

According to the study of Anakwe et al. (13), a significant correlation was found between forearm circumference and grip strength. Our study showed that there is a significant correlation between grip strength with upper extremity

Table 1. Anthropometric measurement and bioimpedance analysis results of young adults

	Male (n=55)	Female (n=55)	p
Triceps Skinfold Thickness (mm)	7.44±2.97	12.84±6.15	<0.001
Biceps Skinfold Thickness (mm)	4.5 (3.5-7.5)	8.1 (5.0-12.0)	<0.001
Arm Circumference (mm)	183.07±132.44	140.68±109.04	0.070
Forearm Circumference (mm)	28.00 (26.90-29.00)	23.00 (22.00-24.00)	<0.001
Wrist Circumference (mm)	18.00 (17.50-19.00)	15.90 (15.00-16.00)	<0.001
Upper Extremity Length (mm)	759.10±38.88	714.51±33.84	<0.001
Arm Length (mm)	361.00 (354.50-376.50)	337.00 (324.00-349.00)	<0.001
Forearm Length (mm)	278.71±36.65	273.98±36.82	0.501
Hand Length (mm)	182.00 (176.50-188.24)	166.00 (159.00-172.28)	<0.001
Third Finger Length (mm)	77.03 (73.68-82.17)	73.59 (70.27-76.69)	<0.001
Palm Length (mm)	108.57±14.28	97.05±8.52	<0.001
Hand Width (mm)	87.62±11.14	76.17±4.01	<0.001
Wrist Medio-lateral Diameter (mm)	60.51±4.60	53.00±5.34	<0.001
Wrist Dorso-volar Diameter (mm)	40.47 (37.31-43.70)	32.70 (29.02-34.41)	<0.001
Tip Pinch (kg)	5.03 (3.81-5.99)	3.36 (2.86-3.90)	<0.001
Key Pinch (kg)	8.85 (7.44-10.16)	5.13 (3.99-5.81)	<0.001
Palmar Pinch (kg)	6.34±1.67	4.93±1.32	<0.001
Weight (kg)	74.40 (65.90-83.90)	54.60 (51.20-63.50)	<0.001
Height (cm)	178.00 (173.00-181.00)	167.00 (162.00-170.00)	<0.001
Body Mass Index (kg/m ²)	23.93±3.87	20.83±4.03	<0.001
Lean Mass (%)	83.59 (77.59-88.24)	78.64 (72.68-80.68)	<0.001
Muscle Mass (%)	79.46 (73.72-83.79)	74.67 (68.96-76.54)	<0.001
Fat Mass (%)	16.41 (11.44-22.42)	21.36 (19.33-27.33)	<0.001
Total Body Fluid (lt)	60.91 (55.73-64.56)	56.71 (52.60-58.78)	<0.001
Intracellular Fluid (kg)	26.90 (24.80-28.70)	18.20 (17.20-19.90)	<0.001
Extracellular Fluid (kg)	18.00 (16.60-19.00)	12.70 (12.00-14.10)	<0.001
Basal Metabolic Rate (kcal)	1855.38±199.04	1362.64±138.78	<0.001
Protein (%)	16.66 (16.06-17.56)	15.94 (14.56-16.62)	<0.001
Mineral (%)	6.09 (5.60-6.48)	5.64 (5.18-5.89)	<0.001
Obesity Degree (%)	9.01 (-3.26-22.74)	-5.24 (-14.87-7.93)	<0.001
Waist/Height ratio	0.48 (0.44-0.53)	0.42 (0.39-0.47)	<0.001
Waist/Hip ratio	0.88±0.05	0.76±0.06	<0.001
Phase Angle	6.38±0.52	5.30±0.77	<0.001
Grip Strength (kg)	41.62±8.67	25.31±5.67	<0.001

Table 2. Correlation results between grip strength and anthropometric measurements with BIA of young adults

	r	p
Triceps Skinfold Thickness	-0.323	0.001
Biceps Skinfold Thickness	-0.194	0.042
Arm Circumference	0.096	0.317
Forearm Circumference	0.680	<0.001
Wrist Circumference	0.016	0.870
Upper Extremity Length	0.349	<0.001
Arm Length	0.416	<0.001
Forearm Length	0.095	0.322
Hand Length	0.460	<0.001
Third Finger Length	0.235	0.013
Palm Length	0.415	<0.001
Hand Width	0.452	<0.001
Wrist Medio-lateral Diameter	0.487	<0.001
Wrist Dorso-volar Diameter	0.438	<0.001
Tip Pinch	0.579	<0.001
Key Pinch	0.728	<0.001
Palmar pinch	0.604	<0.001
Weight	0.520	<0.001
Height	0.225	0.018
Body Mass Index	0.327	<0.001
Lean Mass	0.348	<0.001
Muscle Mass	0.346	<0.001
Fat Mass	-0.351	<0.001
Total Body Fluid	0.357	<0.001
Intracellular Fluid	0.733	<0.001
Extracellular Fluid	0.675	<0.001
Basal Metabolic Rate	0.687	<0.001
Protein	0.197	0.039
Mineral	0.039	0.686
Obesity Degree	0.345	<0.001
Waist/Height ratio	0.283	0.003
Waist/Hip ratio	0.535	<0.001
Phase Angle	-0.555	<0.001

anthropometric measurements and BIA. Also, we found a significant correlation between muscle mass and grip strength, which was higher in males ($r=0.346$).

As a result of the study by Seethamma et al. (14), it was found that grip strength was directly related to the anthropometric measurement of hand length and forearm circumference, and therefore grip strength was associated with hand length and forearm circumference regardless of age and gender. While we obtained similar results to Seethamma et al. (14), contrary to the results of our study, Nefesoğlu et al. (15) reported that grip strength was associated with forearm length, arm circumference, and wrist circumference.

Contrary to our results, Narin et al. (6) reported that grip and finger grip strength were higher in individuals with longer forearms and more circumference measurements. In our research, a significant result was found between the palm length and grip strength, as in Narin et al. (6). In the analysis performed by Öktem et al. (11), no significant correlation was found between the arm length measured in

both arms and the grip strength. However, a highly significant correlation was found in our study ($r=0.416$).

The study of Fallahi et al. (16) on grip strength, found that individuals for all age groups with larger hand circumference or longer palm length had stronger grip strength. In our research, a significant result was found the palm length and hand width with grip strength, as in this study. Our study is the first, conducted to examine the dorso-volar and medio-lateral diameter of the wrist and its effect on grip strength in both genders among sedentary young adults (0.438, and 0.487, respectively).

In another study on young adults, forearm circumference, third finger length, and height showed a significant positive correlation with grip strength (17). Similarly, we found that forearm circumference, height, third finger, and palm length are positively correlated with grip strength. In numerous studies, the effect of many anthropometric parameters on grip strength was investigated, but none of them searched the effect of pinches strength. Tip, key and palmar pinches are positively correlated with grip strength in our study ($r=0.579$, $r=0.728$, and $r=0.604$, respectively). In the current study, there is a significant and positive correlation between waist/hip ratio with grip strength, contrary to Hutashut et al.'s (18) study. Also, a significant and negative correlation was found between waist-height ratio and BMI with grip strength (19). Contrary to this study, we found a positive correlation between BMI and waist/height ratio with grip strength.

In a study conducted in prepubertal children, triceps and biceps skinfold thickness was found to be significantly higher in females (20). Our triceps and biceps skinfold thickness outcomes are also similar to results in the literature ($r=-0.323$, and $r=-0.194$, respectively).

Stenholm et al. (21) reported that long-term exposure to obesity was associated with poor grip strength in adulthood. In the group we studied in this study, a negative significant correlation was found between BMI and obesity degree with grip strength.

In another study, lean body mass was associated with stronger grip strength in both genders, but mainly in males, while the fat mass index was associated with weaker grip strength as in our study (22).

In a study by Bittencourt et al. (23) examining the correlation between phase angle and functionality in the elderly female population, a significant correlation was found between phase angle and grip strength ($r=0.177$). We obtained a more significant correlation ($r=-0.555$) in our study than in Bittencourt et al. (23).

According to our results, adipose parameters such as skinfold thickness and fat mass were found to be higher in females. This may be due to hormonal reasons and a sedentary lifestyle or less physical activity. Obesity is becoming a global problem in young adults as well as adults. In order to prevent this, routine examinations including grip strength can be developed in the early control of obesity based on the highly significant values in our study. Nutritional content may be one of the reasons for the positive and significant association between grip strength with protein and mineral ratio in our study. The total body fluid, intracellular and extracellular fluid had a positive and significant correlation with grip strength in the current study. These fluid parameters can contribute to the treatment of edema.

CONCLUSION

In conclusion, we believe that the data we collect as a result of the current study will be beneficial in many areas, especially in the early diagnosis and treatment of diseases. Furthermore, this study can be helpful for rehabilitation and sports branches. The assessment of grip strength is crucial for identifying artistic and athletic prowess, monitoring neuromuscular illnesses, assessing potential side effects following hand surgery, and formulating treatment plans.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Kırşehir Ahi Evran University (10.05.2022, 2022-09/99).

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A New Parameter for Erythropoiesis: The Relationship of Immature Reticulocyte Fraction Values in Cord Blood with Clinical Factors and Reference Values for Newborns

Eritropoez için Yeni Bir Parametre: Kordon Kanında İmmatür Retikülosit Fraksiyon Değerlerinin Klinik Faktörlerle İlişkisi ve Yenidoğan için Referans Değerleri


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ABSTRACT

Aim: The immature reticulocyte fraction (IRF) is a new parameter for the reticulocyte maturity index, representing an independent parameter of erythropoiesis that may be useful to better assess erythropoietic activity in neonates. In this study, the relationship between IRF values and clinical features in newborns was investigated and reference values were obtained.

Material and Methods: Newborns between 28-40 weeks of gestation were included in this prospective study. At birth, maternal venous and cord blood samples were obtained for measurements of complete blood count (CBC), blood gases, and plasma concentrations of various biochemical parameters.

Results: A total of 123 newborns, 99 term and 24 preterm, were included in the study. When the laboratory characteristics of the premature and term babies were compared according to their gestational weeks, while the median IRF value of cord blood was higher in term babies than in premature babies ($p=0.039$), other laboratory findings did not differ significantly. The median IRF value was 0.52 (range, 0.15-1.00) in term infants and 0.34 (range, 0.16-0.76) in preterm infants. IRF reference values for the term and preterm newborns were determined in cord blood. Moderately positive correlations were observed between the IRF levels and both the RDW ($r=0.423$, $p<0.001$) and the CRP ($r=0.389$, $p<0.001$) levels.

Conclusion: The results of this study showed that newborns' IRF values were not affected by maternal variables and changed with the week of birth. The results of this study might be considered a guide for future studies using IRF value in newborns.

Keywords: Cord blood; newborn; immature reticulocyte fraction.

ÖZ

Amaç: Olgunlaşmamış retikülosit fraksiyonu (immature reticulocyte fraction, IRF), retikülosit olgunluk indeksini gösteren yeni bir parametredir ve yenidoğanlarda eritropoietik aktiviteyi daha iyi değerlendirmek için yararlı olabilecek bağımsız bir eritropoez parametresini temsil eder. Bu çalışmada yenidoğanlarda IRF değerleri ile klinik özellikler arasındaki ilişki araştırılmış ve referans değerler elde edilmiştir.

Gereç ve Yöntemler: Bu prospektif çalışmaya 28-40 gebelik haftaları arasında olan yenidoğanlar dahil edildi. Doğumda, tam kan sayımı (complete blood count, CBC), kan gazları ve çeşitli biyokimyasal parametrelerin plazma konsantrasyonlarının ölçümleri için maternal venöz ve umbilikal kord kan örnekleri alındı.

Bulgular: Çalışmaya 99 term ve 24 preterm olmak üzere toplam 123 yenidoğan dahil edildi. Prematüre ve term bebeklerin gebelik haftalarına göre laboratuvar özellikleri karşılaştırıldığında, kordon kanı ortanca IRF değeri term bebeklerde prematüre bebeklere göre daha yüksek bulunurken ($p=0.039$), diğer laboratuvar bulguları anlamlı farklılık göstermedi. Ortanca IRF değeri term bebeklerde 0,52 (aralık, 0,15-1,00) ve prematüre bebeklerde 0,34 (aralık, 0,16-0,76) idi. Term ve preterm yenidoğanlar için kordon kanında IRF referans değerleri belirlendi. IRF düzeyleri ile hem RDW ($r=0,423$, $p<0,001$) hem de CRP ($r=0,389$, $p<0,001$) düzeyleri arasında orta düzeyde pozitif korelasyonlar gözlemlendi.

Sonuç: Bu çalışmanın sonuçları, yenidoğanların IRF değerlerinin maternal değişkenlerden etkilenmediğini ve doğum haftası ile değiştiğini göstermiştir. Bu sonuçlar yenidoğanlarda IRF değerini kullanarak yapılacak olan sonraki çalışmalar için bir rehber olarak kabul edilebilir.

Anahtar kelimeler: Kordon kanı; yenidoğan; immatür retikülosit fraksiyonu.

Presented as a poster at the 24. National Neonatology Congress (April 17-20, 2016; Antalya, Türkiye)

INTRODUCTION

Erythropoiesis in newborns can differ compared to older children and adults. The commonly used forms of erythrocyte series in the clinic are reticulocytes and mature erythrocytes. The reticulocyte or non-nucleated erythrocyte counts that contain immature RNA provide useful information about erythrocyte synthesis and circulating capacity in response to the ability of bone marrow to respond to a physiological condition, such as anemia. With the latest technological advances, the newest measurement parameter is the immature reticulocyte fraction (IRF) which can be useful in the evaluation of erythropoietic activity in newborns. IRF may be an economical alternative to the measurement of plasma transferrin receptor and serum erythropoietin levels. We also investigated the place of IRF in the early diagnosis of sepsis. There are only a few studies available about IRF because it is a new parameter and there are no international standards, like for other complete blood count parameters (1-2).

The aims of this study were to assess the relationship between the cord blood IRF values and the clinical characteristics of newborns and to obtain reference values for IRF from the cord blood samples.

MATERIAL AND METHODS

In this prospective study, 123 newborns who were born with normal or cesarean section in Kırıkkale University Medical Faculty Hospital between February 2015 and June 2015 with antenatal follow-up and over 28 weeks of gestation were enrolled. Babies with a gestational age of less than 28 weeks, a major congenital anomaly, and hydrops fetalis were excluded from the study.

Umbilical cord blood samples of the infants were studied prospectively. Maternal age, chronic illnesses, obstetric complications, maternal drug abuse, the number of previous pregnancies and deliveries for the mother, maternal blood group, type of delivery, gestational age of the baby, resuscitation, Apgar scores, birthweights, and gender were recorded.

In the delivery room, umbilical cord blood samples were taken by the pediatrician immediately after umbilical cord clamping. Then, 2 ml of cord blood was taken up in tubes containing K3 EDTA for a sample of the complete blood count. The complete blood count, reticulocytes, and IRF measurements were automatically performed on the Beckman Coulter LH 780 device. Additionally, 1 ml of heparinized injectors was prepared for blood gas measurements. The Siemens RAPIDLab 348 instrument was used to determine the blood gas parameters. Blood for the CRP (2 ml) was centrifuged for five minutes at 3,500 rpm, and the serum was separated. In a Beckman Coulter AU 480-680, the CRP levels were quantitatively determined using an immuno-nephelometric method with the appropriate kit.

Local ethics committee approval was obtained before the study (Clinical Research Ethics Committee of Kırıkkale University (23.02.2015, 04/02).

Statistical Analysis

Statistical analyses were performed with the SPSS v.15.0 statistical program (SPSS, Inc., Chicago, IL, USA). For categorical variables, frequencies and percentages were used for descriptive statistics, and the chi-square and

Fisher's exact tests were used for comparing groups. The distributions of the measured data were analyzed by Kolmogorov-Smirnov and Shapiro-Wilk tests and were confirmed using histograms. The mean and standard deviation were provided for normally distributed variables, and the median and minimum-maximum values were provided for not normally distributed variables. The IRF reference values were created using the 2.5 and 97.5 percentiles. The significance of the inter-group differences was tested using the Independent samples t-test and Mann-Whitney U test. Correlations between the variables were analyzed using Pearson or Spearman correlations, respectively, according to whether they were distributed normally or not. The statistical significance level for all tests was set to <0.05.

RESULTS

The study group consisted of 99 term and 24 preterm infants. The demographic characteristics of the infants were shown in Table 1. In comparing the laboratory characteristics of the infants according to gestational week, the umbilical cord blood median IRF value in term infants was higher than for preterm infants ($p=0.039$). The other laboratory findings were not significantly different between preterm and term infants (Table 2).

When the IRF value of umbilical cord blood was analyzed according to the individual variables for both the term and preterm infants, there was no significant relationship between gender, birth percentiles, mode of delivery, the need for resuscitation, hospitalization, gestational complication, antenatal steroid, and multiple pregnancies (Table 3).

The IRF reference values for the term infants have a lower limit (2.5 percentile) of 0.17, an upper limit (97.5 percentile) of 0.89, and a median of 0.52. For the preterm infants, the lower limit (2.5 percentile) was found to be 0.16, the upper limit (97.5 percentile) to be 0.73, and the median to be 0.34 (Figure 1).

