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EDITORIAL

EDİTÖRYAL

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What is neuroplasticity? Why it is important?: Types and its basic mechanisms

Nöroplastisite nedir? Neden Önemlidir?: Türleri ve temel mekanizmaları.

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ABSTRACT

The human brain is interconnected in a plastic manner to form functional brain networks. This dynamic and flexible neuronal reorganization process is essential for an efficient regeneration process in the central nervous system, especially when it comes to memory, learning, and posttraumatic conditions. Here, we tried to define basic principles and the mechanisms underlying neuroplasticity process in the brain which serve as an important template for future studies on neurorehabilitation in neurodegenerative diseases, such as stroke, Alzheimer's Disease and traumatic brain injury.

Keywords: Activity-dependent plasticity; Developmental plasticity; Post-lesional plasticity; Long-term Potentiation; Learning; Memory

ÖZ

İnsan beyni, işlevsel beyin ağları oluşturmak için plastik bir şekilde birbirine bağlıdır. Bu dinamik ve esnek nöronal yeniden düzenleme süreci, özellikle hafıza, öğrenme ve travma sonrası durumlar söz konusu olduğunda, merkezi sinir sisteminde verimli bir yenilenme süreci için gereklidir. Bu yazıda, inme, Alzheimer Hastalığı ve travmatik beyin hasarı gibi nörodejeneratif hastalıklarda nörorehabilitasyon üzerine gelecekteki çalışmalar için önemli bir şablon görevi gören beyindeki nöroplastisite sürecinin altında yatan temel prensipleri ve mekanizmaları tanımlamaya çalıştık.

Anahtar Kelimeler: Aktiviteye bağlı plastisite; Gelişimsel esneklik; Lezyon sonrası plastisite; Uzun Vadeli Potansiyasyon; Öğrenme; Hafıza

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Introduction

Several hundred million nerve cells in the human brain are interconnected in a convergent and divergent manner to form functional brain networks [1]. However, even in adults, these networks are not rigidly organized but show a dynamic reorganization process. This is especially important in the regeneration/reorganization process during the learning process or after brain trauma which is called neuroplasticity [2].

Basics

Contrary to the traditional concept inferring that CNS neurons are post-mitotic cells and exert no division and axonal growth, studies in the last decade focusing mainly on brain trauma patients have suggested that the human nervous system has a unique growth regeneration potential [3].

These findings opened a new window suggesting a dynamically organized nervous system. Today, this paradigm shift away from the statically organized nervous system is a fundamental principle of how the CNS responds to trauma and the learning process. For instance, the neuroplasticity process plays a pivotal role in functional improvements in neurorehabilitation, which say that rehabilitation might optimize functional recovery through modulating the neuroplasticity [4]. In addition to learning and trauma processes, neuroplasticity also plays an essential role during neurodevelopment by modulating the convergent organization of nerve cells in the embryonic period, which are then fine-tuned under environmental influence stimuli until the early adolescence period. This "vulnerable phase" is critical in the context of many neuropsychiatric diseases, such as early childhood traumas [5]. The second form of neuroplasticity is activity-dependent plasticity, which is related to hippocampal reorganization during the learning and memory process [6]. Here, the hippocampus plays a critical role in regulating the neuronal network and shaping the general framework of neurocognitive processes. Another activity-dependent neuroplasticity process depends on the sensorimotor cortex, which shows a rapid cortical reorganization process during motor learning. For example, in violin players, finger movements training leads to a dynamic cortical reorganization that can not be explained with the classical model of somatotopia, suggesting that the classical map-like representation of the body surface is dynamically overlapping [7]. The third form of neuroplasticity is evident when the neuroplasticity process is functionally effective beyond the traditional limits ("cross-modal plasticity"). This phenomenon is not restricted to one sensory modality but also use stimuli from other modalities to become functionally effective beyond the classical rehabilitation limits [8]. The most impressive example for cross-modal plasticity is the visual cortex's potential in overtaking the processing of tactile stimuli in blind people [9].

In contrast to trans-modal plasticity, post-lesional plasticity is observed after peripheral or central nervous system injury [10]. Hence, this process is classified in another category and includes a maladaptive neuroplasticity process: The failure to use the injured parts of the body can lead to significant deterioration in function, which is related to the dysregulation in the size of the cortical representations (phantom phenomena) [11]. However, the good news is that this period is open to applying novel neurorehabilitation and training paradigms so that the cortical representation can be increased and neurological function improved [10].

Mechanisms

As mentioned above, the central part of neuroplasticity is synaptic regulation. This includes the reversal of the strengthening and weakening of other synaptic connections, essential for a flexible neuronal reorganization. This process's electrophysiological correlates are called LTP (longer-lasting potentiation) [12] and long-term depression (LTD) [13], which describes strengthening and weakening of synaptic connections, respectively. Shortly, these mechanisms are mainly responsible for very rapid (minutes, hours) and long-lasting neuroplasticity processes (over weeks and months) [14], requiring the expression of genes and new synthesis of proteins and neurotrophins, such as BDNF [15] for permanent structural changes in the synapses and specific brain areas.

Summary

The human brain is plastic. In other words, it is not statically but dynamically organized. This astonishing potential of the brain is not only active during the development (development plasticity) where it adapts its function and structure to the environment but also during motor training and cognitive learning, which lead to a divergent reorganization process (activity-dependent neuroplasticity). Also, after peripheral or central nervous system injuries, there is a functional and structural reorganization (post-lesional plasticity) of neuronal networks. Specific mechanisms of neuroplasticity include short-term synaptic and long-term structural changes in the CNS. These flexible plasticity periods enable us to apply modern neurological rehabilitation strategies to optimize neuroplasticity and improve neurological function.

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RESEARCH ARTICLE

ARAŞTIRMA

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Determination of effects of chemical agents on liver fibrosis models frequently used in different doses and time periods

Karaciğer Fibrozis Modellerinde Sık Kullanılan Kimyasal Ajanların Farklı Doz ve Zaman Dilimindeki Etkilerinin Belirlenmesi

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ABSTRACT

Aim: In this study, it was aimed to reveal a more effective model depending on the dose and time by evaluating histopathological properties and biochemical parameters, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, triglyceride, cholesterol in carbon tetrachloride and thioacetamide (CCl_4 and TAA) models.

Method: Rats were divided into three groups for each model and intraperitoneally (i.p.) injected with CCl_4 (0.5 ml/kg, 1.0 ml/kg, 2.0 ml/kg) and TAA (100 mg/kg, 200 mg/kg, 300 mg/kg) for 4, 6 and 8 weeks, three times weekly, respectively.

Results: In the biochemical investigation, ALT and AST values in the only 0,5 ml CCL4 of groups for 6 and 8 weeks and were found to have significant differences compared to the control groups ($p < 0.05$), while the other biochemicals parameters values did not reveal significant difference in the groups ($p > 0.05$). According to the results of the histopathology in the liver tissues, both the control groups showed a normal histological feature. The hepatofibrotic alterations were remarkable in the CCl_4 and TAA models fibrosis depending on the increasing dose and time in all of the groups.

Conclusion: Our results showed that the dose and time were reached up to until the cirrhosis for eighth week. These results would be a helpful reference for hepatofibrotic studies.

Keywords: TAA, CCl_4, Liver, Fibrosis

ÖZ

Amaç: Bu çalışmada, karbon tetraklorür ve tiyoasetamid (CCl_4 ve TAA) modellerinde alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), albümin, trigliserit ve kolesterol gibi biyokimyasal parametreler ve histopatolojik özellikler değerlendirilerek doz ve zamana bağlı olarak daha etkin modelin ortaya çıkarılması amaçlanmıştır.

Yöntem: Her bir model için ratlar 3 gruba ayrılmıştır ve intraperitoneal (i.p.) olarak CCl_4 (0.5 ml/kg, 1.0 ml/kg, 2.0 ml/kg) ve TAA (100 mg/kg, 200 mg/kg, 300 mg/kg) 4, 6 ve 8 hafta boyunca hafta da üç kez enjeksiyon yapılmıştır.

Bulgular: Biyokimyasal araştırmalar sonucunda ALT ve AST değerleri, sadece 0,5 ml CCL_4 6. ve 8. hafta gruplarında kontrol gruplarına göre istatistiksel olarak anlamlı fark göstermiştir ($p<0.05$). Diğer biyokimyasal parametrelerin değerleri kalan gruplar arasında anlamlı farklılık göstermemiştir ($p>0.05$). Histopatolojik sonuçlara bakıldığında karaciğer dokusunda, kontrol gruplarının her ikisinde de karaciğer, nor-mal histolojik yapısını göstermiştir. Diğer bütün gruplarda, artan zaman ve doza bağlı olarak her iki modelde göze çarpan hepatofibrotik değişiklikler gözlemlenmiştir.

Sonuç: Doz ve zamana bağlı olarak sekizinci haftaya ulaşan gruplarda siroz geliştiği gözlemlenmiştir. Bu sonuçlar hepatofibrotik çalışmalar için yarayışlı birer referans olabilecektir.

Anahtar Kelimeler: TAA, CCl4, Karaciğer, Fibrozis.

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INTRODUCTION

Fibrosis is defined as excessive collagen regulation due to only minor clinical symptoms or new fiber formation that causes disruption in cell function [1]. Liver fibrogenesis is the ultimate common consequence of liver damage, a critical factor leading to liver dysfunction, and may be important in the pathogenesis of other chronic problems, portal hypertension [2] and biliary cirrhosis [3,4]. Hepatic fibrosis is a disease characterized by the accumulation of the extracellular matrix (ECM) following liver damage and can be treated by early diagnosis. If the damage to the liver is acute or with limited destruction, it can return to its normal structure. In case of ECM accumulation, it becomes permanent by replacing it with the parenchyma for wound treatment. This process results in cirrhosis in case of advanced fibrosis, with a high mortality rate [5]. Experimental animal models and cell culture methods will be helpful in understanding potential reversal of hepatic fibrosis and the mechanism underlying the activation of hepatic stellate cells [6]. CCL_4 and TAA are the most commonly used chemical agents in fibrotic studies, due to the fact that they are easy to apply and reproducible [7]. CCL_4 is metabolized by cytochrome P450 enzymes in the liver and converts to the highly reactive trichloromethyl (CCl_3), therefore it causes inflammation and fibrosis in the liver. The chronic application of CCl_4 has long been one of the most widely accepted models of acute-onchronic liver failure (ACLD), although it can also be used in shorter protocols for acute liver injury studies. In general, CCl_4 is administered to rats or mice through intraperitoneal injection or inhalation [8]. TAA is the second most-used model of hepatotoxin-induced ACLD after the CCl_4 and it has recently been used frequently, in both mice and rats. TAA is an organosulfur compound used in textile, paper, leather production and laboratories. It causes chronic liver damage as it affects protein synthesis, RNA, DNA and gamma glutamyl transpeptidase activity [9]. In this study, by inducing at the same time two different chemotoxins, CCl_4 and TAA, in animal models, it was possible to observe liver pathological features.

MATERIAL AND METHODS

Experimental animals

All animal procedures and experimental protocols were approved by the Experimental Animals Ethics Committee of Erciyes University, Turkey (12/89-12/08/2012). In total, 72 male Wistar-Albino rat of about 8 to 10 weeks of age, with an average body weight of 200 to 250 g, were procured from Laboratory Animal Unit of Experimental and Clinical Research Center of Erciyes University. They were held under controlled conditioning (25±1 °C constant temperature, 55% relative humidity, 12 h dark/ light cycles), while food and water were allowed ad libitum during the study period. The rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment.

Treatment Schedule of Chemical Agencies

Properties of the Control Groups

Eighteen (18) rats were randomly divided into two groups as control groups.

Group 1: negative olive oil control group (n=9), 9 rats were randomly divided into three groups: Group 1.1. (n=3) olive oil was injected i.p. (intraperitoneal) three times a week for four weeks, Group 1.2. (n=3) olive oil was injected i.p. three times a week for six weeks and Group 1.3. (n=3) olive oil was injected i.p. three times a week for eight weeks.

Group 2: negative saline solution control group (n=9), 9 rats were randomly divided into three groups: Group 2.1. (n=3) saline solution was injected i.p. three times a week for four weeks, Group 2.2. (n=3) saline solution was injected i.p. three times a week for six week and Group 2.3. (n=3) saline solution was injected i.p. three times a week for eight weeks.

Properties of the CCL_4 Groups

Group 3: (n:9); 9 rats were randomly divided into three groups: Group 3.1. (n=3) 0.5 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for four weeks, Group 3.2. (n=3) 0,5 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for six weeks and Group 3.3. (n=3) 0,5 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for eight weeks.

Group 4: (n:9); 9 rats were randomly divided into three groups: Group 4.1. (n=3) 1 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for four weeks, Group 4.2. (n=3) 1 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for six weeks and Group 4.3. (n=3) 1 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for eight weeks.

Group 5: (n:9); 9 rats were randomly divided into three groups: Group 5.1. (n=3) 2 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for four weeks, Group 5.2. (n=3) 2 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for six weeks and Group 5.3. (n=3) 2 ml/kg CCl_4 in 20% olive oil was injected i.p. three times a week for eight weeks.

Properties of TAA Groups

Group 6: (n:9); 9 rats were randomly divided into three groups: Group 6.1. (n=3) 100 mg/kg TAA was injected i.p. three times a week for four weeks, Group 6.2. (n=3) 100 mg/kg TAA was injected i.p. three times a week for six weeks and Group 6.3. (n=3) 100 mg/kg TAA was injected i.p. three times a week for eight weeks.

Group 7: (n:9); 9 rats were randomly divided into three groups: Group 7.1. (n=3) 200 mg/kg TAA was injected i.p. three times a week for four weeks, Group 7.2. (n=3) 200 mg/kg TAA was injected i.p. three times a week for six weeks and Group 7.3. (n=3) 200 mg/kg TAA was injected i.p. three times a week for eight weeks.