Table 1. Distribution of infant and mother characteristics in the study group by gestational week

	Term (n=99)	Preterm (n=24)
Gender, n (%)		
Female	48 (48.5)	11 (45.8)
Male	51 (51.5)	13 (54.2)
Birth percentile, n (%)		
SGA	15 (15.2)	4 (16.7)
AGA	78 (78.8)	20 (83.3)
LGA	6 (6.1)	0 (0.0)
Type of delivery, n (%)		
Vaginal delivery	27 (27.3)	5 (20.8)
Cesarean section	72 (72.7)	19 (79.2)
Resuscitation, n (%)	5 (5.1)	3 (12.5)
Hospitalization, n (%)	45 (45.5)	11 (45.8)
Maternal preeclampsia, n (%)	7 (7.1)	3 (12.5)
Maternal diabetes, n (%)	6 (6.1)	1 (4.2)
Multiple pregnancies, n (%)	1 (1.0)	3 (12.5)
Antenatal steroid, n (%)	0 (0.0)	2 (8.3)
Mother's smoke, n (%)	13 (13.1)	1 (4.2)

SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age

Table 2. Laboratory findings from cord blood samples for both term and preterm infants

	Term (n=99)	Preterm (n=24)	p
RBC (x10 ⁶ uL)	4.48±0.65	4.59±0.73	0.459
HGB (g/dl)	15.7±2.1	15.9±2.5	0.647
HCT (%)	47.0 (9.0) [34-67]	48.5 (12.0) [33-69]	0.638
MCV (fl)	107.3±4.9	108.9±4.4	0.511
MCHC (g/dl)	32.0 (3.0) [30-36]	31.0 (3.0) [28-35]	0.315
RDW (%)	17.0 (2.0) [13-22]	17.0 (2.8) [13-20]	0.805
WBC (x10 ³ uL)	12100 (4600) [6000-27500]	12600 (5250) [5900-25100]	0.898
PLT (x10 ³ uL)	230.86±58.84	216.67±51.73	0.281
RTC (%)	3.71 (0.73) [2.32-6.68]	3.50 (1.02) [2.23-5.98]	0.098
IRF	0.52 (0.30) [0.15-1.00]	0.34 (0.28) [0.16-0.76]	0.039
pH	7.26 (0.11) [6.83-7.49]	7.26 (0.13) [7.10-7.41]	0.783
BE (mmol/L)	-4.7 (2.7) [-20-1.4]	-4.8 (4.1) [-8.9- -1.1]	0.568
HCO₃ (mmol/L)	21.2±3.0	21.3±2.0	0.814
PCO₂ (mmHg)	46.8±10.3	45.8±11.5	0.667
PO₂ (mmHg)	23.0 (9.0) [16-62]	24.5 (9.3) [16-55]	1.246
CRP (mg/L)	0.65 (1.15) [0-4]	1.07 (1.21) [0.01-3.8]	0.213

RBC: red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, WBC: white blood cell, PLT: platelet, RTC: reticulocyte count, IRF: immature reticulocyte fraction, pH: potential hydrogen, HCO₃: bicarbonate, PCO₂: partial pressure of carbon dioxide, PO₂: partial pressure of oxygen, CRP: C-reactive protein, descriptive statistics were presented as mean±standard deviation or median (interquartile range) [minimum-maximum]

Table 3. Cord blood immature reticulocyte fraction values according to the other variables in term and preterm infants

	Term	p	Preterm	p
Gender				
Female	0.56 (0.29) [0.17-0.78]	0.760	0.31 (0.33) [0.16-0.59]	0.353
Male	0.51 (0.29) [0.15-1.00]		0.36 (0.27) [0.16-0.76]	
Birth percentile				
SGA	0.42 (0.32) [0.21-0.86]	0.859	0.45 (0.40) [0.31-0.76]	0.176
AGA	0.53 (0.31) [0.15-1.00]		0.33 (0.28) [0.16-0.62]	
LGA	0.52 (0.24) [0.30-0.67]		-	
Cesarean section	0.52 (0.29) [0.17-0.92]	0.753	0.36 (0.10) [0.16-0.76]	0.114
Resuscitation	0.43 (0.29) [0.26-0.58]	0.492	0.30 (-) [0.25-0.31]	0.558
Hospitalization	0.46 (0.30) [0.17-0.92]	0.947	0.35 (0.26) [0.25-0.76]	0.090
Maternal preeclampsia	0.43 (0.25) [0.31-0.86]	0.929	0.31 (-) [0.25-0.62]	0.972
Maternal diabetes	0.41 (0.28) [0.30-0.59]	0.628	0.30 (-) [0.30-0.30]	0.595
Multiple pregnancies	0.71 (-) [0.71-0.71]	0.121	0.33 (-) [0.30-0.58]	0.887
Antenatal steroid	0.50 (0.33) [0.26-0.86]	0.780	0.53 (-) [0.31-0.76]	0.209
Mother's smoker	0.58 (0.35) [0.17-0.92]	0.265	0.42 (-) [0.42-0.42]	0.860

SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age, descriptive statistics were presented as median (interquartile range) [minimum-maximum]

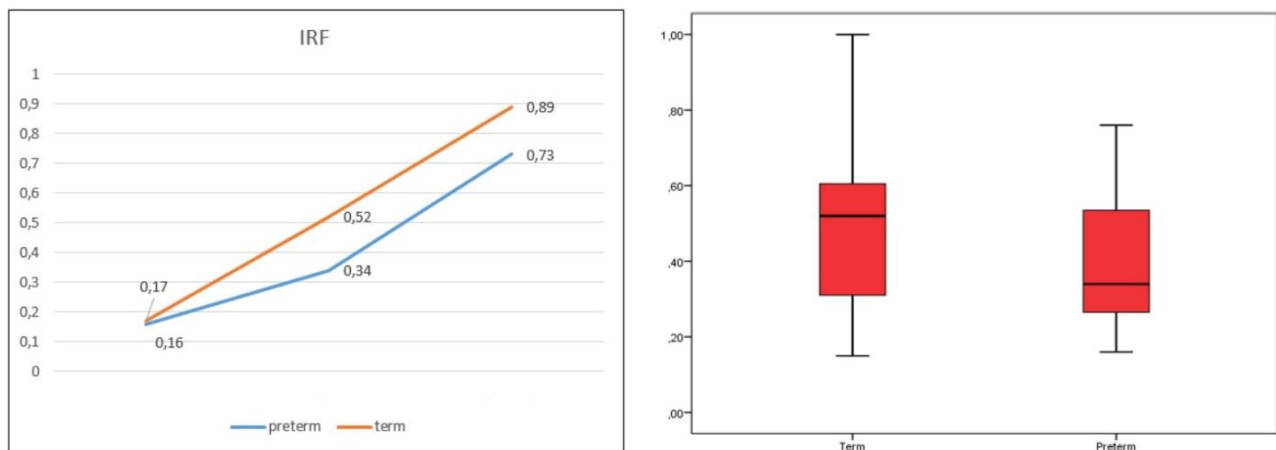


Figure 1. Immature reticulocyte fraction values in term and preterm groups

There were strong positive correlations between the IRF levels and MCHC values in the preterm and also in the term infants ($r=0.775$, $p<0.001$, and $r=0.674$, $p<0.001$, respectively). In addition, moderately strong positive correlations were observed between the IRF levels and both the RDW ($r=0.423$, $p<0.001$) and the CRP ($r=0.389$, $p<0.001$) levels (Table 4).

DISCUSSION

In our study, the median IRF value was 0.52 for term infants and 0.34 for preterm infants, as taken from a cord blood sample. The IRF values for the preterm infants were lower than for the term infants, however, there was no difference in the reticulocyte count between preterm and term babies.

There are a few studies in which different study protocols were applied and different results were obtained in newborns with IRF. However, peripheral venous blood samples were used in the majority of these studies.

Maconi et al. (3) found that the median IRF value for a sample of 98 newborns was 27.45, and the 2.5 and 97.5 percentiles were 7.64 and 41.21, respectively. There was no difference between term and preterm infants in terms of IRF values in that study. However, a different device was used for the analysis of the IRF. Ianni et al. (4) reported the reticulocyte index for a normal healthy term newborn at 24 hours of life. They found that the median IRF value for a sample of 120 was 44.7, and the 2.5 and 97.5 percentiles were 35.9 and 52.8, respectively. In a different study by Makela et al. (5), preterm infants were followed up to their 16th week with consecutive measurements from birth. In the study, the median IRF value was 0.36, and the reference values were 0.13 and 0.59 in the first measurements after birth. The reference range found in that study is similar to the reference range of our study, which

could be due to the similar gestational age. In the study by Schiza et al. (6), the reticulocyte indices of late preterm infants were examined, and the role of the IRF value in erythropoiesis was investigated. They found that the IRF values were inversely proportional to the postnatal age while directly proportional to the gestational age. Additionally, Ringoringo et al. (7) demonstrated the reference range of IRF for born-term babies aged 1-4 months. Although IRF 5-95 percentile level was 5.95-22.35 in the first month; it diminished to 2.94-12.87 in the fourth month.

Christensen et al. (8) examined the reticulocyte parameters in a large population and showed that the IRF value may be an early and sensitive marker for bone marrow erythropoietic activity. In that study, the IRF values were lower in preterm infants than in term infants, like our results, though in that study, peripheral blood specimens were used, and the IRF values had been studied during the postnatal period.

The results of that study showed that the cord blood IRF levels were not affected by maternal variables, though changed with gestational age. In our study, the IRF values were higher in the term infants than in the preterm infants when only the resuscitation rates were different. Predictably, the preterm group had a higher rate of resuscitation. Nucleated erythrocytes were measured as an indicator of both acute and chronic hypoxia, and the increase in the immature erythrocyte count is reported to be an indirect indicator of hypoxemia (9-11).

However, we found that the IRF values were lower in preterm infants in our study, which is contrary to the hypothesis that these changes result from hypoxia. In addition, the fact that there were no significant differences between the groups in terms of Apgar scores and blood gases also supports the theory that the difference observed between the groups was not related to hypoxia.

Sepsis is one of the causes of morbidity and mortality in newborns. Türkmen et al. (12) compare the critical patients of pediatric age with the healthy control group, IRF level was reported higher in the critical and septic patient groups. In our study, we studied CRP in order to investigate whether the IRF of cord blood has a place in the early diagnosis of sepsis. In addition, moderately strong positive correlations were observed between the IRF levels and the CRP values.

In our study, we think that lower IRF values in preterm infants compared to term infants may be related to low EPO levels in preterm infants. This speculation was based on the strong correlation observed between IRF and MCHC values in term and preterm infants. In the preterm infants, besides the lower IRF levels, the association between the IRF levels and the MCHC values was assumed to be the result of lower IRF levels associated with lower EPO concentrations. However, the lack of similarity between the groups in terms of reticulocyte counts suggests that the IRF values are much more sensitive markers compared with reticulocyte counts. A study to support this assumption was conducted in Japan by Butthep et al. (13). In this study, patients with α and β thalassemia were compared with carriers and healthy subjects, and it was found that EPO levels were positively correlated with the immature reticulocyte fraction. In the USA, in the study by Warwood et al. (14), the IRF was examined in infants with anemia who were born younger

Table 4. The correlations between the cord blood immature reticulocyte fraction levels and hematological indices

	Term		Preterm	
	r	p	r	p
RBC	0.077	0.448	-0.218	0.306
HGB	0.192	0.057	0.004	0.983
HCT	-0.025	0.856	-0.249	0.240
MCV	-0.246	0.014	-0.177	0.407
MCHC	0.674	<0.001	0.775	<0.001
RDW	0.423	<0.001	0.309	0.142
WBC	0.067	0.511	-0.164	0.444
PLT	-0.107	0.292	0.353	0.091
RTC	0.280	0.005	0.318	0.130
pH	0.251	0.012	0.278	0.188
BE	0.279	0.005	0.174	0.417
HCO₃	0.016	0.879	0.051	0.814
PCO₂	-0.096	0.347	-0.267	0.207
PO₂	-0.198	0.050	-0.306	0.146
CRP	0.389	<0.001	0.235	0.269

RBC: red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, WBC: white blood cell, PLT: platelet, RTC: reticulocyte count, pH: potential hydrogen, HCO₃: bicarbonate, PCO₂: partial pressure of carbon dioxide, PO₂: partial pressure of oxygen, CRP: C-reactive protein

than 32 weeks and weighing less than 1500 grams and were followed up in the neonatal intensive care unit, and it was observed that the IRF value increased after darbepoetin administration, and a greater increase was observed at higher doses. In a study from Finland, a correlation between the IRF levels and the MCHC and MCV values in cord blood was reported, which is similar to the results found in our study (15). In our study, it can be speculated that the higher IRF values in term babies may be related to EPO. The absence of this difference between reticulocyte values suggests that IRF value is a much more sensitive marker than reticulocyte. However, the fact that EPO levels were not studied in our study is one of the limiting factors. Evaluation of EPO levels in future studies will be useful in demonstrating this relationship.

The study has several limitations. The first limitation of our study is that the EPO levels of the newborns were not studied. The other limitation is the small sample size and the relationship between IRF value and anemia in neonatal follow-up and the lack of follow-up on whether there is a need for transfusion or not. Therefore, there is a need for new studies by increasing the sample size, studying EPO levels, and examining the relationship between IRF value and neonatal anemia and transfusion need.

CONCLUSION

IRF values are significantly higher in term infants than in preterm infants, and the reference IRF values in the cord blood have been established for term and preterm newborns. We suggest that the results of this study can be a guide for the next new research into using IRF values in newborns.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Kırıkkale University (23.02.2015, 04/02).

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The Effect of Changes in the Radio-Ulnar Angle on Wrist Scores on the Treatment of Intra-Articular Fractures of the Distal Radius with Wrist Fixator

Distal Radius Eklem İçi Kırıklarının El Bilek Fiksatorü ile Tedavisinde Radio-Ulnar Açıldaki Değişikliklerin El Bilek Skorlamasına Etkisi

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ABSTRACT

Aim: This study was aimed to evaluate the effect of radial inclination (RI) angle, assessed with radiological measurement, on the clinical outcomes of intra-articular fractures of the distal radius (IFDR) treated with closed reduction and penning type dynamic wrist fixator.

Material and Methods: A total of 120 patients who underwent closed reduction and dynamic wrist fixation due to IFDR between 2012 and 2020 were retrospectively reviewed. The effects of the changes in RI on clinical outcomes according to Mayo wrist scoring (MWS) criteria were investigated by measuring the RI angles in anteroposterior wrist radiographs of the patients at postoperative 2nd, 6th, and 10th weeks.

Results: According to the AO fracture classification, 77 (64.2%) of the patients had type C1 and 43 (35.8%) of the patients had type C2 fracture. The patients comprised 69 (57.7%) females and 51 (42.3%) males, and the mean age was 50.08±15.36 years. Fixators were removed at six weeks. RI angles measured at post-op week 10 were 20°-30° in 83 (69.1%) patients, 10°-19° in 32 (26.6%) patients, and 0°-9° in 5 (4.3%) patients. According to MWS, 39 (32.5%) patients were evaluated as excellent, 76 (63.3%) patients as good, and 5 (4.2%) patients as moderate. A statistically significant positive correlation was observed between RI values and MWS ($r=0.369$, $p<0.001$).

Conclusion: Dynamic wrist fixation with closed reduction is a simple and minimally invasive method, and provides successful clinical and functional results in the treatment of IFDR. We consider that optimal RI angles are effective in clinical and functional outcomes.

Keywords: Radius; external fixation; intra-articular fractures; orthopedic surgery.

ÖZ

Amaç: Bu çalışmada kapalı redüksiyon ve penning tipi dinamik el bilek fiksatorü ile tedavi edilen radiusun distal intra-artiküler kırıkları (RDIK)'nda radyolojik ölçüm ile değerlendirilen radial inklınasyon (RI) açısının klinik sonuçlara olan etkisinin değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: 2012 ve 2020 yılları arasında RDIK nedeniyle kapalı redüksiyon ve dinamik el bilek fiksatorü uygulanan 120 hastanın dosyaları geriye dönük olarak incelendi. Hastaların post operatif 2., 6. ve 10. haftalarda radyolojik olarak el bileği ön/arka grafilerinden RI açıları ölçülerek, açısal değerlerdeki farklılıkların, Mayo el bilek skorlaması (MEBS) kriterlerine göre klinik sonuçlara olan etkisi araştırıldı.

Bulgular: AO kırık sınıflamasına göre, 77 (%64,2) hastanın tip C1, 43 (%35,8) hastanın ise tip C2 kırığı vardı. Hastaların 69 (%57,7)'u kadın ve 51 (%42,3)'i erkek olup ortalama yaş 50,08±15,36 yıldı. Fiksatorler altıncı haftada çıkarıldı. Post op 10. haftada ölçülen RI açıları, 83 (%69,1) hastada 20°-30°, 32 (%26,6) hastada 10°-19° ve 5 (%4,3) hastada ise 0°-9° idi. MEBS'e göre, 39 (%32,5) hasta mükemmel, 76 (%63,3) hasta iyi ve 5 (%4,2) hasta ise orta olarak değerlendirildi. RI değerleri ile MEBS arasında pozitif yönde istatistiksel olarak anlamlı bir korelasyon gözlemlendi ($r=0,369$; $p<0,001$).

Sonuç: Kapalı redüksiyon ile dinamik bilek fiksator uygulaması basit ve minimal invaziv bir yöntemdir ve IFDR tedavisinde başarılı klinik ve fonksiyonel sonuçlar sağlar. Optimal RI açılarının klinik ve fonksiyonel sonuçlarda etkili olduğunu düşünüyoruz.

Anahtar kelimeler: Radius; eksternal fiksator; eklem içi kırıklar; ortopedik cerrahi.

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INTRODUCTION

Intra-articular fractures of the distal radius (IFDR) are commonly encountered traumas in orthopedics. These fractures are caused by low-energy trauma most often attributed to underlying osteoporosis in elderly individuals, and due to high-energy trauma in young and active individuals (1,2). IFDR occurs as a result of falling on the open hand while the elbow is in extension and the wrist is in dorsiflexion (3). Treatment of intra-articular fractures is much more difficult and complex than extra-articular fractures. These types of fractures have a poor prognosis as they are unstable and pave the way for the development of arthrosis and are unstable. The aim of treatment is to achieve painless wrist and hand functions by providing anatomical reduction of the joint surfaces as much as possible (4,5). Many conservative and surgical methods are used in the treatment of IFDR. The main purpose of wrist fixator applications is to bring together fractures fragments and to bring the joint cartilage, palmar inclination, and radial height to an acceptable anatomical level by providing the appropriate tension of structures such as tendons and ligaments around the wrist with the effect of ligamentotaxis (6). Closed repositioning and external fixation applications yield very successful results as adequate cartilage restoration can be achieved with appropriate reduction without opening the fracture line or disturbing the blood supply of the fracture fragments.

The aim of this study was to evaluate the effects of radial inclination values, obtained with 10-week postoperative radiographic measurements, on hand-wrist scores in patients who underwent closed reduction and external fixation for IFDR.

MATERIAL AND METHODS

Patients

A total of 162 patients who applied to our clinic due to IFDR underwent closed reduction followed by dynamic wrist fixator (penning tip) application between March 2012 and April 2020. Non-invasive ethics committee approval was obtained from Sakarya University Faculty of Medicine on 16.04.2021 with document number 257. 42 of the patients who did not regularly attend follow-ups and patients with additional trauma and open fracture lines were excluded from the study. Accordingly, 120 patients were included in this study. All patients were operated on and followed up in the orthopedics and traumatology clinic of Sakarya Yenikent State Hospital by a single orthopedic surgeon.

Surgical Procedure

All patients underwent operations under the axillary block and 1 gr IV cephazolin sodium was administered to the patients before the operation. Tourniquets were not applied. In regards to surgical technique, traction was applied to the fracture site for 3-5 minutes while the patients were lying in the supine position. Then, the fracture was reduced by adapting the dynamic wrist fixator under fluoroscopy with the placement of two Schanz screws in the proximal base of the second metacarpal and two in the radius diaphysis. The wrist fixator system was locked by applying 20 degrees of ulnar deviation and semiflexion to the wrist. The distraction of the wrist fixator was performed to provide the effect of ligamentotaxis. The patients were checked with fluoroscopy and the bases of

screws were applied with dressing and then wrapped with an elastic bandage.

Follow-up

Radial inclination angles were measured from the anteroposterior (AP) wrist radiographs of the patients taken at post-op 2, 6, and 10 weeks. All patients were evaluated with Mayo wrist score (MWS) at post-op week 10. The effects of radial inclination angles on the wrist score were evaluated.

Statistical Analysis

In this study, statistical analyzes were performed with Number Cruncher Statistical System (NCSS) 2007 Statistical Software (Utah, USA) package program. In addition to descriptive statistical methods (mean, standard deviation, frequency, percentage), the distribution of the variables was examined with the Shapiro-Wilk normality test. One-way analysis of variance was used to assess intergroup comparisons of the normally distributed variables and the Tukey multiple comparison test in the subgroup comparisons. The chi-square test in the comparisons of qualitative data, categorical variables were also analyzed with the Fisher-Freemant-Halton test, and the Pearson correlation test was used to determine the relations of variables between each other. The results were evaluated at a significance level of $p < 0.05$.

RESULTS

A total of 120 patients were included in the study and the mean age was 50.08 ± 15.36 years. The patients consisted of 69 (57.7%) females and 51 (42.3%) males. Fracture types were evaluated as C1 in 77 (64.2%) patients and C2 in 43 (35.8) patients according to the Arbeitsgemeinschaft für Osteosynthesefragen (AO) classification. The operation was performed on the left side in 64 (53.3%), and on the right side in 56 (46.7%) patients. The mean time between hospitalization and operation was 16.5 ± 4.2 hours. The mean operating time was 14.0 ± 3.7 minutes. The mean follow-up period was 4.3 ± 2.1 months. Post-op mean hospital stay was 2.7 ± 1.3 days. The fixators were removed at the end of the post-op 6th week at the latest, and passive and then active physical therapy exercises were initiated in patients (Figure 1 and Figure 2). The complete union was observed in all cases included in the study. Delayed union or nonunion was not observed. The mean union time was 6.7 ± 2.4 weeks. Complications of superficial pin tract infection were observed in eight patients, and complications of loosening of Schanz screws were observed in four patients. These complications did not affect stability and improved with appropriate treatments. In total, the mean radial inclination (RI) was 20.32 ± 4.51 and the mean MWS was 91.12 ± 5.20 (Table 1).

Comparison of the MWS in the RI groups for ages of ≤ 50 , > 50 , and all study groups were presented in Table 2.

In the ≤ 50 age group, a statistically significant difference was observed between the mean MWS of the RI 0° - 9° , 10° - 29° , and 20° - 30° groups ($p < 0.001$). The mean MWS of the RI 0° - 9° group was found to be statistically significantly lower than the mean MWS of the RI 10° - 29° and 20° - 30° groups ($p = 0.001$, $p < 0.001$), Mean MWS of RI 10° - 29° group was found to be statistically significantly lower than the mean MWS of the RI 20° - 30° group ($p = 0.018$).

In the >50 age group, a statistically significant difference was observed between the mean MWS of the RI 0°-9°, 10°-29° and 20°-30° groups (p<0.001). The mean MWS of the RI 0°-9° group was significantly lower than the mean MWS of the 10°-29° and 20°-30° groups (p=0.001, p<0.001). No statistically significant difference was observed between the mean MWS of the RI 10°-29° and RI 20°-30° groups (p=0.981).

In all patients, a statistically significant difference was observed between the mean MWS of the RI 0°-9°, 10°-29° and 20°-30° groups (p<0.001). The mean MWS of the RI 0°-9° group was significantly lower than the mean MWS of the 10°-29° and 20°-30° groups (both p<0.001). No statistically significant difference was observed between the mean MWS of the RI 10°-29° and 20°-30° groups (p=0.194, Table 2).

In the study group, there was a statistically significant difference between the MWS distributions of the RI groups (p<0.001). Excellent MWS was not observed in the RI 0°-9° group, while the rate of excellent MWS was high in the RI 20°-30° group (Table 3).

DISCUSSION

Since intra-articular fractures cause degenerative disorders in the joints, they are usually complicated fractures (6,7). Anatomical restoration of the joint is significant in these types of fractures. The aim of IFDR treatment is to achieve a functional hand and wrist after union by providing

anatomical restoration. Many methods, ranging from conservative to surgical, are used in the treatment of these fractures (7,8). The closed reduction and external fixation technique helps in reduction as it stabilizes the carpal ligaments with the effect of ligamentotaxis without opening the fracture line. Although the effect of ligamentotaxis is effective in providing height and inclination, may be insufficient in the anatomical restoration of joint surfaces (9). However, closed wrist fixators minimize the damage of intra-articular fractures by reducing the load on the bones and joints, even if anatomical repositioning cannot be achieved in the joint (9,10). Dynamic



Figure 1. Treatment with fixator application

Table 1. Descriptive characteristics of the patients

Age, n (%)	
≤50 Age	60 (50.00)
>50 Age	60 (50.00)
Gender, n (%)	
Male	51 (42.50)
Female	69 (57.50)
Side, n (%)	
Right	56 (46.67)
Left	64 (53.33)
Radial inclination, n (%)	
0°-9°	5 (4.17)
10°-29°	32 (26.67)
20°-30°	83 (69.17)
Mayo wrist scoring, n (%)	
Medium	5 (4.17)
Good	76 (63.33)
Excellent	39 (32.50)
AO classification, n (%)	
C1	77 (64.17)
C2	43 (35.83)

AO: Arbeitsgemeinschaft für Osteosynthesefragen

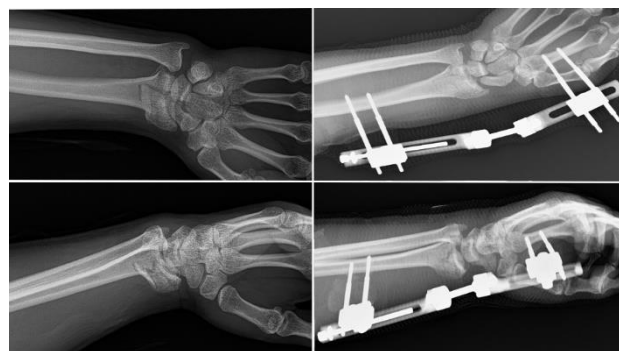


Figure 2. Wrist fixator application, pre-op and post-op images

Table 2. Comparison of Mayo wrist score according to the radial inclination in age groups

Age group	0°-9° (n=2)	10°-29° (n=18)	20°-30° (n=40)	p
≤50 years	77.50±3.54 (45.73-109.27)	89.17±4.93 (86.72-91.62)	92.38±3.58 (91.23-93.52)	<0.001
Age group	0°-9° (n=3)	10°-29° (n=14)	20°-30° (n=43)	p
>50 years	80.00±10.00 (55.16-104.84)	92.14±3.78 (89.96-94.33)	91.86±4.88 (90.36-93.36)	0.001
Age group	0°-9° (n=5)	10°-29° (n=32)	20°-30° (n=83)	p
Whole Group	79.00±7.42 (69.79-88.21)	90.47±4.64 (88.80-92.14)	92.11±4.29 (91.17-93.04)	<0.001

descriptive statistics were reported as mean±standard deviation (95% confidence interval)

Table 3. Comparison of the distribution of Mayo wrist score in radial inclination groups

Age group		0°-9° (n=2)	10°-29° (n=18)	20°-30° (n=40)	p
	Mayo wrist score, n (%)				
≤50 years	Medium	2 (100)	1 (5.56)	0 (0.00)	<0.001
	Good	0 (0.00)	15 (83.33)	22 (55.00)	
	Excellent	0 (0.00)	2 (11.11)	18 (45.00)	
Age group		0°-9° (n=3)	10°-29° (n=14)	20°-30° (n=43)	p
	Mayo wrist score, n (%)				
>50 years	Medium	2 (66.67)	0 (0.00)	0 (0.00)	0.001
	Good	1 (33.33)	10 (71.43)	28 (65.12)	
	Excellent	0 (0.00)	4 (28.57)	15 (34.88)	
Age group		0°-9° (n=5)	10°-29° (n=32)	20°-30° (n=83)	p
	Mayo wrist score, n (%)				
Whole Group	Medium	4 (80.00)	1 (3.13)	0 (0.00)	<0.001
	Good	1 (20.00)	25 (78.13)	50 (60.24)	
	Excellent	0 (0.00)	6 (18.75)	33 (39.76)	

wrist fixators are an effective treatment method for rapid healing of the fracture and for the clinical and functional recovery of wrist activity, due to ease of application, shorter operating time, and minimal invasiveness (11). In our study, we observed a correlation between RI angles and clinical and functional results in IFDR treatment; clinical and functional results were inadequate in patients with inclination angles below 10°, and clinical and functional results were adequate or even excellent in cases with RI above 10°.

Güler et al. (12) reported that radiological results and clinical results may not always be consistent in the treatment of IFDR, but the general standpoint is that patients with good radiological findings will achieve good clinical and functional results. Seyfettinoğlu et al. (13) reported that volar plate applications yield successful results in distal radius fractures, but this method may cause some complications and adversely affect the results. Dario et al. (14) conducted a study of 51 patients who were treated with a volar plate and reported that the most important radiographic parameters to be restored were volar tilt and ulnar variance, and that RI angle did not affect the results. Thuysbaert et al. (15) stated that the two most important radiographic parameters in the evaluation of clinical and functional success in IFDR treatment are ulnar variance and RI angles. Ünal et al. (16) reported that volar plate applications yielded satisfactory mid-term results, despite longer operating time and hospital stay compared to external fixation. Özden et al. (17) stated that intra-articular fractures can be evaluated and by arthroscopic methods and corrected. Stirling et al. (18) stated that the RI angle is one of the most important parameters in the evaluation of bone displacement in distal radius fractures. Gereli et al. (19) stated that volar plates are superior to EFin IFDR, but there is no difference in terms of clinical and functional results in the midterm and long term. Sun et al. (20) reported that radial length and RI angle had no effect on correction in 85 patients with IFDR who were treated with EF, while volar tilt was effective in correction, and EF had no chance of treatment success in multi-fragmented fractures. In our study, we achieved the opposite of this result, and we obtained satisfactory results in regard to clinical and functional aspects of the treatment of IFDR. In their study consisting of 46 patients,

Çopuroğlu et al. (21) used external fixators to treat IFDR by providing mild flexion and reduction in ulnar deviation, avoiding excessive distraction; they reported that they achieved complete radiological and clinical union in all patients at the end of postop 7 weeks.

Our study had some limitations. Due to the wide age distribution of the patients, elderly patients with porotic bones and young patients were examined in the same study. In addition, factors affecting the accuracy of the results such as comorbidities, smoking, and additional drug use were not evaluated in the study. The absence of a randomized control group was another limitation of our study.

CONCLUSION

EF application with closed reduction is an effective surgical method in the treatment of IFDR and achieves successful clinical and functional results by providing restoration close to anatomical repositioning on displaced joint surfaces due to the effect of ligamentotaxis. Optimal RI angles in the postoperative period are directly correlated with clinical and functional outcomes. Achieving normal RI angles is critical for clinical and functional success. Considering that this study will shed light on future studies, we believe that more comprehensive studies are needed.

Ethics Committee Approval: The study was approved by the Ethics Committee of Sakarya University Faculty of Medicine (16.04.2021, 257).

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Author Contributions: Idea/Concept: BA; Design: MOY; Data Collection/Processing: BA, MOY; Analysis/Interpretation: BA; Literature Review: BA, MOY; Drafting/Writing: BA; Critical Review: BA, MOY.


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Effect of Percutaneous Endobiliary Radiofrequency Ablation in Malignant Bile Stenosis

Malın Safra Yolu Darlıklarında Perkütan Endobilyer Radyofrekans Ablasyonun Etkisi


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ABSTRACT

Aim: This study aimed to demonstrate the feasibility of the percutaneous endobiliary radiofrequency ablation (ERFA) method, which is used to increase stent patency in malignant biliary strictures.

Material and Methods: A total of 25 patients, 9 (36%) female and 16 (64%) male, who developed malignant biliary stenosis secondary to various advanced tumors and underwent ERFA and metallic stenting after percutaneous biliary drainage were retrospectively evaluated. The types of malignancies causing obstruction and the follow-up after the procedure were evaluated to demonstrate the median survival and stent patency values of the patients. Stent patency and survival rates were calculated using the Kaplan-Meier method.

Results: The results of the study demonstrated that 17 (68%) out of these 25 patients displayed a significant decrease in their first-week postoperative bilirubin values, with a reduction of greater than 50% compared to the pre-biliary drainage values. The study determined that this treatment approach's overall clinical success rate was 68%. Stent occlusion developed within 180 days in 8 (32%) out of the 25 patients who underwent ERFA and metallic stenting. Additionally, 18 (%72) patients died as a result of malignancy progression. The mortality rates at post-treatment 30 and 180 days were determined to be 24% and 72%, respectively. The median survival and stent patency times were 65 and 70 days, respectively.

Conclusion: Percutaneous ERFA and metallic stenting have the potential to improve survival and stent patency, especially in selected patient groups with distal biliary stenosis. Randomized controlled studies are needed to confirm these results.

Keywords: Malignant biliary obstruction; percutaneous endobiliary radiofrequency ablation; biliary drainage; biliary stenting.

ÖZ

Amaç: Bu çalışmanın amacı, malign biliyer darlıklarda stent açıklığını artırmak için kullanılan perkütan endobilyer radyofrekans ablasyon (endobiliary radiofrequency ablation, ERFA) yönteminin uygulanabilirliğini ortaya koymaktır.

Gereç ve Yöntemler: Çeşitli ilerlemiş tümörlere sekonder malign biliyer stenoz gelişen ve perkütan biliyer drenaj sonrası ERFA ve metalik stent uygulanan 9 (36%) kadın ve 16 (64%) erkek olmak üzere toplam 25 hasta geriye dönük olarak değerlendirildi. Hastaların ortanca sağkalım ve stent açıklığı değerlerinin gösterilmesi için obstruksiyona sebep olan malignitelerin türleri ile işlem sonrası takipleri değerlendirildi. Stent açıklığı ve sağkalım oranları Kaplan-Meier yöntemi kullanılarak hesaplandı.

Bulgular: Bu çalışmaya dahil edilen 25 hastadan 17'si (%68) işlem sonrası ilk hafta bilirubin değerlerinde pre-bilyer drenaj değerlerine göre %50'den fazla azalma ile anlamlı bir düşüş gösterdi. Bu tedavi yaklaşımının genel klinik başarı oranı %68 olarak tespit edildi. ERFA ve metalik stent uygulanan 25 hastanın 8'inde (%32) 180 gün içinde stent tıkanıklığı gelişti. Ayrıca 18 (%72) hasta malignite progresyonu sonucu hayatını kaybetti. Tedavi sonrası 30 ve 180 gündeki ölüm oranları sırasıyla %24 ve %72 olarak belirlendi. Ortanca hayatta kalma ve stent açık kalma süreleri sırasıyla 65 ve 70 gündü.