Group 8: (n:9); 9 rats were randomly divided into three groups: Group 8.1. (n=3) 300 mg/kg TAA was injected i.p. three times a week for four weeks, Group 8.2. (n=3) 300 c mg/kg TAA was injected i.p. three times a week for six weeks and Group 8.3. (n=3) 300 mg/kg TAA was injected i.p. three times a week for eight week.

The blood samples were collected from the heart of every 3 animals at the end of the fourth, sixth, eighth weeks for all groups, which were then sacrificed. Blood samples were used for biochemical investigation and following sacrification, liver tissue was removed and examined for histological parameters.

Evaluation of Serum Biochemical Analysis

Serum was separated by centrifugation (3000xg, 15 min) following clotting of the blood. Serum AST, ALT, albumin, cholesterol and triglyceride levels were determined using a Cobas 8000 (Erciyes University)

Evaluation of Histopathological Parameters

Tissues samples were fixed in neutral 10% buffered formalin (pH 7.2) at room temperature. After fixation, tissues were dehydrated through graded alcohol solutions and embedded in paraffin. Sections (5 µm thickness) were stained with Masson trichrome and examined under a light microscope (Zeiss Axiolab) for histopathological analysis. The degree of fibrosis of liver sections was graded numerically based on the following criteria: 0, no fibrosis; I, slight fibrosis, fibrosis located in the central liver lobule; II, moderate fibrosis, widened central fibrosis; III, severe fibrosis, fibrosis extended to the edge of liver lobule; IV, liver cirrhosis.

Statistical Analysis: Compliance with the normal distribution of data and variance homogeneity were assessed by the Shapiro-Wilk and Levene test, respectively. Comparisons between groups were evaluated using the Kruskal-Wallis H tests and one way variance analysis. Multiple comparisons were evaluated using the Tamhan T2 and Siegel-Castell tests. Data analyses were evaluated using the IBM SPSS Statistics 20.0 commercial package programs (IBM Inc., Chicago, IL, USA) and significance level was assumed at roughly $p<0.05$, $p<0.001$.

RESULTS

Comparisons of Final Body

Throughout the experiments, the body weight of most treated animals decreased regularly. The animals started to die from the 4th week of treatment and continued to do so until the last day of cessation of observation. In total, 12 rats died during the whole observation period, 3 died after the cessation of 1 ml/kg CCl_4 at the end of 6th week and 6 died after the cessation of 2 ml/kg CCl_4 at the end of 4th week. Three died after the cessation of 300 mg/kg TAA at the end of 6th week. After the injection of chemical agents, the maximum weight loss was observed in group 5.1.

among the experimental groups. But the weight loss was found to be significantly decreased in group 3.2., group 4.1., group 6.2. and group 7.3 when compared with the control groups ($p<0.05$). In addition, the body weight changes were significantly different between CCl_4 and TAA model ($p<0.05$) (Table.1).

Table 1. The average weight changes in control and experiment groups

Groups	Initial weight (g) Mean ± SD	Weight after treatment(g) Mean ± SD	Body weight change (g)
1.1	223.6 ± 0.5	225.3 ± 0.5	+2
1.2	225.3 ± 1.5	227.0 ± 2.0	+2
1.3	225.6 ± 1.5	227.6 ± 1.5	+2
2.1.	222.3 ± 2.5	223.6 ± 2.0	+1
2.2.	222.0 ± 1.0	223. 6 ± 1.1	+1
2.3.	225.0 ± 1.0	226.6 ± 0.5	+1
3.1.	235.6 ± 2.0	232,3 ± 2.3	-3
3.2.	242.0 ± 2.0	237.3 ± 2.5	-5
3.3.	247.0 ± 1.0	241.0 ± 1.0	-6
4.1.	245.0 ± 1.0	240.3 ± 1.5	-5
4.2.	247.6 ± 0.5	241.0 ± 1.0	-6
5.1.	249.3 ± 0.5	241.0 ± 1.0	-8
6.1.	247.6 ± 0.5	244.0 ± 2.0	-3
6.2.	248.0 ± 1.0	243.0 ± 1.0	-5
6.3.	247.6 ± 0.5	241.0 ± 1.0	-6
7.1.	244.6 ± 0.5	241.3 ± 0.5	-3
7.2.	246.3 ± 0.5	243.0 ± 1.0	-3
7.3.	248.3 ± 0.5	243.6 ± 1.5	-5
8.1.	249.0 ± 1.0	245.6 ± 1.1	-4
8.2.	249.0 ± 1.0	242.3 ± 1.5	-7

Values are expressed as n (%) ($p<0.05$).

Comparisons of Serum Biochemistries

The serum albumin, cholesterol and triglyceride levels were increased by two chemotoxins injections including TAA and CCl_4 at all of the experimental groups, but this increase was not significantly compared with the control groups ($p>0.05$) and it was not significant among of the experimental groups either ($p>0.05$).

Both the serum ALT and AST were markedly increased at the end of the fourth week, after cessation of CCl_4. ALT and AST levels at group 3.2. that experimental group of CCl_4 model were significantly increased, compared with control groups ($p<0.05$). By the 6th week following cessation of TAA with group 6.3., the serum ALT was also markedly increased and this notable increase was found significantly different, compared with the control groups ($p<0.05$). AST levels at group 7.3. increased in comparison to the control groups but this increase was not found statistical significantly among them and the experimental groups ($p> 0.05$). AST levels at the groups 8.1 and 8.2 were increased compared to the control groups and this increase was found statistically significantly different ($p<0.05$). ALT levels at the other TAA groups except for group 7.3. were not found statistical significantly compared to the control groups. The level of serum ALT and AST are shown in Table. 2.

Table 2. Changes of serum ALT and AST levels and ± SD

Groups	n	AST (U/L)	ALT (U/L)
1.1.	3	88±1.5	42±1
1.2.	3	88±1	43±1
1.3.	3	92±2	70±1.5
2.1.	3	97±1.1	53±1.5
2.2.	3	97±2	66±0.5
2.3.	3	67±5.2	54±1.5
3.1.	3	102 ±9.5	75.6±7
3.2.	3	240± 138	210±133
3.3.	3	151±31	156±20.1
4.1.	3	131±7.6	117±3
4.2.	3	130±4.5	112±3.2
5.1.	3	211±3.2	142±37.4
6.1.	3	86±10.1	64±8
6.2.	3	121±10.2	67±9
6.3.	3	73±4.7	61±8.5
7.1.	3	88±4.5	63±12
7.2.	3	88±14.1	70±9.2
7.3.	3	146±43.5	86±8.5
8.1.	3	106±3.5	68±10.4
8.2.	3	129±34.7	78±10.4

Values are expressed as n (%) ($p<0.05$).

Histopathological Changes

The masson trichrome-stained histopathological appearance revealed normal hepatocytes morphology and intact hepatic lobules architecture in untreated control rats. According to the histopathological findings, liver tissue of normal control groups exhibited normal parenchymal structure features and normal architecture of hepatocytes radiating chord from the central vein (Figure 1).

CCl_4 intoxicated rats showed that collagen

deposition accumulates around the vena centralis, portal areas and blood vessels. Due to increased of CCL4 dose, the degree of fibrosis was increased as well. In addition to fibrosis, it was observed in notable necrosis and inflammation (Figure 2).

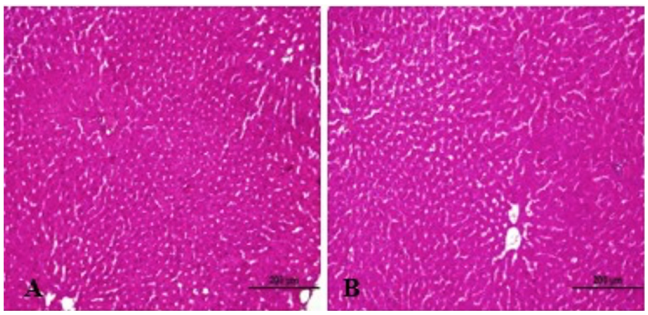

Figure 1: Section of liver obtained from control groups; group1 and 2: Group 1, no marked pathological changes A; group 2, no marked pathological changes and B; (×20).

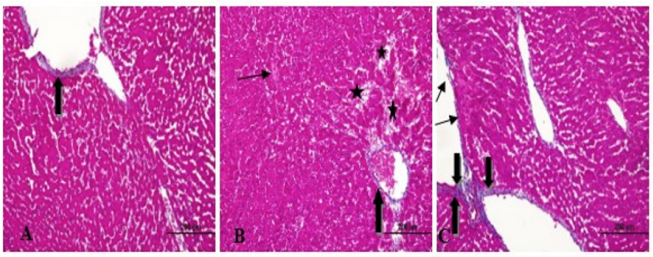

Figure 2: Liver histopathology of CCl_4-treated rats: Group 3.2. fibrosis (black thick arrows presented the fibrosis) tissue can be seen, it extended to the edge of liver lobule A; group 4.1. hemorrhagic necrosis (black thin arrows presented the necrosis) and inflammation (stars shapes presented the inflammation) B; group 5.1. necrosis, inflammation (black thin arrows presented the necrosis) and wide infiltration of inflammatory cells around the central veins (black thick arrows presented the fibrosis) C; (×20).

TAA intoxicated rats showed a higher degree of fibrosis and hepatic damage compared to the CCl_4 groups. Disruption of hepatic cell cord and infiltration of inflammatory cells were observed. Increased vacuolization and acidophilus bodies in the cytoplasm were also seen in the liver section.

The CCl_4 and TAA group showed notable bridging necrosis, inflammation and wide infiltration of inflammatory cells, around the central veins. From the masson trichrome staining, fibrotic changes (Figure 3) were most pronounced in the TAA group.

Each sample of models of CCl_4 and TAA showed enlarged portal tracts and severe fibrosis deposition. Compared with model CCl_4, liver cirrhosis IV and fibrosis III were apparent respectively in 13 and 11 of 24 samples in model TAA. Fibrosis III and liver cirrhosis IV were apparent respectively in 6 and 11 of 18 samples in model CCl_4. The fibrosis scores of liver sections for both CCl4 and TAA models are shown in Table 3.

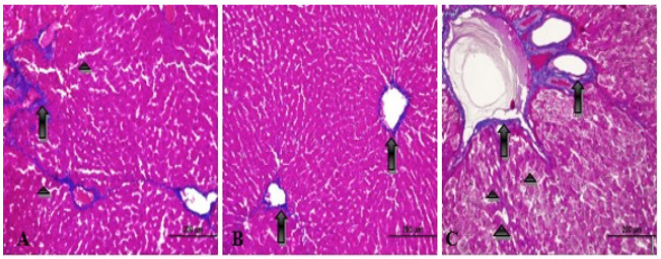

Figure 3: Liver histopathology of TAA-treated rats: Group 6.1. fibrosis (triangle shape presented vacuolization and acidophilus bodies in cytoplasm) tissue can be seen, it extended to the edge of liver lobule fibrosis (black thick arrows presented the fibrosis) A; group 7.1. hemorrhagic necrosis and inflammation B; group 8.2. triangle shape presented vacuolization and acidophilus bodies in cytoplasm and black thick arrows presented the fibrosis C; (×20).

Table 3. Histopathological semiquantitative scores of collagen deposition in the liver

Groups	n	0	I	II	III	IV
Control Groups	18	18	0	0	0	0
CCl_4 Model	18	0	0	1(5.6)	6(33.3)	11(61.1)
TAA Model	24	0	0	0	11(45.8)	13(54.2)

Values are expressed as n (%) ($p<0.001$).

The results showed that the degree of fibrosis scores were markedly increased on fourth, sixth and eighth weeks after cessation of CCl_4 and TAA. Fibrosis III and IV degrees, compared with the control group, were significantly increased and there were also marked differences between the two models ($p<0.001$).

DISCUSSION

Various animal models have been developed for liver diseases; fibrosis, CCl_4 and TAA are chemotoxin models [10,11]. Because of their applicability and reproducibility, they are the most commonly used chemotoxin models for inducing liver fibrosis [12]. Differences in the etiology and fibrosis degrees between these models have been reported in previous studies [13,14]. However, there are very few studies comparing the comprehensive properties of the two fibrosis models simultaneously [7]. Due to the dissimilar characteristics of chemotoxins, they were injected

intraperitoneally at different doses for different periods. In both models, fibrous septa transitions were seen around portal triads, and scoring showed severe fibrosis and liver cirrhosis in III and IV, respectively. These two hepatotoxic agents are known to be agents that increase oxidative stress causing damage to hepatitis [15]. ALT and AST values were elevated that inducing with CCl_4 forming the most potent free radical. Liver enzymes are markers of inflammation in hepatic damage [16]. These enzymes including ALT and AST have been elevated with chemotoxins. Both the serum ALT and AST were markedly increased at the end of fourth week after cessation of CCl_4 0,5 ml and also six rats have been seen severe fibrosis histopathologically at CCl_4 groups. At the end of the 6th and 8th weeks and group 5.1, it was observed that 11 rat had cirrhosis at these groups. ALT and AST enzyme levels have been elevated in TAA model. Although not as high as in CCl_4 groups, the number of rats that turn into cirrhosis is higher in this model. Histopathologically severe fibrosis was most common seen at group TAA 300 mg. At the both of models was seen liver enzymes increasing at the end of sixth week. It was observed pathologically differences for each model. This is because each chemotoxin has different properties. In addition, it has been shown that caused by the differences between the histological properties of liver effected, oxidative stress, liver enzymes and fibrotic changes. Among these models, it was observed that 300 mg TAA group had cirrhosis and 100 mg TAA group had severe fibrosis in the short term. In the CCl_4 model, it was seen histopathological changes which fibrosis characteristics on the 6th week at 0.5 ml CCl_4 group. When the other groups were examined, it was observed that the deaths due to the high doses and frequency of administration. In this study, 0.5 ml CCl_4 and 100 mg of TAA groups were found to be suitable to create an experimental animal model.