Sonuç: Perkütan ERFA ve metalik stentleme, özellikle distal biliyer stenozu olan seçilmiş hasta gruplarında sağkalımı ve stent açıklığını iyileştirme potansiyeline sahiptir. Bu sonuçları doğrulamak için randomize kontrollü çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Malign biliyer darlık; perkütan endobilyer radyofrekans ablasyon; biliyer drenaj; biliyer stent.

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INTRODUCTION

Malignant biliary obstruction may result from a primary tumor of the bile duct epithelium and from metastatic tumors invading these ducts. Palliation is provided through biliary stenting in this patient group with a life expectancy of less than 6-12 months (1). Stenting via percutaneous biliary drainage is an effective and safe treatment method, especially in the patient group where endoscopic access is not possible (2). Metallic stents are more cost-effective and are associated with shorter hospital stays and fewer reinterventions. The most critical factor limiting the effectiveness of stent therapy is stent occlusion due to tumor growth (3). Covered stents have been developed to prevent this complication, but migration problems have developed in these stents, and as a result, sufficient efficacy has not been achieved (4).

The use of endobiliary radiofrequency ablation (ERFA) has recently become more common as a supplemental treatment for alleviating symptoms associated with malignant biliary obstructions. By utilizing radiofrequency energy to generate heat and cause cellular damage, this technique can be carried out through either an endoscopic or a transhepatic method and has the potential to delay stent blockages by reducing tumor load along the obstructed bile duct (5). Recent endoscopic and percutaneous studies have shown that ERFA can be used safely in the biliary system with low complication rates (6-8).

In this study, we reported our single-center experience and aimed to demonstrate the feasibility, safety, and effectiveness of the percutaneous ERFA method and stenting procedure in the palliative treatment of malignant biliary obstructions.

MATERIAL AND METHODS

Study Design and Patient Selection

This retrospective study obtained approval from the local ethics committee (Clinical Research Ethics Committee of Ankara Numune Training and Research Hospital, date: 01.11.2018, number: E-18-2297). Patients who were considered inoperable due to malignant biliary obstruction in general surgery and medical oncology clinics and referred to the interventional radiology clinic for palliative treatment and treated with percutaneous transhepatic ERFA and metallic stenting between April 2017 and April 2018 were included in the study. Between these dates, a total of 28 patients were treated with percutaneous transhepatic ERFA and stenting in our interventional radiology clinic. All patients had hyperbilirubinemia, biliary obstruction symptoms, and pathological diagnoses at admission. None of the patients received radiotherapy. None of the patients had contraindications to the procedure such as irreversible coagulopathy, systemic infection, sepsis, or pregnancy. The follow-ups of two patients could not be accessed, and one patient was excluded from the study due to a Whipple operation in the second month after the procedure. As a result, 25 patients were included in the study.

Patient Demographics

Twenty-five patients with the age range of 44 to 83 (mean 65.0±10.8) years, comprising 9 (36%) females, and 16 (64%) males were evaluated. Etiologies of malignant biliary obstruction were detected radiologically and histopathologically. Evaluation of percutaneous

transhepatic cholangiography (PTC) images before the procedure revealed total and near-total stenosis in all of the 25 patients, and the diameter was defined as 0 mm. Patient demographics and tumor characteristics are summarized in Table 1.

Technique

Assessments of complete blood count and biochemical and blood coagulation parameters were performed prior to each procedure. All patients were informed about the details and possible complications of the percutaneous transhepatic ERFA and stenting procedure, and their informed consent was obtained. Operations were planned according to ultrasonographic evaluation. Patients fasted for 8-12 hours before the procedure. Broad-spectrum antibiotics (IV 1 gr 3rd generation cephalosporin) prophylaxis were administered to all patients before the procedure.

Procedures were performed in an angiographic suite with a flat-panel detector-based system (Innova 4100, General Electric Medical Systems, USA). The procedure was performed under standard sterile conditions with the anesthesia team providing sedoanalgesia. ELRA™ (STARmed, Korea) bipolar radiofrequency catheter and radiofrequency generator VIVA Combo™ (STARmed, Korea) were used for the ERFA procedure. The ELRA™ catheter is a disposable catheter with a probe diameter of 7Fr (2.31 mm) and a catheter length of 40 cm for the percutaneous procedure, which can be advanced over a 0.035" guidewire. Four annular electrodes of 3 mm in length with a distance of 2 mm between them after a 7 mm gap at the distal end were placed on the catheter. This RFA catheter can ablate tissue of a length of 18±3 mm and a radius of 2.1±0.3 mm with 7W power and 120 seconds of bipolar activation. In addition, the catheter has a temperature sensor that keeps the tissue at the target temperature (75-80 °C) and provides ablation without carbonization (7).

Table 1. Demographic data and tumor characteristics of the patients

Age (years), mean±SD	65±10.8 (44-83)
Gender , n (%)	
Female	9 (36%)
Male	16 (64%)
Primary tumor , n (%)	
Pancreatic carcinoma	11 (44%)
Cholangiocarcinoma	6 (24%)
*Type 1	1
*Type 2	1
*Type 4	4
Ampulla of Vater tumors	2 (8%)
Gallbladder carcinoma	1 (4%)
Metastases	5 (20%)
Histopathological subtype , n (%)	
Adenocarcinoma	24 (96%)
Squamous cell carcinoma	1 (4%)
Infiltrated bile duct segment , n (%)	
Proximal	9 (36%)
Distal	16 (64%)
Malignant stricture diameter (mm)	0
Malignant stricture length (mm), mean±SD	36.3±11.0

SD: standard deviation

ERFA was performed in a separate session in all patients after internal/external biliary decompression was achieved with the appropriate technique. A hydrophilic 0.035" Amplatz Super Stiff™ (Boston Scientific, USA) guidewire was advanced through the internal/external drainage catheter and fixed, and the catheter was removed. A 7F vascular sheath was placed over the guidewire to facilitate balloon and stent interventions. The level and length of the stenosis segment were determined with cholangiograms obtained by sending contrast material from the side of the vascular sheath. The ELRA™ catheter was placed over the guidewire under the guidance of fluoroscopy. The VIVA Combo™ RF generator was activated by setting it to 7W power and the target ablation temperature value to 80 °C. After every 120 seconds of activation, 60 seconds waited, and washing was done from the vascular sheath. Then, ablation was achieved by moving it proximally to cover the entire stenosis segment. After ablation was completed, a cholangiogram was taken for leakage control and the ERFA catheter was removed. A self-expandable metallic stent was placed over the guidewire to cover the stenosis segment 1-2 cm proximally and distally. Balloon dilatation was performed in cases when the stent patency was insufficient. A follow-up cholangiogram was repeated to evaluate stent placement and biliary drainage. If patency was achieved after the follow-ups, the guidewire and vascular sheath were removed. However, if adequate patency was not achieved, the follow-up catheter was left in place. The catheter was removed after normal bile flow was observed in the follow-up cholangiograms performed a few days after the procedure (Figure 1).

After the procedure, patients' pain medication was arranged, and their follow-up was provided in the relevant clinic. After discharge, the patient's clinic, biochemistry, and imaging were performed at regular intervals according to the oncology protocol. These results were evaluated through the hospital information management system.

Evaluation of Treatment

Observation of easy transition of the contrast agent into the duodenum after percutaneous ERFA and metallic stent application was interpreted as technically successful. One week after percutaneous ERFA and metallic stenting, a decrease of more than 50% in total bilirubin level compared to before biliary drainage was interpreted as clinically successful. Complications that developed in the first 30 days after the percutaneous ERFA procedure were classified as early complications and those that developed 30 days after the procedure were classified as late complications. The time until death from the percutaneous ERFA procedure was defined as survival. Recurrence of jaundice and a total bilirubin value above 2 mg/dL after percutaneous ERFA procedure were defined as stent occlusion and the elapsed time was determined as stent patency.

Statistical Analysis

Analyzes were performed using the IBM SPSS Statistics v.22.0 (SPSS Inc. USA) statistical analysis program. The Shapiro-Wilk test was used to evaluate the conformity of the data with normal distribution. Normally distributed data were expressed as mean±standard deviation, while data that did not conform to normal distribution were expressed as median (minimum-maximum). Frequencies were presented with percentages. The period between the

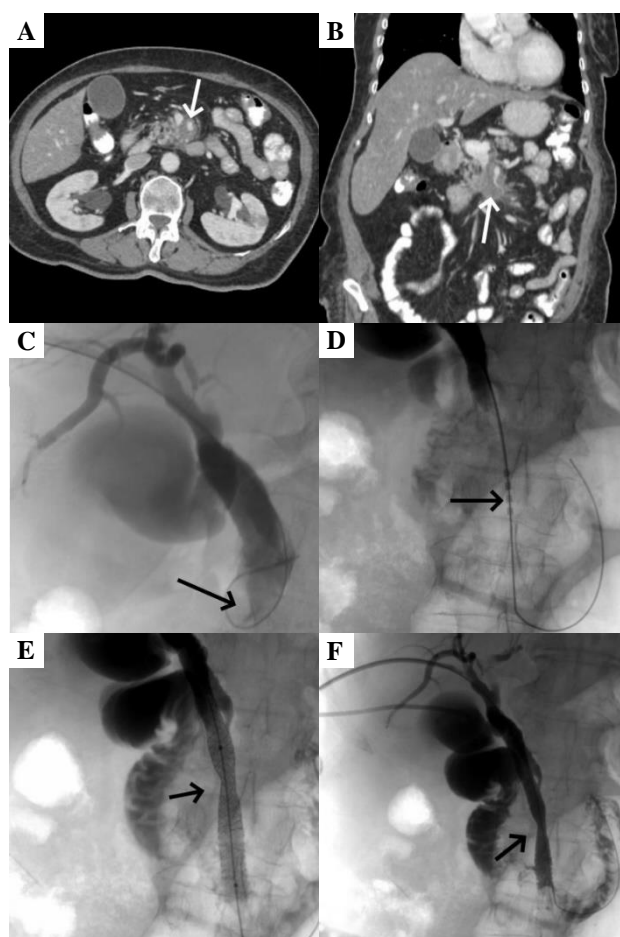


Figure 1. Pancreatic adenocarcinoma. **A, B)** Axial and coronal CT scans show a pancreatic mass surrounding the SMA and SMV in a 77-year-old female patient **C)** The level of stenosis in the PTC obtained with the right approach **D)** ERFA was applied in the next session. **E)** A metallic stent was then applied **F)** The stent was opened in the follow-up cholangiogram

radiofrequency ablation application time and time of death was recorded in days, and the Kaplan-Meier method was used for survival analysis. The Kaplan-Meier curve was used to determine the relationship between stent patency and survival. Log-rank test was used to compare the survival curves between groups. For both tests, $p < 0.05$ was considered statistically significant.

RESULTS

All 25 patients were treated in two separate sessions in our interventional radiology clinic. Percutaneous biliary drainage was applied in the first session, while ERFA and metallic stenting were performed in the second session. The median time between the two sessions was 5 (range, 1-50) days. ERFA and metallic stenting were performed in 4 (16%) of the 25 patients. Balloon dilatation was performed in 21 (84%) patients who did not achieve adequate stent patency. Evaluation of PTC images before ablation revealed nearly complete stenosis in all 25 patients. After ablation, the open bile duct diameter was measured as 5.3 ± 0.7 mm. Follow-up cholangiogram demonstrated the transition of the contrast agent into the duodenum in all 25 patients who underwent ERFA and stenting and the technical success was found to be 100%.

In the 6-month follow-up after the ERFA application, a progressive decrease was detected in patients' total and direct bilirubin values (Figure 2). The slight increase in the second month after the stent procedure observed in the graph was interpreted as the development of stent occlusions. Bilirubin values decreased below 50% of the baseline value before biliary drainage in 17 (%68) of 25 patients one week after ERFA and metallic stenting. Clinical success was determined as 68% (Table 2). Six (%24) patients died at the end of the first month due to disease progression. The mortality rate in the first 30 days after the procedure was 24%. Bilirubin value decreased below 2 mg/dL in 11 (57.9%) of the remaining 19 patients.

Stent occlusion developed in 1 (%4) patient in the early period (first 30 days) after ERFA and metallic stenting, and the hepatic abscess was observed in also 1 (%4) patient. In the late period (30 days - 6 months following the procedure), stent occlusion developed in 7 (%28) patients, and the hepatic abscess was observed in 2 (%8) patients (Table 3).

As of April 2019, 24 (%96) of the 25 patients included in the study died, accordingly, the median survival time was 65 (95% CI: 32.59-97.40) days (Figure 3). Looking at the stent patency, the median value of stent patency time was 70 (95% CI: 0.27-139.72) days (Figure 4).

Table 2. ERFA success and bilirubin values

Ablation energy (watts)	7-10
Duration of ablation (sec)	90-120
Malignant stricture diameter (mm)	
Before	0
After	5.3±0.7
Technical success , n (%)	25 (100%)
Total/Direct mean bilirubin levels (mg/dL)	
Before PBD	
Total	16.21
Direct	14.07
Before ERFA Day 1	
Total	10.92
Direct	9.48
After ERFA Day 1	
Total	10.27
Direct	8.65
After ERFA Week 1	
Total	7.86
Direct	6.89
After ERFA 1-month	
Total	4.67
Direct	3.97
Clinical success , n (%)	17 (68%)

ERFA: endobiliary radiofrequency ablation, PBD: percutaneous biliary drainage

Table 3. Complications after the procedure, n (%)

Early (0-30 days) , n (%)	
Stent occlusion	1 (%4)
Abscess	1 (%4)
Delayed (30 days-6 months) , n (%)	
Stent occlusion	7 (%28)
Abscess	2 (%8)

ERFA: endobiliary radiofrequency ablation

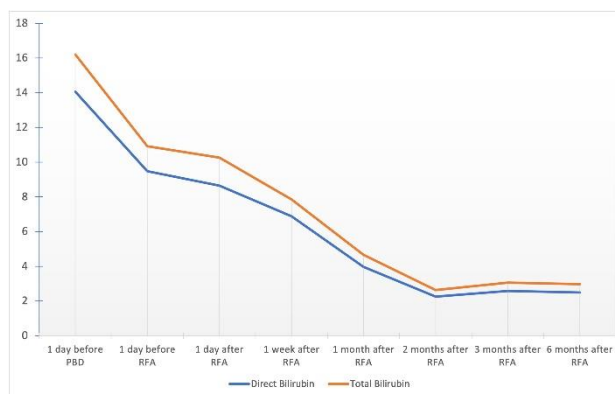


Figure 2. Total and direct bilirubin values after ERFA application in a 6-month period

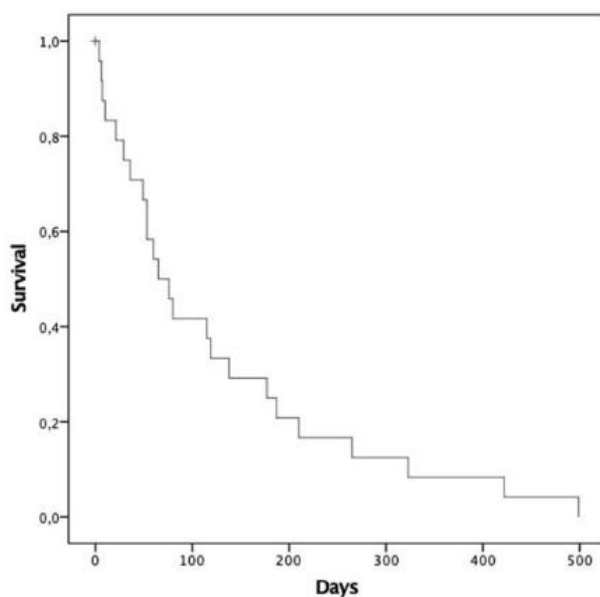


Figure 3. Kaplan-Meier survival curve of the patients

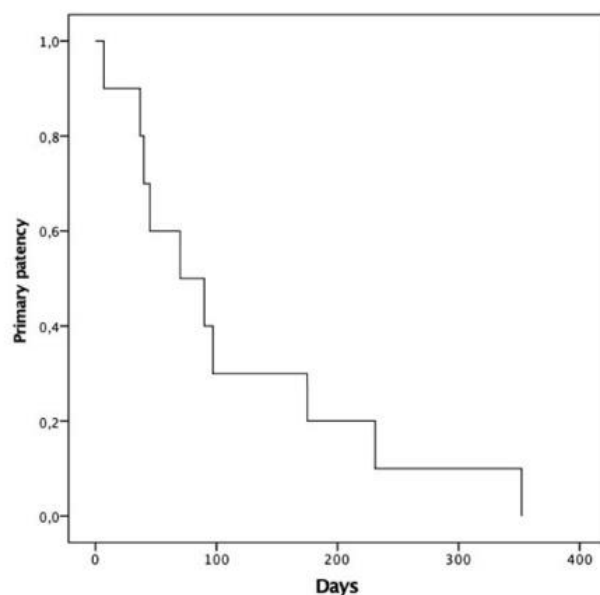


Figure 4. Kaplan-Meier stent patency curve of the patients

There was no statistically significant effect of the gender variable on overall survival ($p=0.556$). The median survival time of patients diagnosed with cholangiocarcinoma, one of the histopathological variables, was statistically significantly higher than patients with other histopathological diagnoses ($p=0.035$).