Limitation of Study: This study was pilot study. The small number of rats is an important limitation, in particular for the evaluation of the analysis of both groups. For more reliable results of each group, they should be examined in large-scale studies. We believe that this study should be a guide for larger ones to be carried out in the future.

CONCLUSION

In this study, it is provided to test the compounds that can be used as therapy for fibrosis or to interpret the background to evaluate fibrosis content. It will be a useful reference by saving time for researchers in the process of creating fibrosis model using animal models.

Conflict of Interest: The author has no conflict of interest related to this article.

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RESEARCH ARTICLE

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The Investigation of Endomyocardial Biopsy Results, Plasma pro-BNP Levels and Non-invasive Parameters for Diagnosing of Acute Rejection in Patients Who Undergo Cardiac Transplantation

Kalp Transplantasyonu Yapılan Hastalarda Akut Rejeksiyon Tanısında Endomiyokardiyal Biyopsi Sonuçları, Plazma pro-BNP Seviyeleri ve Non-invazif Parametrelerin Araştırılması

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ABSTRACT

Aim: Heart failure is a disease with high mortality and morbidity, reducing the patient's quality of life. Each year 10% of heart failure patients progress to end-stage heart failure. Cardiac transplantation is the gold standard treatment method in these patients, however acute rejection is the most important factor affecting the success of this treatment. In this study, it was aimed to evaluate endomyocardial biopsy results, serum pro-BNP and non-invasive parameters in patients with acute rejection following cardiac transplantation.

Patients and Methods: Twenty patients who underwent cardiac transplantation in our center were included in the study. The patients were divided into two groups, namely acute rejection (n: 10) and without rejection (n: 10). Echocardiography, electrocardiography (ECG), endomyocardial biopsy results, serum reactive proteins (CRP), sedimentation rate and serum pro-BNP levels, were evaluated among the patients and compared between the groups.

Results: Endomyocardial biopsies obtained from patients with acute rejection revealed grade 1 rejection in 6 (60%) patients, grade 2 in 3 (30%) patients and grade 3 rejection in 1 (10%) patients. CRP and sedimentation rate were found to be similar between the groups (p> 0.05). High pro-BNP levels were found in patients with rejection (4843.20 ± 6690.10 pg / ml) when compared to the control group (496.30 ± 216.20 pg / ml) (p: 0.001). In addition, higher pro-BNP levels were detected with progressing of rejection grade (p: 0.03). The highest pro-BNP level was found in a patient with Grade-3 rejection (15211 pg / ml, p: 0.000).

Conclusion: Our results show that serum pro-BNP levels are associated with acute rejection. In addition, higher pro-BNP levels were found to be associated with advanced rejection levels.

Key Words: Cardiac transplant, acute rejection, endomyocardial biopsy, pro-BNP

ÖZ

Amaç: Kalp yetersizliği, mortalite ve morbiditesi yüksek olan hastanın yaşam kalitesini düşüren bir hastalıktır. Kalp yetmezliği hastalarının her yıl %10'u son dönem kalp yetmezliğine ilerlemektedir. Bu hastalarda kalp nakli altın standart tedavi yöntemidir. Ancak nakil hastalarında akut rejeksiyon tedavinin başarısını etkileyen en önemli faktördür. Bu çalışmada kalp nakli sonrası akut rejeksiyon olan hastalarda endomiyokardial biyopsi sonuçları, serum pro-BNP ve non-invazif parametrelerin değerlendirilmesi amaçlanmıştır.

Hastalar ve Yöntem: Merkezimizde kalp nakli yapılan 20 hasta çalışmaya dâhil edil-erek, akut rejeksiyon gelişen (n:10) ve gelişmeyen (n:10) olarak iki gruba ayrılmıştır. Hastalar arasında ekokardiyografi, elektrokardiyografi (EKG), endomiyokardiyal bi-yopsi sonuçları, serum reaktif protein (CRP), sedim ve serum pro-BNP seviyeleri değerlendirilmiş ve gruplar arasında karşılaştırılmıştır.

Bulgular: Akut rejeksiyon gelişen hastalardan alınan endomiyokardiyal biyopsilerde 6(%60) hastada grade 1, 3(%30) hastada grade 2, 1(%10) hastada grade 3 rejeksiyon bulguları, CRP ve sedimantasyon oranı gruplar arasında benzer olarak saptanmıştır (p>0.05). Rejeksiyon olan hastalarda (4843,20 ± 6690,10 pg / ml) kontrol grubu ile karşılaştırıldığında (496,30 ± 216,20 pg / ml) yüksek pro-BNP seviyeleri tespit edilmiştir (p:0,001). Bunun yanı sıra rejeksiyon grade ilerledikçe daha yüksek pro-BNP seviyelerine ulaşılmıştır (p:0,03). Bu bağlamda grade-3 rejeksiyon olan hastada en yüksek pro-BNP düzeyi bulgusu elde edilmiştir (15211 pg / ml, p:0,000).

Sonuç: Bulgularımız serum pro-BNP düzeylerinin akut rejeksiyon ile ilişkili olduğunu göstermiştir. Ayrıca daha yüksek pro-BNP düzeyleri ile rejeksiyon seviyeleri ilişkili olarak bulunmuştur.

Anahtar Kelimler: Kalp nakli, akut rejeksiyon, endomiyokardiyal biyopsi, pro-BNP

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INTRODUCTION

Heart failure (HF) disrupts quality of life and progresses to higher mortality and morbidity rates. Despite the advanced medical treatment strategies and recently developed assisting devices, the prognosis of HF is still quite negative [1,2]. HF is a progressive disease that has an undesirable clinical course that cannot be recovered to previous condition, and every hospital consultations with acute exacerbation result in a decreased survival rate. Timely diagnosis and controlled treatment may delay quick progression and the bridging of treatment strategies can provide additional time to heart transplantation, which is the gold standard treatment for end stage HF. However, in the end, these patients require transplantations [2-4].

Heart transplantation provides effective cardiac functions if patients pass through the postoperative period uneventfully. The main issue following heart transplantation is acute organ rejection, which occurs during the first year of transplantation. The next process following the first year is related to immunosuppressive treatments, which can result in infections, hypertension, renal failure, malignancies and transplant vasculopathy. Five-year survival rates were reported at 70–80% in patients with heart transplantation that received a triple immunosuppressive regimen [2]. The endomyocardial biopsy sample evaluations, echocardiographic, electrocardiographic examinations and serum blood parameters are important for follow up, during the postoperative first year [5,6].

The natriuretic peptides were reported as a pathological neurohormonal activation of various disorder, also especially in disrupted cardiac functions. B-type natriuretic peptide or brain natriuretic peptide (BNP) was reported as a biomarker for acute cardiac events. Acute loading condition related with increased ventricular wall tension, leads to pro-BNP releasing. Therefore, this biomarker was investigated for different kinds of cardiovascular diseases such as pulmonary hypertension, cor pulmonale, ventricle hypertrophy, overload situations in circulation, hypertension, acute coronary syndrome, atrial fibrillation, etc. [7].

In this study we aimed to evaluate the echocardiography, electrocardiography (ECG), endomyocardial biopsy results, serum reactive protein (CRP), sedimentation rate, serum pro-BNP and biochemical parameters in heart transplant patients with acute rejection. Thereafter we compared the results with heart transplantation patients undergoing a healthy progression.

MATERIAL and METHOD

Study Design and Patient Selection

After obtaining an ethical approval from the Human Ethical Committee of Akdeniz University (Approval No.2011,06-82-107/87), a retrospective analysis for cardiac transplantation cases was designed. Thirty patients who underwent cardiac transplantation in cardiovascular surgery clinic of Akdeniz University School of Medicine were recorded retrospectively. Patients who had regular periodical endomyocardial biopsies, simultaneous echocardiography and electrocardiography follow-up records and regular blood parameters follow-up records [cytomegalovirus (CMV) antigen test, sedimentation, C-reactive protein (CRP) test, troponin and pro-BNP (Brain natriuretic peptide) tests], were included in the study. Patients without regular control tests and under treatment due to an active infection, were also excluded from the study.

Following the inclusion and exclusion criteria, a total of twenty patients were included in the study. Demographic findings, echocardiography and electrocardiography results as well as endomyocardial biopsy reports, were evaluated. Thereafter, patients were divided into two groups, based on biopsy reports of 6th month endomyocardial biopsy samples, as the tissue rejection (study) group (n: 10) and the normal biopsy group (n: 10).

Clinical Management

All cardiac transplant patients received triple immune-suppressive treatment in the early postoperative period. This regimen includes calcineurin inhibitors (cyclosporine or tacrolimus) + mycophenolate mofetil (MMF) or Everolimus + oral prednisolone treatments. The drug dosages were determined as follows: Cyclosporine: 2.5

or 5 mg/kg/day (per oral: PO), Tacrolimus: 5 mg twice daily (PO), MMF: 0.5 or 1 gr twice daily, Everolimus: 0.75 or 1.5 mg twice daily (PO). The endomyocardial biopsies (EMB) were taken as follows: one fifteen days during the postoperative 2nd months, one a month during the postoperative 2 to 6th months, one a year after postoperative 6th month. We evaluated the 6th month biopsy results in our study.

The EMB was taken from the right ventricle through the catheterizing right jugular vein. After placement of 8 F introducer sheath to the right jugular vein, the 7 F EMB forceps (Sparrow Hawk®) was progressed into the sheath. After progressing forceps tip (biotom) to the right atrium laterally, biotom was progressed with 90 degrees of rotation for passing through the tricuspid valve and the biotom was directed to the right ventricle apex. When reaching to selected areas 4 to 6 pieces of the sample with a size of 3x2x2 mm were collected with biotom. All collected samples were stored at 10% formalin and 4% buffered formalin solutions and send to pathological examination.

The EMB samples obtained were classified according to 2004 ISHLT (the International Society for Heart and Lung Transplantation) grading guidance, as follows: Grade 0: Non-rejection, Grade 1: Mild rejection, Grade 2: Moderate rejection, Grade 3: Severe rejection [8].

The M-mode and two-dimensional evaluation were made by echocardiography (GE VIVID®). The left ventricle mass and pericardial effusion were evaluated and ejection fraction (EF) and pulmonary artery pressure (PAP) were measured. In the echocardiogram evaluations, the following findings were accepted in favor of rejection: more than 4 mm thickening in the interventricular septum and posterior wall, increased myocardial echogenicity, newly developed pericardial effusion or increment of previous effusion, more than 20% percent decrease in halving time of mitral valve flow pressure, more than 20% decrease in isovolumetric relaxation time of left ventricle (LV), and more than 10% decrease in LV ejection time [9,10].

In the electrocardiogram evaluations, the following findings were accepted in favor of rejection: newly developed arrhythmia, prolonged P wave, more than 20% decrease in QRS voltage of chest derivations, more than 2 mm of ST variances and T wave negativity.

Simultaneous CMV antigen and CRP tests, troponin and pro-BNP were evaluated. The pro-BNP evaluation was made from 10 ml of antecubital vein blood samples stored in EDTA containing tubes. The samples were centrifuged at 1500 rpm for 5 minutes under +4 degree and the top phase plasma levels of the centrifuged samples were taken to another tube. NT-proBNP levels were measured with the electrochemiluminescence immunoassay method and commercially available kits (Roche Diagnostics, Indianapolis, Indiana), by using the Elecsys® 1010 autoanalyzer device from these samples.

Statistical Analysis

The statistical analyses were made using a software program (SPSS for windows v.18). The continuous variables were analyzed using an independent T test, and the categorical variables were compared using the Chi-Square test. The Mann-Whitney U tests were performed for the comparison of groups. The $p<0.05$ results were considered as statistically significant.

RESULTS

Demographical and clinical findings

Twenty patients who underwent heart transplantation were included to the study. The patients were divided into two groups, one with rejection (Study group, n:10) and one without rejection (Control group, n:10). The demographical findings of the two groups were reported in Table 1.

The groups were similar in regards to age, gender, accompanying disease, postoperative drug usage (Cyclosporine, Tacrolimus, Mycophenolate Mofetil (MMF), Everolimus) ($p>0.05$). Similarly, there was no difference between the follow-up periods of the two groups ($p>0.05$).

The higher rejection rates were found in patients with 0 (Rh+) blood groups when compare with control subjects (p: 0.03). The distribution of blood groups in heart transplant patients was summarized in Table 2. The symptoms were found

as follows: 5 (50%) non-specific, 2 (20%) with cardiac irritation, 1 (10%) with low cardiac output complaints, in patients with rejection.

Table 1. The Demographical Findings

Variable	Study Group	Control Group	**p
Gender (male,%)	9(90%)	8(80%)	0.62
Age (mean±SD), years	43.40±6.86	38.20±8.27	0.78
Follow-up, months	11.70±6.96	13.10±6.62	0.08
Diabetes (n,%)	4(40%)	3(30%)	0.70
Hypertension (n,%)	3(30%)	4(40%)	0.70
Cyclosporine (n,%)	6(60%)	5(50%)	0.65
Tacrolimus (n,%)	4(40%)	5(50%)	0.62
*MMF (n,%)	8(80%)	7(70%)	0.84
Everolimus (n,%)	2(20%)	3(30%)	0.71

*MMF: Mycophenolate Mofetil, **p: p<0.05 is considered as statistically significant

Table 2. The Distribution of Blood Groups

Blood Groups	Study Group n(%)	Control Group n(%)	P
0 (Rh +)	6 (60%)	1 (10%)	0.03
A (Rh +)	2 (20%)	4 (40%)	0.68
B (Rh +)	1(10%)	3(30%)	0.81
AB (Rh +)	1(10%)	2 (20%)	0.97

Histopathological Findings from Biopsy Samples

Biopsies performed after the 6th month of transplantation were found as a grade 1 rejection in 6 (60%) patients, a grade 2 rejection in 3 (30%) patients, and one patients (10%) with low cardiac output symptoms had a grade 3 rejection. The histopathological findings are illustrated in Image 1.