DISCUSSION

In our study, we successfully catheterized 25 patients and performed ERFA followed by metallic stenting. There was an early regression in bilirubin levels. In the first week after ERFA and stenting, total bilirubin regressed to below 50% of the pre-procedural value in 17 of the 25 patients, and clinical success was determined as 68%. In addition, the total bilirubin value decreased below 2 mg/dl in 11 of the 19 patients who were alive and could be followed up in the first month, and our success in the first month was 57.9%. When we look at the literature, nearly 100% of first-month success is observed, and it was found to be significantly lower in our study (6,9). We thought that this was due to inadequate bile duct drainage despite intervention due to complete obstruction of the lumen at the level of the stenosis by advanced tumor invasion.

The mean diameter of the stenosis before the procedure was measured as 1.47 ± 0.17 mm in a study by Acu et al. (9) and as 1.35 ± 0.63 mm in a study by Wu et al. (10), which differs from our study. In our study, the evaluation made with PTC before the procedure revealed almost complete occlusion in the lumen in all patients. The mean diameter of the open lumen after the procedure was 5.23 ± 1.50 mm in Acu et al.'s (9) study and 6.69 ± 2.48 mm in Wu et al.'s (10) study, which is similar to our present study (5.3 ± 0.73 mm).

The literature demonstrates that two separate sessions are conducted, in which biliary drainage is performed in the first session, followed by ERFA and stenting in the second session (9). Thus, it aims to increase the procedure's success by providing biliary decompression. This method was applied in all cases in our study. After ERFA, self-expanding metallic stents of different sizes were placed, and the transfer of contrast material to the duodenum was evaluated. Technical success was found to be 100%, similar to other studies, and was found to be compatible with the literature.

There are case reports in the literature stating that the biliary perforation develops in the early period after ERFA, and stenting is recommended in the early period of treatment (11). A study by Lee et al. (7) reported that the procedure was performed safely in 30 patients without bile leakage or vascular injury, with the target temperature controlled by an ELRA™ catheter. With this technique, overheating and carbonization are prevented, and thus significant vascular structure or biliary tract injury is avoided. With the ELRA™ catheter, which we also used in our study, no bile leakage or vascular damage was detected after ERFA.

In a study by Akıncı et al. (12), balloon dilation was applied after the ERFA procedure in refractory bilioenteric anastomosis strictures. Although metallic stent was not used in this study, it demonstrated that balloon dilation can be performed safely after RFA. In our study, 24 of 25 patients underwent ERFA and balloon dilation after stenting.

In a study conducted by Acu et al. (9), stent occlusion developed in 3 patients and hepatic abscess in 1 patient within six months out of 20 patients they followed. Our present study observed that 8 out of 25 patients developed stent occlusion, and 3 patients developed a hepatic abscess in a six-month period.

Few publications in the literature have compared ERFA with bare metallic stenting (13-17). Cui et al. (15) reported that stenting with ERFA provides a therapeutic benefit in stent patency compared to stent placement alone, especially in patients with cholangiocarcinoma. The author suggests that cholangiocarcinoma inherently responds better to ERFA because of its intraluminal spread. Similarly, in our study, the median survival time in patients diagnosed with cholangiocarcinoma was statistically significantly higher than the median survival time of patients with other histopathological diagnoses. Cui et al. (15) stated that ERFA and stenting prolong the stent patency. However, no advantage over overall survival has been demonstrated. In studies with control groups conducted by Uyanık et al. (14) and Kallis et al. (16), significant prolongation was observed in stent patency and overall survival.

In the study by Acu et al. (9), the median total survival time was 76 days, and the median stent patency time was 133 days. In the study by Mizandari et al. (6), the median survival was 89.5 days, and the median stent patency was 84.5 days. Wu et al. (10) reported a median survival of 181 days, and median stent patency was 149 days. The patient groups in these studies were mixed, like ours, but the Habib™ was used as the ERFA catheter. In a controlled study by Kallis et al. (16), the Habib™ catheter was used in a selected patient group consisting of only pancreatic carcinomas and it was reported the median survival was 472 days and stent patency was 226 days. Lee et al. (7) conducted a study in a selected group of patients who developed extrahepatic malignant biliary stenosis located 2 cm from the hilum. In the aforementioned study, the median survival was 383 days, and the median stent patency was 236 days. In the study by Uyanık et al. (14), the distribution of patients was mixed, with a median survival of 246 days and median stent patency of 223 days. In these two studies, ELRA™ was used as the ERFA catheter, as in our study. In our study, the median total survival time was 65 days, and the median duration of stent patency was 70 days. In our comparison with the literature, we are significantly behind in stent patency and survival. Although many reports in the literature show that radiofrequency ablation improves stent patency and survival, few studies show that it is ineffective. The study by Oh et al. (18) showed that it did not increase survival and patency.

Evaluation of the results of our present study in the context of the literature, clinical success, stent patency, and survival values were found to be significantly lower. Complete obliteration of the lumen at the level of stenosis and a significantly higher level of total bilirubin before biliary drainage in our patients compared to the literature are thought to affect our results (Table 4). In the context of the literature, we see that the results of ERFA applied in malignancies involving the distal bile ducts are better compared to a mixed patient group, as in our study (7,16). Finally, palliative percutaneous biliary drainage solutions

Table 4. Overview of the available literature on ERFA

Study	Method	Year	n	Catheter	Etiology	Median Stent Patency	Median Survival	Preprocedural Total Bilirubin (mg/dL)
Mizandari et al. (6)	Percutaneous	2013	39	Habib™	Mixed	84.5	89.5	7.54±3.6
Wu et al. (10)	Percutaneous	2015	47	Habib™	Mixed	149	181	14.03±5.8
Kallis et al. (16)	ERCP	2015	23	Habib™	Pancreas	472	226	14.27±2.83
Acu et al. (9)	Percutaneous	2018	21	Habib™	Mixed	133	76	15.09±8.59
Lee et al. (7)	ERCP	2019	30	ELRA™	Distal	236	383	Not reported
Uyanık et al. (14)	Percutaneous	2021	30	ELRA™	Mixed	223	246	9.3±2.8
Oh et al. (18)	ERCP	2022	28	ELRA™	Mixed	140	311	Not reported
Current Study	Percutaneous	2019	25	ELRA™	Mixed	70	65	16.2±8.5

ERFA: endobiliary radiofrequency ablation, ERCP: endoscopic retrograde cholangiopancreatography

in our interventional radiology clinic are applied in complex patient groups when ERCP is unsuccessful. This situation causes a limitation in selecting patients suitable for ERFA and reduces the procedure's success.

Our study had some limiting factors. The retrospective nature of this study made it difficult for the researcher to master all possible factors that would affect the outcome. In addition, the absence of a control group prevented a comparison of the effect of ERFA on stent patency and survival. Another limitation of our study was the small patient population. The lack of homogeneity among the factors that may affect the results, such as malignancy types and stages, is another limitation of our study.

CONCLUSION

Compared with the literature, the conclusion of our study, we observed that the application of ERFA and metallic stenting before the development of complete obliteration in the lumen, especially in selected patient groups with distal malignant biliary stenosis gives very satisfactory results. In the context of the literature, we observed that the application of ERFA in the patient group with unsuccessful ERCP and relatively low life expectancy does not contribute to stent patency and survival. In order to observe these effects more clearly, homogeneous, randomized controlled prospective clinical studies are required.

Ethics Committee Approval: The study was approved by the Ethics Committee of Ankara Numune Training and Research Hospital (01.11.2018, E-18-2297).

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Oral Lichen Planus in Childhood with Unique Histological Finding: A Case Report

Çocukluk Çağında Benzersiz Histolojik Bulgusu Olan Oral Liken Planus: Olgu Sunumu

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ABSTRACT

Oral lichen planus is a chronic inflammatory disease that targets adults. Over the years, few reports documented solitary cases of children being affected by oral lichen planus. In this report, a case of an 8-year-old, medically uncompromised boy who complained of a severe burning sensation in the tongue with a history of one year of remission and exacerbation was presented. When examined, the tongue showed a large irregular superficial non-indurated ulcer occupying the whole anterior 2/3 of the tongue sparing the tip and was surrounded by white radiating lines. The ulcerative lesion showed a peculiar butterfly pattern. Histopathological examination of the incisional biopsy showed intraepithelial civatte bodies and dense subepithelial lymphocytic infiltrate arranged in a pseudo-lymphomatous shape. Although the clinical and histological pictures suggest lichen planus, the atypical arrangement of lymphocytes brought up a question mark. This report demonstrates a peculiar clinical picture of a rare disease combined with a unique histopathologic finding.

Keywords: Civatte bodies; juvenile oral lichen planus; oral lichenoid lesions; tertiary lymphoid follicle.

ÖZ

Oral liken planus yetişkinleri hedef alan kronik inflamatuvar bir hastalıktır. Yıllar boyunca, oral liken planustan etkilenen tekil çocuk vakaları az sayıda raporla belgelenmiştir. Bu raporda, 8 yaşında, medikal açıdan herhangi bir sorunu olmayan, dilde şiddetli yanma şikayeti ile başvuran, bir yıldır remisyon ve alevlenme öyküsü olan bir erkek çocuk olgusunu sunulmuştur. Dil muayenesinde dil ucu hariç anterior 2/3'lük bölümün tamamını kaplayan, etrafı beyaz yayılan çizgilerle çevrili, büyük, düzensiz, yüzeysel, sertleşmemiş ülser saptandı. Ülseratif lezyon tuhaf bir kelebek paterni gösterdi. İnsizyonel biyopsinin histopatolojik incelemesinde intraepitelyal civatte cisimcikleri ve psödo-lenfomatöz bir şekilde düzenlenmiş yoğun subepitelyal lenfositik infiltrat görüldü. Klinik ve histolojik tablo liken planusu düşündürse de, lenfositlerin atipik dizilimi bir soru işareti yaratmıştır. Bu rapor, benzersiz bir histopatolojik bulguya sahip nadir bir hastalığın kendine özgü klinik tablosunu göstermektedir.

Anahtar kelimeler: Civatte cisimcikleri; juvenil oral liken planus; oral likenoid lezyonlar; üçüncül lenfoid folikül.

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INTRODUCTION

Lichen planus (LP) is a chronic inflammatory disease that affects the skin, nails, and mucous membranes. Oral lichen planus (OLP) generally affects patients of middle age or older (1). However, it has rarely been detected in pediatric patients (1-9).

Like adult OLP, OLP in childhood (OLPc) is diagnosed clinically and histopathologically -but in individuals <20 years old-; after excluding oral lichenoid reaction through evidence negating mucosal contact with dental restorative materials or exposure to medications that are known to induce oral lichenoid reactions (10).

Although OLP is relatively rare in childhood, it often presents in asymptomatic forms. This suggests that cases of OLPC are commonly unnoticed and thus, pass undiagnosed; and if symptomatic, it is suggested to be misdiagnosed (11). Consequently, OLPC is believed to have a higher prevalence but is under-reported for the aforementioned causes. Such few reports hinder drawing sound conclusions about OLPC regarding its demographic preference of age, sex, race, predisposing factors, clinical picture, treatment, and prognosis (12). In this report, a case of a child affected by OLP with a characteristic clinical picture and a unique histopathological presentation was presented.

CASE REPORT

An 8-year-old Caucasian boy presented with a severe burning sensation in the tongue that progressed gradually through the past year, with a course of remission and exacerbation. The pain was precipitated by eating salty and spicy food but nothing relieved it. Other than the oral complaint, the child had no significant medical, family, or dental history. However, he adopted poor nutritional habits of depending on candies and chips only.

Extraoral clinical examination revealed multiple small round pigmentations on the forearms marking for healed lesions that the patient reported were itchy (Figure 1). Intraorally, a large butterfly-shaped superficial non-indurated

ulcer was detected, occupying the whole anterior 2/3 of the tongue sparing the tip, and extending to the lateral borders of the tongue bilaterally. The ulcer was covered by a pseudomembrane and surrounded by a depapillated tongue that appeared as an erythematous atrophic area covered by white radiating lines. Besides, the buccal mucosa showed linear melanotic pigmentation bilaterally along the occlusal plan (Figure 2).

The differential diagnosis list included oral lichenoid reaction to preservatives in candies, OLP, and systemic lupus erythematosus. Therefore, further investigations were performed; showing mild normocytic normochromic anemia and a normal level of C-reactive protein (CRP). On the other hand, histopathological examination of the incisional biopsy taken from the tongue dorsum revealed severe atrophy of the epithelium, intraepithelial civatte bodies, subepithelial clefting, and very dense subepithelial lymphocytic infiltrate with pseudo-lymphomatous arrangements giving the shape of a lymphoid germinal center (Figure 3).

The clinical and histopathologic pictures were more consistent with OLPC. The parents and patient were instructed that they should start a healthier diet and also refrain from consuming candies. Besides, a topical antifungal (Miconazole 20 mg/g) was prescribed for one week to clear away candidal superimposition. Afterward, topical steroids (Triamcinolone acetonide 0.1%) were



Figure 1. Discolored skin lesions on the forearms

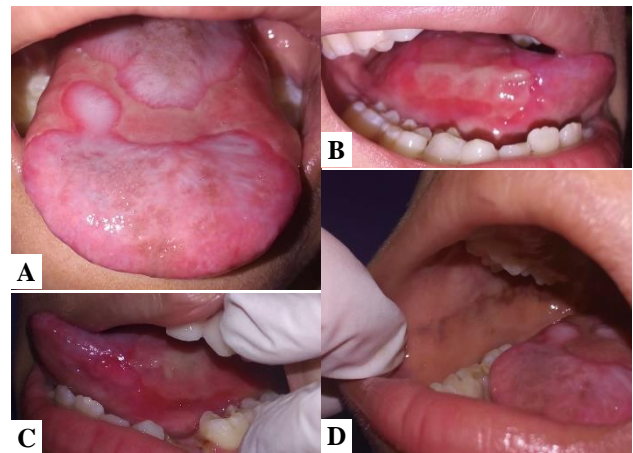


Figure 2. The clinical picture of the lesions appearing on the **A)** dorsal surface of the tongue, **B)** right lateral border of the tongue, **C)** left lateral border of the tongue, and **D)** buccal mucosa

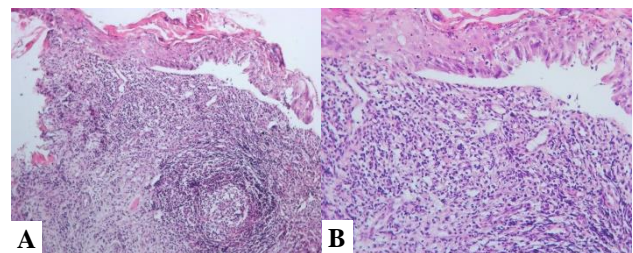


Figure 3. Histopathological picture of the specimen in magnification of **A)** x100 showing the tertiary lymphoid follicle, and **B)** x200 showing the intraepithelial changes

prescribed to be used 4 times daily, after meals and at bedtime; just in the same regimen as the topical antifungal. Unfortunately, the parent and the patient did not comply with the diet instructions. However, after one month of applying topical steroids, the pain was relieved. Consequently, the father did not show up at follow-up appointments as long as his son was not complaining. He only then came seeking treatment through the subsequent periods of exacerbation. This pattern extended for 7 months, where the father only showed at the peaks of the exacerbation (at 1, 3, and 7 months); while refraining from following up during the healing or remission periods. Afterward, the patient was lost to follow-up.

DISCUSSION

The occurrence of OLP in children is rare; where the total prevalence of OLP ranges between 0.1 and 1.2%; yet the prevalence of OLPc counts only as 0.03% (1). This case report presented an 8-year-old child with a peculiar ulcerative form of OLPc that -histopathologically- shows a lymphoid follicle-like structure.

The complaint of the presented patient started at 7 years of age; which falls within the target age range of OLPc between 6 and 17 years (1).

Just like OLP, the exact etiology of OLPc is unknown; but other autoimmune diseases and positive family history are frequently associated with the incidence of OLPc (3). However, in the presented case, there was no history of OLP in any of the family members, nor was there a history of autoimmune diseases or allergy to any type of drug or food.

Clinically, most commonly (70.5%) the juvenile OLP manifests as reticular lesions and least commonly (5.8%) in the ulcerative form (1). Moreover, skin lesions are reported in only 12.6% of cases of OLPc (1). In the presented case, extraoral lesions were detected on the flexor surfaces of the arms; besides the ulcerative form of the intraoral lesion. Such a picture highlights the uncommon presentation of the herein-reported case.

Intraorally, a typical clinical picture of the presented case (extensive butterfly-shaped ulcer affecting the tongue dorsum and its lateral borders sparing the tip) has been previously reported only twice (1,13). The two affected children were females, 3 and 9 years old. The 3-year-old child had no significant medical history nor had she any extraoral manifestations (13). Similarly, the 9-year-old child had no extraoral symptoms. However, she had a history of autoimmune polyendocrinopathy candidosis ectodermal dystrophy (APECED) (1). In the presented case in this report, other than having extraoral lesions, the boy did not have any symptoms of endocrinal abnormalities or ectodermal dystrophy.

Another similar ulcerative lesion was also reported but affecting only the mid-dorsum of the tongue of an otherwise healthy 12-year-old boy; with no other intra- or extraoral manifestations (14).

Therefore, differential diagnosis of the case included OLPc, oral lichenoid lesion (OLL), and lupus erythematosus. Normal levels of CRP, together with the absence of other symptoms, excluded lupus erythematosus from the list.