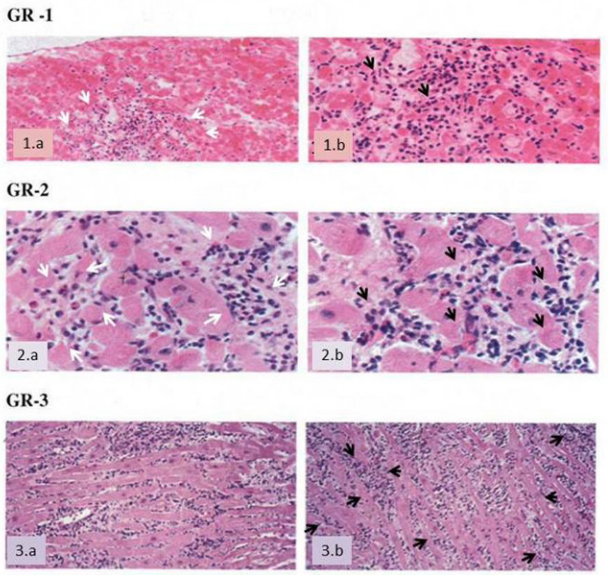
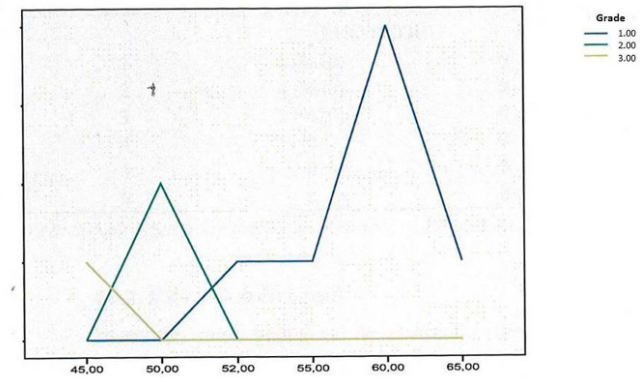


Image 1. Rejection grades in endomyocardial biopsy samples: GR1: Mild rejection; 1.a. Single foci of myocytes injury (white arrows), 1.b.Few perivascular lymphocyte infiltrations (black arrows). GR2: Moderate rejection; 2.a.More than two foci of myocytes injury are associated with at least a moderate cellular infiltrate (white arrows), increased cellular infiltration, 2.b. Increased perivascular lymphocyte infiltrations (black arrows). GR3: Severe rejection; 3 a. The diffuse myocyte injury with erased foci areas, 3 b. Widespread inflammatory cells (black arrows).

Echocardiography

Ejection fractions (EF) were found as 60.00±4.08 % and 55.70±6.27 % in the control and study groups respectively (p: 0.19). The distributions of EF values were presented in Graphic 1. Higher PAP values (38.90±4.97mmHg) were found in the study group when compared with the control group (28.60±2.63mmHg) (p: 0.05). The echocardiography score in the study group were found as score 1 in four patients with increased myocardial echogenicity, score 2 in four patients with increased myocardial echogenicity and more than 4 mm thickening in interventricular septum, score 3 in two patients with myocardial echogenicity, more than 4 mm thickening in interventricular septum and more than 10% decrement in left ventricular ejection time (p: 0.02). There was no pericardial effusion in either groups. Mild-moderate tricuspid insufficiency was detected in 5 patients with rejection.



Graphic 1. The distribution of ejection fraction in regards of rejection fraction in study group

Electrocardiography

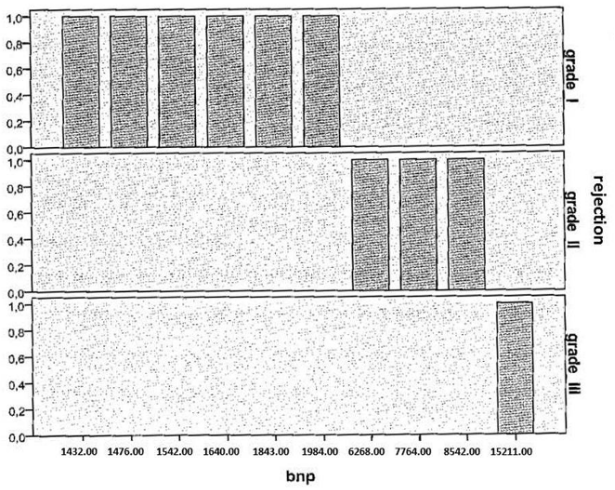
Atrial fibrillation was detected in two (20%) patients who admitted to hospital with cardiac irritation findings. The endomyocardial biopsy findings of these two patients were grade 2 rejections. An amount of 20% decrement in QRS complexes of chest derivations were found in patient with grade 3 rejection who had low cardiac output symptoms.

Additional abnormal electrocardiography finding was not detected in other patients.

Biochemical Findings

The cytomegalovirus tests were negative for all patients. The serum leukocyte numbers were found as 9.65±2.72/mm3and 8.240±1.710/mm3, in the study and control groups respectively (p: 0.19). Erythrocyte sedimentation rates were found as 24.11±8.17 mm/h and 20.32±7.14mm/h in rejection and control groups respectively (p: 0.32). In regards to CRP level, the control group (1.33±0.35 mg/L) and rejection group (3.58±1.18mg/L) were found to be similar (p: 0.11). Only one patient with grade 3 rejection had a more than 12.45 /mm3 white blood cell count (WBC). Additionally, this patient had a 32 mm/h sedimentation rate and higher CRP level (4 mg/L). Although, the troponin levels were found as 0.18±0.02 ng/ml in the rejection group, there was no statistical difference when compared with the control group's (0.09±0.05 ng/ml) troponin levels (p: 0.13).

Markedly higher pro-BNP levels were found in rejection patients (4843.20±6690.10 pg/ml) when compared with the control group's pro-BNP levels (496.30±216.20 pg/ml) (p: 0.001). Furthermore, the distributions of pro-BNP levels in regards of rejection grade in the study group were found to be statistically different (p: 0.03). The highest pro-BNP level (15211 pg/ml) was detected in a grade 3 rejection patient (p: 0.000). The distribution of pro-BNP levels in regards to rejection grades was reported with a bar graph in Graphic 2.



Graphic 2. Bar graph of pro-BNP levels in regards to rejection grades in study group.

DISCUSSION

Our results indicate that pro-BNP levels were found to be higher in acute rejection patients with heart transplantation. Moreover, higher pro-BNP levels were related to an advanced rejection grade. On the other hand, the incremental pro-BNP levels were found to be related with the impaired ventricle functions, although exact discriminative changes were detected with mainly an EMB examination.

The success of heart transplantation is evaluated based on early-mid-long term survival results and quality of life. Although the recent advances that reduce the risk of early complications (early graft insufficiency, infection, rejection) and mortality rates in recent years, the main determinant of a successful transplantation is a one-year survival rate. The early mortality and morbidity risks are related to acute rejection and infections, whereas the late risks are related with coronary artery disease and immunosuppressive treatment associated with renal insufficiency and secondary malignancies [11]. We included twenty heart transplantation patients who underwent heart transplantation and we detected ten acute rejection cases from this group. None resulted in a one-year mortality.