Despite the clinical differences between childhood and adult forms of OLP, they both have the same histopathologic picture. They are seen as basal cell degeneration and band-like sub-epithelial lymphocytic infiltration (6,15). In the presented case, an extra finding was added to the list; namely the presence of a lymphoid-follicle-like structure.

Such benign lymphoid hyperplasia forming extranodal lymphoid lesions in a site that normally lacks secondary lymphoid tissues is referred to as pseudolymphomas. If detected, it is considered a "tertiary" lymphoid follicle. These follicles represent an aggregation of lymphocytes and dendritic cells where B-cells undergo maturation and immunoglobulin isotype switching (16).

In case of OLLs, T-lymphocyte aggregates may organize forming an extrafollicular T-cell zone where B-cells become activated, together with antigen-specific T-cells. This finding draws attention to the presence of the antigen in close proximity as it is frequently reported by oral lichenoid contact lesions related to amalgam restorations (16).

Leaving OLPc and OLL in the differential diagnosis list, candies and their preservatives were suspected to have a role in the disease. This suspicion was corroborated by a previous report which stated that allergy to flouring agents and preservatives may induce lichenoid reaction (17). In the presented case, the presence of skin lesions together with the widespread oral lesions on the tongue and buccal mucosae were not pointing to support the diagnosis of lichenoid reaction.

Therefore, the case was diagnosed as OLPc, and treatment was planned using topical steroids. Like adult OLP, symptomatic OLPc is primarily treated by topical corticosteroids. Other topical treatments have provided successful results as well including topical tacrolimus, tretinoin, and cyclosporin. However, the safety of the long-term use of these topical medications was not evaluated in pediatrics. On the other hand, systemic steroids are not favorably used due to their significant effect on the growth and metabolism of children (1,15).

Using topical steroids, luckily, OLPc was reported to resolve completely after the treatment in 38.2% of the cases (1). However, this conclusion is believed to be reached based on reports that lack long-term follow-up. In the presented case, OLPc followed a chronic clinical course similar to OLP with periods of remission and exacerbation.

The good news about OLPc is the absence of reports -to date- indicating malignant transformation (1). Nevertheless, in the presented case, the parents were instructed to perform regular follow-ups as long as the lesion persists. This was planned in accordance with recommendations suggesting one or two visits per year for persistent lesions and even more frequent visits for symptomatic OLPc (1,12,14).

CONCLUSION

Although information lacks about its real prevalence, sporadic reports are accumulating to document OLPc cases. The diagnosis of OLPc should not be overlooked in suspicious lesions under the preconception of being a rare disease in childhood.

Informed Consent: All the management steps, together with the publication of this data and images, were performed after obtaining a written signed informed consent from the patient's father (guardian). The case was managed in accordance with the ethical standards of the ethics committee of the Faculty of Dentistry, Cairo University; and with the Declaration of Helsinki and its revision.

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Unpredictable Event during EUS-Guided Hepaticojejunostomy

EUS Kılavuzluğundaki Hepatikojejunostomi Sırasında Öngörülemeyen Olay


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ABSTRACT

Endoscopic ultrasound (EUS)-guided biliary drainage is a therapeutic alternative method in patients with cholestasis where the ampulla of Vater cannot be reached because several reasons. An unfortunate complication during EUS-guided biliary drainage was presented in this report. A 53-year-old male patient presented with jaundice and pruritus. He had a history of total gastrectomy due to gastric cancer. An abdominal tomography scan detected a tumoral mass in the common bile duct due to the recurrence of gastric cancer. We decided to perform EUS-guided hepaticojejunostomy because biliary drainage cannot be obtained by conventional methods and percutaneous drainage impairs patient comfort. A double pigtail plastic stent was placed from the jejunum into the segment-3 bile duct towards the hilus. After that echoduodenoscope was removed to the outside gently. We noticed that the stent was stuck in the echoduodenoscope elevator. We reached into the bile duct again and placed a double-pigtail plastic stent successfully.

Keywords: Cholestasis; extrahepatic cholestasis; endoscopic ultrasound; EUS.

ÖZ

Kolestazi olan hastalarda ampulla Vateri'ye çeşitli nedenlerde erişilemediği durumlarda endoskopik ultrason (EUS) kılavuzluğunda biliyer drenaj alternatif bir terapötik yöntemdir. Bu raporda, EUS kılavuzluğunda biliyer drenaj sırasında talihsiz bir komplikasyonun bildirilmektedir. 53 yaşında erkek hasta sarılık ve kaşıntı şikayeti ile başvurdu. Mide kanseri nedeniyle total gastrektomi öyküsü mevcuttu. Abdomen tomografisinde mide kanseri nüksüne bağlı gelişen peritoneal karsinomaza bağlı koledokta tümöral kitle saptandı. Biliyer drenajın konvansiyonel yöntemlerle sağlanamaması ve perkütan drenajın hasta konforunu bozması nedeniyle EUS eşliğinde hepatikojejunostomi yapılmasına karar verildi. Jejunumdan segment-3 safra yoluna hilusa uzanacak şekilde double pigtail plastik stent yerleştirildi. Ardından ekoduendoskop yavaşça dışarıya çıkarıldı. Stentin ekoduendoskopun kaldırıcına takıldığı fark edildi. Safra kanalına tekrar ulaşıldı ve bir double-pigtail plastik stent başarıyla yerleştirildi.

Anahtar kelimeler: Kolestaz; ekstrahepatik kolestaz; endoskopik ultrason; EUS.

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INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC) guided implantation of a biliary stent has been established as the procedures of choice for the treatment of biliary obstructions and cholestasis (1,2). However, under some circumstances, both approaches fail to obtain cholangiodrainage. Endoscopic ultrasound (EUS)-guided biliary drainage was an alternative method for patients who have failed ERCP or surgically altered anatomy (3,4). In this case report, we wanted to share the unexpected situation experienced while performing a EUS-guided hepaticojejunostomy.

CASE REPORT

A 53-year-old man was referred to our hospital with 6 weeks history of jaundice and pruritis. He had a history of total gastrectomy with the Roux-en-Y procedure due to gastric cancer 1 year ago. He had no drug use, herbal medicine, or alcohol. Marked jaundice, cachexia, and right upper quadrant tenderness to deep palpation were detected on physical examination. Laboratory findings were increased total bilirubin level of 29 mg/dl, elevated liver tests with aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels of 325/374 U/L, alkaline phosphatase (ALP) level of 496 U/L, gamaglutamin transferase (GGT) level of 856 U/L; reduced proteins with the total level of 5.2 g/dL, albumin level of 2.9g/dL. The international normalized ratio (INR) was 1.56. The abdominal US revealed that intrahepatic bile ducts and common bile ducts were greatly enlarged. A contrast-enhanced abdominal tomography scan detected a tumoral mass measuring 33x36x42 mm in the proximal part of the common bile duct. We agreed that malign obstructive jaundice occurred due to the recurrence of gastric cancer with peritoneal carcinomatosis. A magnetic resonance cholangiopancreatography (MRCP) revealed enlargement of the intrahepatic bile ducts and marked narrowing of the proximal choledochus.

Percutaneous biliary drainage and surgical bypass were traditional options for treatment. Surgical hepaticojejunostomy was not suitable for him because of previous total gastrectomy with Roux-en-Y anastomosis. We decided to undergo a EUS-guided hepaticojejunostomy because the patient did not accept percutaneous drainage because of its impairment in quality of life.

The procedure was performed using a standard linear echoendoscope (Pentax-Hitachi Corporation, Japan) in a supine position under the general anesthesia. We reached to efferent jejunum limb by passing through the esophagus. An enlarged left intrahepatic bile duct was selected (Figure 1). The transjejunal puncture was performed by using a 19-gauge needle (EZ Shot 3, Olympus Corporation, Japan) then some bile was aspirated and a contrast agent was injected within the bile duct for confirmation (Figure 2-3). After the 0.35 mm diameter guidewire (visiglide 2, Olympus Corporation, Japan) was sent to the bile duct, the tract was expanded using needle knife sphincterotome and 7 french biliary bougie (Soehendra, Cook Medical, US) over the guidewire. A 7 french 10 cm double pigtail plastic stent (Advenix, Boston Scientific, US) was placed from the jejunum into segment-3 bile duct towards the hilus (Figure 4). Satisfactory bile flow was observed. Then echoduodenoscope was removed to the outside gently. We noticed that the stent was stuck in the echoendoscope elevator (Figure 5). After the stent was separated, we went into an efferent loop again. Ultrasound image quality decreased as expected because some air escaped into the peritoneum and biliary tract. After a lot of effort, we reached and inserted the guide wire into the bile duct again. A single double pigtail plastic stent was placed. The patient was followed closely and discharged two days later. After three weeks, laboratory parameters gradually regressed as follows: AST/ALT levels of 51/54 U/L, total bilirubin level of 4.8 mg/dl, and ALP/GGT level of 134/148 U/L.



Figure 1. Dilated segment-3 bile duct on endoscopic ultrasound



Figure 2. Dilated segment-3 bile duct during contrast injection



Figure 3. Dilated intrahepatic bile ducts on fluoroscopy



Figure 4. Pigtail stent extends from segment 3 to hilus



Figure 5. Double pigtail stent stuck in echoduodenoscope elevator

DISCUSSION

For a long time, hepaticojejunostomy has been the main procedure in the palliation of obstructive jaundice in patients with failed ERCP. However, high rates of morbidity and mortality led to the development of nonsurgical methods for palliative biliary drainage (5). PTC is another choice for biliary drainage. However, some patients require long-term external biliary drains, which poorly affect the quality of life (6).

EUS-guided hepaticogastrostomy was first described in 2003 by Burmester et al. (7) in 4 patients with malignant biliary obstruction who had failed standard ERCP. In case of surgically altered anatomy or inaccessible ampulla of Vater, EUS-hepaticoenterostomy (EUS-HE) is useful as an access point for the treatment of obstructive biliary disease (8). EUS-guided biliary drainage is feasible and effective for patients with malignant biliary obstructions (9). When performed by experienced endoscopists, EUS-HE has technical and clinical success rates of 75-100% and 65-92%, respectively (8,10-11). In this case, we punctured a branch of the left intrahepatic duct transmurally from the jejunum and placed a stent in the intrahepatic biliary system. The procedure has been successful so far. We had a bad experience because we withdrew the scope without seeing the stent completely separated from the duodenoscope. After stent placement in therapeutic EUS procedures, it should be carefully checked for separation from the scope.

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

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Pulmonary Parasitic Embolization Caused by Micro Ruptured Hydatid Cyst: An Autopsy Case

Mikrorüptüre Kist Hidatik Kaynaklı Pulmoner Parazitik Embolizasyon: Bir Otopsi Olgusu


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ABSTRACT

Echinococcosis generally remains asymptomatic for many years, but it can also be fatal if complications like cyst rupture or superinfection occur. It is highly uncommon for a macroscopically non-ruptured cyst to cause death, and its exact mechanism is unknown. In the literature, there are several cases with identical characteristics diagnosed by autopsy. This autopsy case has illustrated the death due to a micro ruptured hydatid cyst. The deceased's lung underwent microscopic inspection, and it revealed broad intraalveolar hemorrhage, edema, severe hyperemia, neutrophil stasis in the interstitial capillary lumens, and a large number of parasites in the capillary lumen. Anaphylaxis against cyst contents that leaked into the bloodstream from liver cysts and non-thrombotic pulmonary embolization were shown to be the causes of mortality. In countries where echinococcosis is endemic, echinococcosis should be considered in all cases of anaphylaxis and sudden death.

Keywords: Anaphylaxis; echinococcus; liver; parasitemia; sudden death.

ÖZ

Ekinokokkoz genellikle uzun yıllar boyunca asemptomatik kalmakla birlikte kist rüptürü, süperenfeksiyon gibi komplikasyonlar nedeniyle ölüme de neden olabilmektedir. Makroskopik olarak non-rüptüre kiste bağlı ölüm oldukça nadir olup mekanizması tam olarak bilinmemektedir. Literatürde otopsi ile tanı konulan benzer özellikte birkaç olgu bildirilmektedir. Bu otopsi olgusunda hidatik kist mikrorüptürüne bağlı olduğu değerlendirilen ölüm sunulmaktadır. Karaciğerinde rüptüre olmayan kist saptanan cesetin akciğerinin mikroskopik incelemesinde yaygın intraalveolar kanama, ödem, ağır hiperemi, interstisyel kapiller lümenlerinde nötrofil stazi, kapiller lümeninde çok sayıda parazit varlığı saptandı. Ölüm nedeninin intakt karaciğer kistlerinden kan dolaşımına sızan kist içeriğine karşı gelişen anafilaksi ve nontrombotik akciğer embolizasyonu sonucu geliştiği düşünülmüştür. Ekinokokkozun endemik olduğu ülkelerde tüm anafilaksi ve ani ölüm olgularında ayırıcı tanıda ekinokokkoz düşünülmesi gerekmektedir.

Anahtar kelimeler: Anafilaksi; ekinokokkoz; karaciğer; parazitemi; ani ölüm.

INTRODUCTION

A serious global problem, cystic echinococcosis (CE), which is brought on by the genus Echinococcus, is endemic in the Middle East, Central Asia, South America, North and East Africa (1). It is transmitted either directly through contact with dogs and dog feces or indirectly through contaminated foods; it also causes disease in people over the years. It can be located almost everywhere in the human body. The liver is where it is most frequently found, followed by the lung. Although it is usually

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asymptomatic, depending on the location and size of the cyst in the body, it might result in symptoms such as coughing, fever, chest pain, dyspnea, abdominal masses, ascites, hepatomegaly, and splenomegaly; in severe circumstances, it may even be a fatal condition (1-3).

The clinical picture of the hydatid pulmonary embolism is nonspecific and it can range from asymptomatic or general clinical indications of a pulmonary embolism due to obstruction of pulmonary artery branches (4). Hydatid pulmonary embolism is a rare but potentially life-threatening complication due to the risk of acute fatal complications such as anaphylactic shock (5).

It is reported that the incidence of sudden death due to hydatid cyst is around 1.1-1.3% (6,7). It is difficult to explain the involvement of a hydatid cyst in the death mechanism, particularly if it is seen intact and discovered at autopsy (8).

The presented case shares traits with those of patients who died from parasite pulmonary embolization and anaphylaxis from a ruptured hydatid cyst in the liver. However, it differs in that no macroscopically ruptured cysts have been observed. This case seeks to critically discuss postmortem approaches and causes of death for the evaluation of cases with hydatid cyst during an autopsy, in the light of the literature.

CASE REPORT

The following sequence of events was described in the account provided by witnesses to the 20-year-old male case who was 180 cm tall and weighed 95 kg before the incident: jogging uphill after overeating, followed by a sudden fainting spell, followed by uncontrollable contractions on the floor that resembled epilepsy, which ultimately resulted in death. There was no trauma story. Since the relatives of the deceased were unreachable, his medical history was unknown. Since the cause of sudden death was undetermined, a forensic autopsy was performed by a specialized medical doctor. The postmortem computerized tomography (CT) examination of the case provides the following overview: according to the GHARBI Liver Hydatid Cyst Classification, Type IV cysts measuring 7.93x11.64 cm (Figure 1) and Type II cysts measuring 5.52x5.09 cm (Figure 2) were found in the liver segment 7 and segment 6, respectively. In the abdomen, there was no free fluid to be seen. No significant rupture was observed in the cysts. Contrast-enhanced postmortem CT examination could not be performed.

During the case's external examination, it was noted that the auricles and nail bed were cyanosed in addition to the fact that bloody fluid was leaking from the nose. As far as the macroscopic findings of the internal examination are concerned; petechiae under the scalp, bloody foamy fluid in the form of smearing in the trachea and both main bronchi, mild edema, and hyperemia (Figure 3) in the larynx were all noticed.

The liver weighed 1510 g. Loose, fluid-filled cysts in the superior posterior of the right lobe and white, spherical, harder cysts in the inferior of the right lobe were detected. The cyst walls were macroscopically intact (Figure 4). The right lung weighed 350 g and the left lung 300 g. Mild congestion was noticed in the lungs. A macroscopic embolism was not observed in the main pulmonary arteries. Manual palpation revealed that the main bronchi

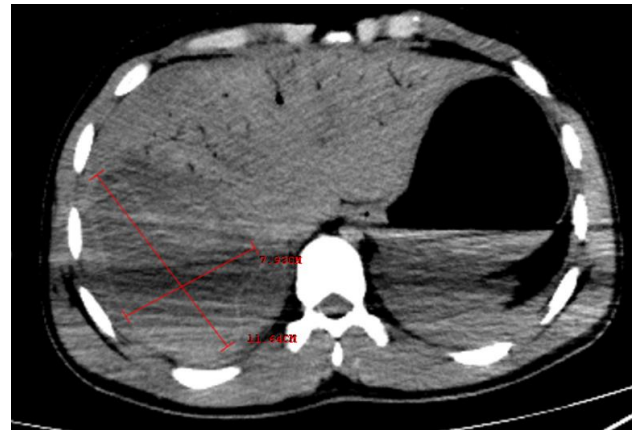


Figure 1. Type IV cyst (7.93x11.64 cm) in liver segment 7

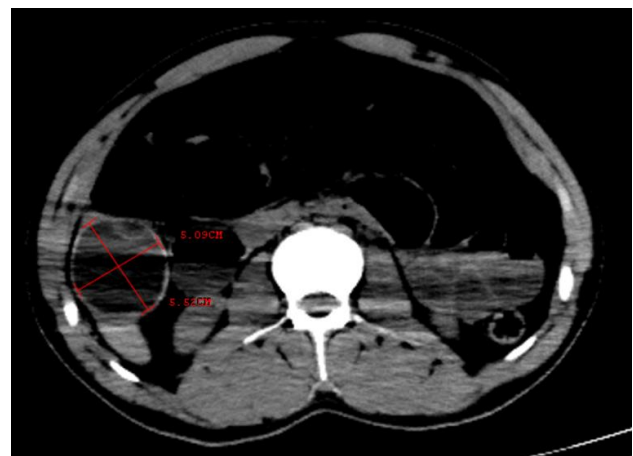


Figure 2. Type II cyst (5.52x5.09 cm) in liver segment 6



Figure 3. Hyperemia and mild edema in the larynx



Figure 4. A cystic structure in the liver's right lobe

of the lung were emitting a bloody and foamy discharge. In the heart, brain, and other organs, macroscopically abnormal characteristics were not found, with the exception of hyperemic appearance. Any macroscopic pathological feature was not observed in the examination of the other systems. A toxic substance was not found in the toxicological analysis.

Samples of tissue were collected from the brain, spleen, kidney, lung, and liver. On the histopathological examination of liver tissue; the wall of an echinococcal cyst, germinative membrane, and multiple daughter sacs were detected. On the histopathological examination of lung tissue; diffuse intraalveolar hemorrhage, edema, severe hyperemia, neutrophil stasis in interstitial capillary lumens, and the presence of many parasites (presence of Echinococcosis) in capillary lumens were detected. As a result, it was determined that the person's death occurred due to parasitemia brought on by micro rupture in the echinococcal cyst located in the liver, along with a potential concomitant anaphylactic reaction.

DISCUSSION

Echinococcosis typically goes unnoticed for many years, but occasionally, it manifests itself at autopsy as a sudden, unexpected death brought on by an undiagnosed hydatid cyst (9). According to Ben Jomaa et al. (8), hydatid cysts were reportedly found in 0.33% (n=26) of the 7808 autopsy cases, and only 15 of these 26 instances had hydatid cysts as a contributing factor to mortality (12 anaphylaxis, 1 hydatid pulmonary embolism, 1 cardiac arrhythmia, and 1 hemothorax). The main causes of death are either complications of hepatic and pulmonary echinococcosis or cardiac hydatid cyst disease. Anaphylaxis, cardiac outflow blockage, pulmonary and cerebral embolism, pulmonary hypertension, and peritonitis are a few of the causes connected to sudden death (9).

Pulmonary artery involvement develops primarily due to the rupture of the right heart cysts. A cyst's contents draining into the venous or arterial system can lead to pulmonary or systemic embolism, chronic pulmonary hypertension, anaphylaxis, or sudden death. The rupture of the cyst inside of the hepatic vein or vena cava related to hepatic focus is among the rare causes of pulmonary embolism (2,10,11). This case fits the definition of an embolism since the cyst's contents travel down the vena cava to the right side of the heart and the pulmonary artery. An important observation for understanding how this process works is the presence of ruptured cyst content in hepatic vascular structures. The most dangerous complication in cases of hydatid cysts in the liver is the opening of the cyst to the blood vessels, which can cause anaphylactic shock and death. Rupture of the cyst and discharge into the bile duct is also a frequent complication. It is also stated that anaphylactic shock, which develops due to the intravascular spread of the cyst content due to minor abdominal trauma, can cause death without a macroscopically visible cyst rupture (3).

According to their examination, Şahpaz et al. (12) concluded that in cases of non-thrombotic pulmonary embolism due to liver hydatid cyst, the cyst was macroscopically well-defined but ruptured due to fresh hemorrhage around the cyst wall and the daughter vesicles inside the hepatic vascular structures. It is highly likely

that micro ruptures have occurred in an unruptured cyst if its contents mix with blood and move to the lung. Especially, when no other cause of death can be noticed, it is suggested that CE plays a part in the etiology and is acknowledged as the mechanism of death (8).

Although anaphylactic reactions often occur due to post-traumatic cyst rupture, recurrent anaphylaxis cases due to unruptured cysts are also rarely reported (13,14). There are very few case reports of patients with an intact hepatic hydatid cyst dying suddenly from anaphylactic shock (3,15). Concerning these cases, researchers have most often pointed to the leakage of intense antigenic cyst content into the blood circulation. While the cyst wall is intact, the cause of this leakage is considered high-intracystic pressure (15,16). According to the literature, a hydatid cyst-induced pulmonary embolism can occur in two ways: either by rupturing in the right atrium or ventricle or by hematogenous dissemination originating from a hepatic focal point. The suggested mechanism behind the latter is the micro rupturing of the cyst inside the inferior vena cava and the subsequent content dissemination into the pulmonary arteries. The research phase for postmortem contrast-enhanced CT examination is now underway. While not being utilized much in practice, it seems to be reliable for identifying cardiovascular causes of death (17). When cysts are not macroscopically ruptured, it can be helpful to demonstrate micro ruptures.

In our case, we concluded that parasite embolization from the intact liver cyst to the lungs and anaphylactic reaction were the causes of death. Especially, exercise and overeating, which will increase intra-abdominal pressure in the patient's history, may account for the formation of micro ruptures due to mechanical effects on the cyst wall. The most common autopsy findings related to anaphylaxis due to cyst hydatid constitute pulmonary congestion, alveolar hemorrhage, bronchial hypersecretion, and laryngeal and/or pharyngeal edema (9). Anaphylaxis is a macroscopically difficult diagnosis to make at autopsy. The most typical signs of anaphylaxis at autopsy are pulmonary congestion, alveolar hemorrhage, upper airway edema, bronchial hypersecretion, and laryngeal and/or pharyngeal edema. As in all cases of suspected fatal anaphylaxis, measurements of serum-specific IgE, serum tryptase and chymase levels can be helpful in cases of suspected anaphylaxis due to cyst rupture (9,18,19). In our autopsy case, serum-specific IgE, serum tryptase and chymase measurements could not be performed. In our case, every additional anaphylaxis-related finding that may be seen during an autopsy was present. Premortem epilepsy-like involuntary contractions expressed by the witnesses of our case could be related to abruptly decreased cerebral blood flow secondary to anaphylaxis as reported by Meyer et al. (20).

CONCLUSION

To conclude, undiagnosed hydatid cysts should be considered in the differential diagnosis in all cases of anaphylaxis and sudden death in regions where echinococcosis is endemic. In cases where cysts are incidentally noticed in any organ, if there is no pathology to explain death macroscopically at autopsy, anaphylaxis, and systemic embolism due to echinococcosis should be considered the causes of death.

Informed Consent: Not applicable because the study is an autopsy case. Permission was obtained from The Council of Forensic Medicine (21.06.2021, 758).

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Isolated Axillary Lymph Node Recurrence from Serous Ovarian Carcinoma: A Case Report

Seröz Over Karsinomunun İzole Aksiller Lenf Nodunda Nüksü: Olgu Sunumu

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ABSTRACT

Ovarian cancer is the most mortal gynecological malignancy among women worldwide. The appropriate treatment protocol is cytoreductive surgery and the main aim is to remove all primary carcinoma focus and all metastatic lesions. A 37 years old woman (gravida 5, para 3) presented with complaints of abdominal distension. During the gynecological examination, bilateral adnexal masses in the solid structure were observed. Staging surgery was performed and 8 cycles of adjuvant chemotherapy regimen were given. A pathological axillary lymph node was detected in the positron emission tomography-computed tomography (PET/CT) imaging at the follow-up 6 months after the end of the chemotherapy regimen. The biopsy result from the axillary lymph node was reported as serous carcinoma metastasis. Metastasis of ovarian carcinoma to the axillary lymph node is uncommon and has a poor prognosis. High-grade ovarian serous carcinoma with axillary lymph node metastasis was presented in this case report.

Keywords: Ovarian neoplasms, cystadenocarcinoma serous, neoplasm metastasis.

ÖZ

Over kanseri, dünya çapında kadınlar arasında en ölümcül seyreden jinekolojik malignitedir. Uygun tedavi protokolü sitoredüktif cerrahidir ve asıl amaç tüm primer karsinom odağını ve tüm metastatik lezyonları çıkarmaktır. 37 yaşında kadın hasta (gravida 5, para 3) karın şişliği nedeniyle başvurdu. Jinekolojik muayenede bilateral solid yapıdaki adneksiyal kitleler izlendi. Hastaya evreleme cerrahisi yapıldı ve 8 kür adjuvan kemoterapi rejimi verildi. Kemoterapi rejimi bitiminden sonraki 6. ay kontrolünde, pozitron emisyon tomografi-bilgisayarlı tomografi (pozitron emission tomography and computed tomography, PET/CT) görüntülemesinde patolojik görünümde aksiller lenf nodu saptandı. Aksiller lenf nodundan alınan biyopsi sonucu seröz karsinom metastazı olarak raporlandı. Over karsinomunun aksiller lenf nodu metastazı oldukça nadirdir ve kötü prognostik bir işaret olarak kabul edilir. Bu vaka raporunda aksiller lenf nodu metastazı olan yüksek dereceli over seröz karsinomu sunulmuştur.

Anahtar kelimeler: Over tümörleri; kistadenokarsinom seröz; tümör metastazı.

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INTRODUCTION

Ovarian cancer is the most mortal gynecological malignancy and the third most common gynecological malignancy after endometrial and cervical cancer (1). Cases are frequently detected in stage III-IV due to their quiet course and late onset of symptoms. The standard treatment is surgical staging and cytoreductive surgery with adjuvant chemotherapy. Distant metastasis is not expended after treatment is completed. Ovarian cancer metastasizes mainly through direct extension and cancer

cells spread to the abdomen. Transcoelomic spread is followed by lymphatic dissemination (2-4). Axillary lymph node metastasis from ovarian cancer is rare, it is reported to have a poor prognosis. There are few case reports of axillary metastasis of ovarian carcinoma (5,6).

In this case, we presented an ovarian carcinoma with metastasis to the axillary lymph node.

CASE REPORT

A 37 years old woman (gravida 5, para 3) presented with abdominal pain and distension. The patient did not smoke and did not have any diseases. Nobody in the family has breast cancer or ovarian cancer. In the gynecological examination performed, bilateral adnexal masses in a solid structure were observed. Diffuse acid fluid was seen during the ultrasonographic examination, which indicated solid masses of 82*73 mm in the left and 85*67 mm in the right adnexa. The patient underwent magnetic resonance imaging (MRI) as an advanced imaging method. MRI showed that the mass in the right adnexa was adjoining to the sigmoid colon and descending colon (Figure 1). Then the patient underwent a colonoscopy to assess the location of the tumor in the colon. As a result of the colonoscopy, no finding suggesting colonic invasion was found. The Ca125 level was 7418 U/ml.

Ovarian cancer was identified, necessitating staging surgery. In exploration, the mass originating from the right adnexa was observed to be attached to the posterior uterine wall it was approximately 10 cm in size. A heterogeneous mass of 8 cm in the size was observed in the left adnexa. There were extensive tumoral implants in the peritoneum and a 4 cm mass invading the rectum was observed. There was a 1-liter discharge of acidic fluid and send for cytological examination. Cytology result was reported as an acid fluid containing malignant epithelial cell groups. The patient had a total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, appendectomy, and pelvic-paraortic lymph node dissection as part of the staging procedure. Low anterior resection was used to remove the colonic mass. Implants in the peritoneum could not be removed completely because they were too much.

As a result of pathology, high-grade serous carcinoma was observed in both adnexa. High-grade serous carcinoma infiltrates in the uterine serosa and bilateral parametrium, detected in the pelvic, paraaortic lymph nodes, and colon. No tumor infiltration was observed in the appendix and omentum. No pathogenic mutation was detected in the genetic tests performed on the patient.

Postoperatively, 8 cycles of carboplatin and paclitaxel chemotherapy regimen were given. There was no pathological finding in the computed tomography (CT) imaging taken at the end of the chemotherapy regimen and the Ca125 value was 11.7 U/ml. 6 months later, in the positron emission tomography-CT (PET/CT) examination, a pathological lymph node in the left axillary region (Figure 2). Biopsy was taken from the axillary lymph node, and the pathology result was reported as high-grade serous carcinoma metastasis. Immunohistochemistry markers of the left axillary node biopsy specimen resulted in positive for P16, P53, and

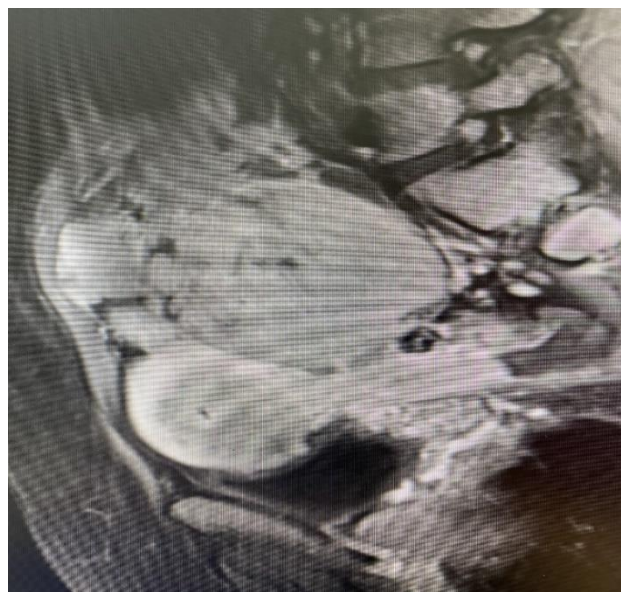


Figure 1. Magnetic resonance image of the uterus and right adnexa

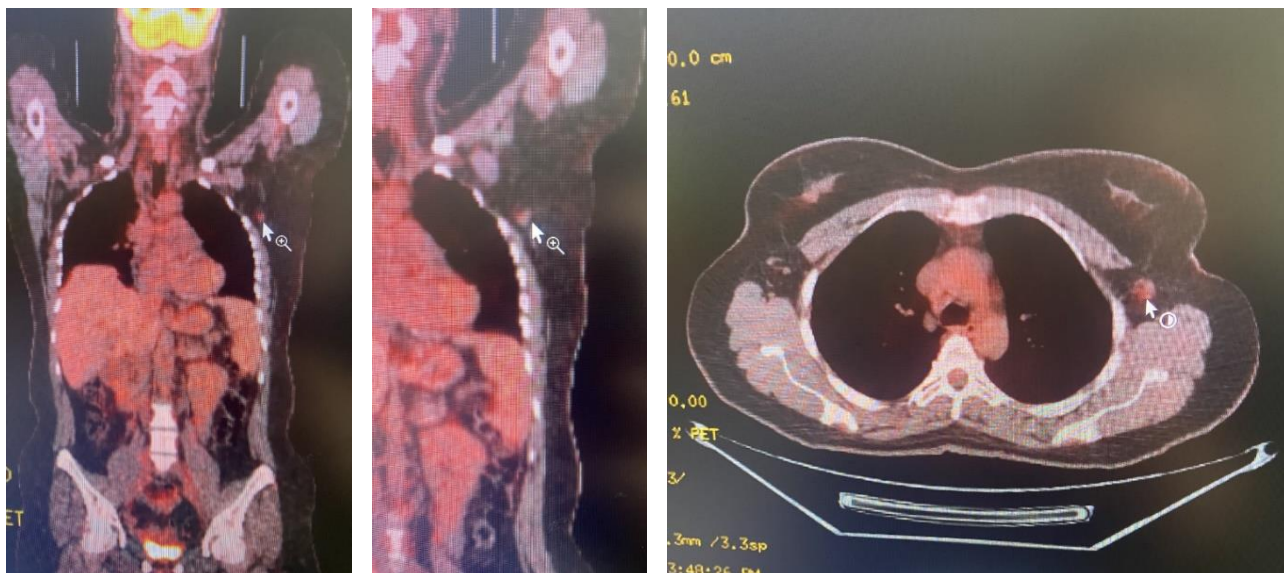


Figure 2. Positron emission tomography-computed tomography examination, a pathological lymph node in the left axillary region

PAX-8, and negative for vimentin and GATA3. This result supported that the tumor originated from the ovary. Serum Ca125 was slightly elevated (26.7 U/ml), the previous value was 6.1 U/ml. Chest and abdominopelvic CT imaging was taken and no lesion was observed. Carboplatin and paclitaxel chemotherapy regimen was started.

DISCUSSION

The peritoneal cavity is where ovarian cancer cells often proliferate, however, they can metastasize to the supraclavicular lymph nodes through the retroperitoneal and diaphragmatic lymphatic systems. Two theories have been put out in an attempt to explain how ovarian cancer spreads to the axillary lymph nodes. Firstly, the cancer cells could travel through the diaphragm via superior diaphragmatic lymph nodes to the internal jugular vein, subclavian vein, or subclavian lymph trunk, and finally to the axilla. The second course is via the deep lymphatic vessels inferior to the diaphragm and the superficial lymphatic vessels inferior to the level of the umbilicus, to the thoracic duct, and finally to the junction of the left subclavian and internal jugular vein (7).

Stage IV tumors indicate distant metastases in ovarian cancer, and these cases typically have poor prognoses. Lymph node metastasis occurs in approximately 14-70% of patients with ovarian cancer and is mainly seen in the pelvic and paraaortic lymph nodes. It has been reported that ovarian cancer metastasizes to axillary lymph nodes with a frequency of approximately 0.03-0.6% (5).

Immunohistochemistry markers can help to identify the axillary lymph node's main. PAX8 is expressed in approximately 90% of malignant ovarian carcinomas, therefore it is an important marker for epithelial ovarian cancers (8). PAX-8 positivity in the axillary lymph node biopsy taken from our patient and the history of advanced ovarian cancer confirms that the metastasis originates from the axillary lymph node.

Secondary cytoreductive surgery is one of the treatment modalities in recurrent ovarian cancer cases (9). However, data on the management of isolated cases of axillary lymph node metastases are limited and the benefit of surgical treatment is uncertain.

It is shown that patients with distant metastases were initially diagnosed with stage III and IV cancer, had high-grade carcinoma, and the median time to metastasis was 20 months (10). It is stated that the time between the diagnosis of ovarian cancer and the documentation of distant metastasis is the most important prognostic factor associated with survival, and the longer this period, the longer the survival time. Especially in ovarian cancer patients with single axillary metastases, survival is better in case of recurrence after a minimum of 26 months after the end of primary treatment (11).

CONCLUSION

Axillary lymph nodes are not a site where ovarian cancer often metastasizes. Cancers that metastasize to axillary lymph nodes more frequently. In particular, the diagnosis of breast cancer should be excluded first. Immunohistochemical markers are useful in excluding breast cancer or other primaries, in demonstrating that the tumor originates from the ovary.

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

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Presence of Accessory Soleus Muscle in Cadaver: Case Report

Kadavrada Musculus Soleus Accessorius Varlığı: Vaka Sunumu


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ABSTRACT

Human anatomy is a variable structure. There are various rare muscle-specific accessory structures. The accessory soleus muscle (ASM) is one of them and was studied in this case report. ASM was seen during routine learning and teaching dissection lessons while dissecting the right leg of a male body. When this muscle was examined, a thick beginning with fascial connections from the soleus muscle, medially in the lower 1/3 of the leg was seen. This unipennate-shaped super numeric muscle had a 3.95 cm width, 25.82 cm length, and 0.82 cm thickness. This muscle, which is seen on radiological images, may often be confused with soft tissue tumors such as ganglion, lipoma, hemangioma, synovioma, and sarcoma, and on the pathology of the muscle may cause pain and tarsal tunnel syndrome-like effects.

Keywords: Accessory soleus muscle; anatomic variation; ankle.

ÖZ

İnsan anatomisi değişken bir yapıya sahiptir. Kasa özgülü çeşitli ve nadir yardımcı yapılar görülebilmektedir. Aksesuar soleus kası (accessory soleus muscle, ASM) bunlardan biridir ve bu olgu sunumunda incelenmiştir. ASM, bir erkek kadavranın sağ bacağına rutin öğrenme ve diseksiyon derslerini öğretme sırasında diseke edilirken görüldü. Bu kas incelendiğinde bacağın alt 1/3'ünde medialde soleus kasından fasiyal bağlantılarla başlayan kalın bir yapı görüldü. Bu unipennate şekilli süper numerik kas 3,95 cm genişliğe, 25,82 cm uzunluğa ve 0,82 cm kalınlığa sahipti. Radyolojik görüntülerde görülmekte olan bu kas sıklıkla ganglion, lipom, hemanjiyom, sinovioma ve sarkom gibi yumuşak doku tümörleri ile karışabilmektedir ve kasın patolojisinde ağrı ve tarsal tünel sendromu benzeri etkilere neden olabilmektedir.

Anahtar kelimeler: Musculus soleus accessorius; anatomik varyasyon; ayak bileği.

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INTRODUCTION

Anatomical variations such as the absence of a muscle, the abnormal presence of extra muscle tissue, abnormal location of a muscle or an unusual course of a muscle are frequently encountered. Accessory muscles are the name given to anatomical variations that represent additional different muscles encountered with the normal complement of muscles. In the literature, most of the data on accessory muscles are based on findings obtained during anatomical dissection, surgical procedures, or clinical examination methods. However, the widespread use of modern cross-sectional

imaging techniques such as ultrasonography (US), computed tomography (CT) and magnetic resonance (MR) imaging has led to more frequent encounters with accessory muscles and their more accurate identification (1). Accessory muscles are usually asymptomatic and overlooked, but may occasionally present with symptoms (1-3). These symptoms are usually palpable swelling caused by the muscle mass in the patient, symptoms that occur with the compression of the neighboring structures (3,4). Cross-sectional imaging can accurately display accessory muscles and help distinguish them from other soft tissue masses (1).

Accessory soleus muscle (ASM), first described by Jean Cruveilhier in his anatomy book titled "Traité d'anatomie descriptive", is a rare anatomical variation in the posteromedial ankle (5). The incidence of this congenital variation varies between 0.7 and 5.5 on average (4). It has been suggested that ASM is formed by the splitting of the soleus muscle (MS) muscle in the early stages of development (6). The ASM originates from the anterior surface of the MS, the fibula, or the linea solei musculi in the tibia (7). According to the insertion point, it has been described that there are 5 types (8). Its innervation is provided by the tibial nerve, and its nutrition is provided by the posterior tibial artery (8,9). The presence of ASM is usually asymptomatic (1-3). With exercises, excessive activities, and the increase in the size of the muscle, the structures in the region may be under pressure, so there may be symptoms such as pain and numbness mimicking tarsal tunnel syndrome (4,10). Sometimes they can be confused with soft tissue (ganglion, lipoma, hemangioma, synovioma, and sarcoma) tumors (11).

CASE REPORT

This case was found during routine learning and teaching dissections on a cadaver used for anatomy practice at the University of Health Sciences, Hamidiye Faculty of Medicine, Department of Anatomy in 2022. The male cadaver's clinical history, family history, and other details were unknown. The cadaver was fixed using 10% formaldehyde. In order to examine the ankle structures, a tendon that terminates in the calcaneus was detected in the posteromedial of the right ankle in the dissected cadaver in accordance with the dissection techniques. When this muscle was examined, a thick beginning with fascial connections from the soleus muscle, medially in the lower 1/3 of the leg has been seen. It ended by attaching to the posteromedial of the calcaneus with a thin tendon at the attachment site (Figure 1). This unipennate-shaped super numeric muscle has a 3.95 cm width, 25.82 cm length, and 0.82 cm thickness. Innervation with the tibial nerve, supply it was through the posterior tibial artery.

DISCUSSION

Various anatomical variations of the posterior leg region have been described in the literature. Anatomic variations have been detected in musculature during anatomical dissection, clinical evaluations, or surgical procedures (12). Variations in the muscles of the posterior leg region can lead to chronic painful conditions of the ankle. ASM is a condition that manifests itself with the increase in clinical problems caused by advanced age (13-17). ASM, whose incidence was reported as 0.7%-5.5% in cadaver studies, is usually unilateral and it has been reported to be more

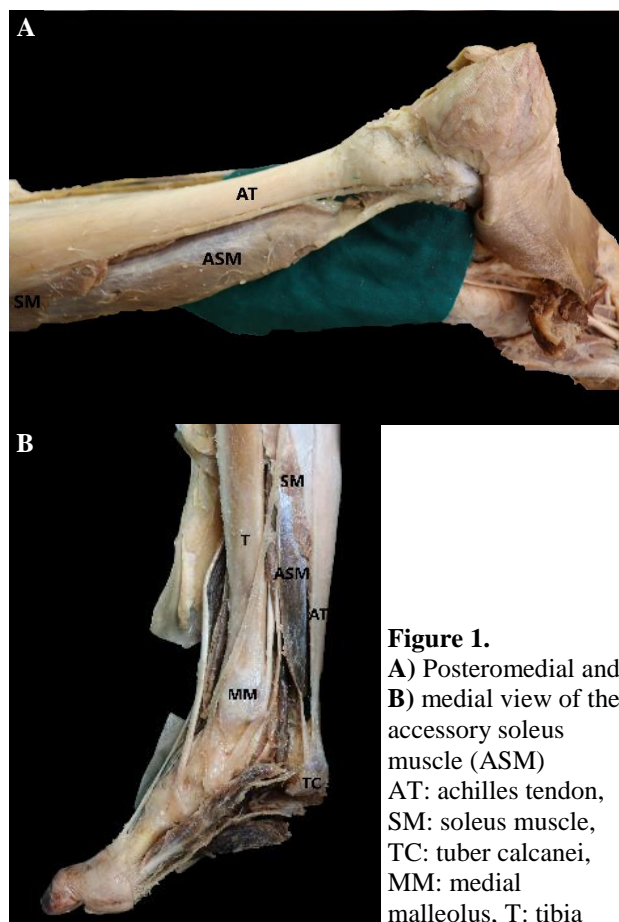


Figure 1.

A) Posteromedial and **B)** medial view of the accessory soleus muscle (ASM)

AT: achilles tendon, SM: soleus muscle, TC: tuber calcanei, MM: medial malleolus, T: tibia

common in males (4,18,19). Reported that the presence of ASM causes nerve compression at a rate of 7-8% in tibial nerve compressions (13).

The soleus muscle (SM) is located deep in the gastrocnemius and the head of the fibula originates from the upper part of the posterior surface of the fibula and the middle part of the posterior aspect of the tibia, forming a short tendon structure and unites with the Achilles tendon. There is a fibrous band between the parts originating from the tibia and fibula. ASM originates from the deep surface of the SM and the line joining the parts of the SM that starts from the two bones. Types starting from the linea musculi solei or fibula in the tibia have also been reported (20). The muscle then runs downward and terminates posteromedially of the calcaneus (1). At the endpoint, the muscle or tendon structure can be seen. Lorentzon and Wirell (21) categorized the endings of the ASM, and Yu and Resnick (8) added a fifth shape to this classification:

1. Insertion along the Achilles tendon
2. Tendinous insertion on its upper surface
3. With muscle tissue on the upper surface of the calcaneus insertion
4. Insertion with muscle tissue on the medial surface of the calcaneus
5. Tendinous insertion on the medial surface of the calcaneus

In another study, they divided attachment sites of ASM into 3 types (22):

A. Proximal attachment to the soleus and distal attachment to the medial surface of the calcaneus by a separate tendon.

B. Proximal attachment to the soleus and distal tendinous attachment to the calcaneal tendon.

C. Proximal attachment to the soleus and distal fleshy attachment to the medial surface of the calcaneus.

According to the division of Hatzantonis et al. (22), this case report fits type A, while according to Yu and Resnick (8), this case reports defines as the 5th type.

In Hatzantonis et al. (22)'s study, six specimens were investigated for ASM attachments, and all six fit type A. In their study, it is indicated that in clinical literature 26.1% of specimens had type A attachments. In unilateral type A attachment of ASM, the accessory muscle measured 3.7 cm in width and 13.5 cm in length (22). In our case report, ASM has been measured at 3.95 cm width and 25.92 cm length. For males, 46.4% of ASM cases are on the right leg, 43.5% left leg, and 10.1% are bilaterally (22). In our case report ASM is seen unilaterally on the right leg of a male body. If the Achilles tendon and ASM are separately attached to the calcaneus, it is generally stated that the ASM is attached to the anterior side of the Achilles tendon. The muscle is usually innervated by the tibial nerve and supplied its oxygenated blood posteriorly to the tibial artery (23). In the presence of ASM, pain especially on exertion has been described. Pain occurs in the later stages of life. The reason for this was shown as the increase in muscle mass in the later periods (4). It is claimed that it is detected more in athletes based on the same reason (8). The mechanisms that will cause pain in the presence of ASM are listed as follows: Increased pressure in the fascia of the muscle, insufficient blood flow to the muscle, and excessive development of the tibial nerve posteriorly (4,8). Even though it does not pass through the tarsal tunnel, there have been researchers who have associated it with tarsal tunnel syndrome (24). In their statement, the researchers suggested that compression of the ASM triggers tarsal tunnel syndrome. On clinical examination, swelling may be detected in the posterior part of the wrist.

In addition to radiography US, CT, and MR imaging methods were used to detect the presence of ASM (3,8,21). In the radiographic imaging method, the image of the Kager fat pad is not formed and is shadowed by the ASM. With these imaging methods, ASM is usually detected in front of the Achilles tendon, superficial to the Flexor retinaculum, between the medial edge of the Achilles tendon and the medial malleolus (1).

In the presence of ASM, in symptomatic cases, it has been reported that treatment methods such as fasciotomy, tendon release, ligation of the artery feeding the muscle, and botulinum toxin injection are applied (25,26). While success was achieved in the short term, the return of symptoms in the long term suggested that surgical excision is the definitive treatment method (1).

CONCLUSION

Painful conditions of the posterior inner region of the ankle should be investigated considering the presence of ASM even if no swelling is detected. MRI examinations applied to patients presenting with such a complaint should be performed by keeping in mind that the muscle may exist. Particularly in the cases with painful conditions that decrease at rest and increase with effort may be associated with ASM.

Informed Consent: Since our study was a cadaveric study, there was no consent form.

Conflict of Interest: None declared by the authors.

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ETHICAL RESPONSIBILITY

- Compliance with The Principles of Helsinki Declaration (<https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/>) is required in all studies including the "human" factor. In this kind of studies, authors must state that they perform the study in compliance with these principles, they have taken approval from the ethics committee of their institution, and the "informed consent" from people participating in the study, in the MATERIAL AND METHODS section.
- If the "animal" factor was used in the study, authors must state that they have protected the animal rights in line with the principles of 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>) and they have taken approval from the ethics committee of their institution, in the MATERIAL AND METHODS section.
- In case reports, informed consent must be taken from patients.
- The information on the ethics committee approval should be indicated together with the name of the committee, approval date, and number, in the MATERIAL AND METHODS section.
- For all research involving human subjects, it must be stated that informed consent was obtained from all participants in compliance with The Principles of Helsinki Declaration (<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>), for participation in the study or use of their tissue (or a parent/legal guardian in the case of children under 18 and patients otherwise considered minors under local legislation). Consent is also required for the procurement of biomaterials for stem cell research and translation, including gamete donors in in vitro fertilization studies. Studies involving vulnerable groups (i.e. individuals who are at higher risk of mistreatment or harm), with potential for coercion or exploitation (for example, prisoners or unconscious patients) or where consent may not have been fully informed (for example, due to a language barrier), will be considered at the Editor's discretion under exceptional circumstances. Scientific research involving vulnerable groups can only be carried out if its aims and scope benefit those groups and meet their specific needs, and authors must be able to demonstrate this for their manuscript to be considered for publication.
- If there is a direct-indirect commercial relation or an institution giving financial support in the study, authors must state that they have no commercial relationship with the commercial product, medicine, company, etc. used, or if any, what kind of a relationship they have (consultant, other agreements), in the cover letter to the editor.
- The authors are responsible for reporting all personal and financial relationships that may be related to the study. It is necessary to state clearly whether there is any conflict of interest related to the submission and/or evaluation of the manuscript.
- Compliance of the manuscripts with the scientific and ethical rules is the responsibility of the authors.

SUBMISSION FILES

Manuscripts must be uploaded to the system as separate files as described below.

Copyright Transfer Form: The Copyright Transfer Form to be obtained from the system during the submission must be signed by all authors in accordance with the authorship order. Submissions without a Copyright Transfer Form signed by all authors are not included in the review process.

Similarity Report: Authors are required to upload the similarity report obtained from a plagiarism software, such as iThenticate, etc. to the system at the submission. In cases where plagiarism is detected, depending on the similarity rate, the editors' right to reject the manuscript and/or request corrections from the authors is reserved.

Cover Letter: Type of the article, the statement that has not been published previously in anywhere before, and/or not in the evaluation process for publication, if any, the people and institutions supporting the study financially, and the relationship of these institutions with authors (if not, there is no relationship) must be stated. The names, academic titles, institutions, contact information, and e-mail addresses of at least two reviewers suggested in relation to the subject of the manuscript and not related to the authors and their institutions should be written. Editors' right to choose the reviewers is reserved.

Title Page: It must include the title of the article (English and Turkish), a short title not exceeding 40 characters, names, academic titles, ORCID® numbers, institutions, e-mail addresses of all authors, and also name, correspondence address, phone number, email address of the corresponding author. If the manuscript has been presented previously in a scientific meeting; the name, date, and place of the meeting (if not, not presented) should be stated.

Main Text: The title of the article (English and Turkish), short title not exceeding 40 characters, Abstract (English and Turkish), Keywords (English and Turkish), Main Text (sectioned according to the type of article submitted), References, Tables, and Figures should be included.

Ethics Committee Approval Document: Ethics Committee Approval Document should be uploaded as a separate file for all research articles.

Note: If there are figures, pictures, or photographs, each of them must be uploaded as separate files.

SECTIONS THAT SHOULD BE USED ACCORDING TO THE TYPE OF ARTICLE

Research Article

TITLE (English and Turkish), SHORT TITLE (not exceeding 40 characters), ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES

ABSTRACTS in both languages (English and Turkish) must be fully compatible with each other, and each should be between 200 and 250 words.

ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".

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" ögesini içeren tüm çalışmalarda Helsinki Deklarasyonu 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" ögesi 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 sunularında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- İnsan ögesini 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 translyonu için biyomateryallerin tedariği için de onam gereklidir. Zorlama veya istismar potansiyeli olan (örneğin, mahkumlar veya bilinci kapalı hastalar) veya rızanın tam olarak bilgilendirilemeyeceği (örneğin, bir dil engeli nedeniyle) savunmasız/hassas grupları (yani, kötü muamele veya zarar görme riski daha yüksek olan bireyler) içeren çalışmalarda Editörün takdiriyle ilgili 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 olmadığını 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, Tablolara ve Şekillere 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. Yazımın 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 KELİMELELER

- 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 cümlelerin 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 cümlelerin 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:

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.