The rejection was classified as mild, moderate and severe in accordance with ISHLT guidelines [8]. The classification is based on EMB sample histopathological evaluations. There were six mild patients, three moderate patients and one severe patient in our series. The acute rejection can usually progress with asymptomatic or nonspecific symptoms. However, cardiac irritation or low cardiac output findings can be detected in progressed cases [12]. In a report from the Turkish population, rejection occurred in 34.2% of heart transplantation cases and EMB samples were used to follow up on progression [13].

Atrial fibrillation (AF) and atrial flutter are the most common arrhythmia types in acute rejection in heart recipient patients, with an incidence of 1% to 2% [14]. The other ECG finding is attenuation of the electrocardiographic QRS complexes for heart failure [15]. Additionally, QRS changes were reported in rejection patients [16]. We

detected AF in two rejection patients with cardiac irritation symptoms and found attenuation of the electrocardiographic QRS complexes in chest derivations of three rejection patients with low cardiac output symptoms. The echocardiographic follow up of heart transplantation depends on evaluation of ventricle functions, strain and mass calculations. Principally, left ventricle (LV) and right ventricle (RV) longitudinal strains are essential for late indication of dysfunction, but not correlating with the rejection grade [17]. Although, myocardial-injury associated graft dysfunction is related to rejection, the grade of damage should confirm with the EMB samples simultaneously. Lack of enhancement in LV ejection fraction and longitudinal strain is associated with poor outcomes after heart transplantation. Ciliberto et al. described the echocardiographic grading for rejection in patients with heart transplantation [9,10]. Additionally, the European Association of Cardiovascular Imaging (EACVI) recommended echocardiographic examination methods of heart recipients for follow up. These methods can be listed as EF, end diastolic and systolic volumes of LV, septal wall thickness, pulmonary artery pressures, pericardial effusion, RV functions, valve regurgitations and longitudinal wall strain evaluations [18]. We did EMB simultaneously with echocardiography during the sixth month. Our findings revealed that EF was similar in patients with rejection and without rejection, although higher PAP values were detected in rejection patients with higher grade. The other findings of our study was the increased myocardial echogenicity: more than 4 mm thickening of interventricular septum and more than 10% decrement in left ventricular ejection time was detected with increment of rejection grade. There was no pericardial effusion in either groups. Mild-moderate tricuspid insufficiency was detected in 5 patients with rejection.

BNP is a pathological hormonal indicator of myocardial response to ventricular stretch. It has a regulatory role for fluid homeostasis and blood pressure by inhibiting to renin–angiotensin–aldosterone system. Thereby, BNP shows cGMP activator, natriuretic, diuretic, vasorelaxant, antifibrotic, and positive lusitropic effects. Therefore, it has been studied as a biomarker in situations that negatively affect heart functions [19,20]. Normal levels of BNP can be affected by hemodynamic alterations and inflammatory reactions. In heart recipients the impaired cardiac functions and allograft vasculopathy leads to incremental serum BNP levels. Despite the higher BNP levels due to increased inflammatory response and disrupted cardiac blood supply, cardiac functions can be found as normal [19,20]. Yin et al. found decreased pro-BNP levels after heart transplantation, however they detected that this decremental values in proBNP levels were reversely affected from early reactions in acute rejection of heart recipients. They suggested that although incremental values can be detected in acute rejection patients, the diagnostic value of proBNP is low [21]. Similarly, Cuppoletti et al. found a lowering in proBNP levels by the duration, after heart transplantation. They found an inverse correlation between acute rejection and proBNP levels and a late increase of NT-proBNP values were observed in their series. Finally, they suggested that isolated proBNP levels are not useful for diagnosing acute rejection after heart transplantation [22]. In our series, proBNP levels were markedly higher in the acute rejection group and the highest proBNP value was detected in a grade 3 rejection patient. However, our sample size was lower than those of previous studies.

Limitations of Study: The main limitation of study is related to low sample size and therefore, the results of our study should be confirmed with larger series. The second limitation is concerning the follow up period. We investigated the EMB biopsies, imaging findings and blood samples at the sixth month. The late response and prolonged systemic response should be investigated after the first year of heart transplantation.

In conclusion, it seems to be that EMB is still the gold standard for evaluation of acute rejection in heart recipients. Although, an alteration of pro-BNP levels seems to be useful for follow up systemic response in acute rejection patients, findings should be confirmed with EMB invasively. The diagnostic accuracy of pro-BNP levels in acute rejection after heart transplantation should be investigated with larger series.

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Ozgur AKKAYA 0000-0001-6460-5066	The author fully involved to all stage of this study and final approved this article.

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Is enoxaparin sodium exactly safe for subcutaneous fibroblast?: A cell culture study

Enoksaparin sodyum subkutan fibroblast için tam olarak güvenli midir?: Bir hücre kültürü çalışması

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ABSTRACT

Aim: Despite relatively low amount in the subcutaneous tissue, fibroblasts play a critical role in the continuity of intercellular connections, maintenance of tissue integrity, and forming a balanced fascial network. Enoxaparin sodium is widely used in the prophylaxis and treatment of deep vein thrombosis. In the present study, we aimed to examine the cytotoxic effects of enoxaparin sodium on fibroblast cells in an in vitro model.
Material and Methods: L929 mouse fibroblast cells were treated with enoxaparin sodium 4000 IU, 2000 IU, 1000 IU, 500 IU, and 250 IU. At 24 hours, cell morphology was evaluated; cell viability was analyzed through methylthiazole tetrazolium assay and propidium iodide/ acridine orange staining was made to support changes in nuclear morphology as the sign of initial apoptosis.
Results: The test results showed that high doses of enoxaparin sodium (4000 IU, 2000 IU) exerted cytotoxic effects and induced apoptotic morphology in initial stage. Compared to the control group, there was no significant difference in the cell viability in Dilutions III, IV, and V.
Conclusion: Based on our results, despite prophylactic dose in the in vitro setting, high-dose enoxaparin showed cytotoxic effects. Long-term high-dose enoxaparin sodium may affect the number of subcutaneous fibroblasts, impairing the skin integrity and subcutaneous tissue healing

Keywords: Enoxaparin sodium, cytotoxicity, apoptosis, skin, fibroblast.

ÖZ

Amaç: Fibroblastlar, subkutan dokuda nispeten düşük miktarlara rağmen, hücreler arası bağlantıların sü-rekliliğinde, doku bütünlüğünün korunmasında ve deri fasyası ile dengeli bir iletişim oluşturmada kritik rol oynarlar. Enoksaparin sodyum, derin ven trombozunun profilaksisinde ve tedavisinde yaygın olarak kullanılmak-tadır. Bu çalışmada, enoksaparin sodyumun fibroblast hücreleri üzerindeki sitotoksik etkilerini in vitro bir mod-elde incelemeyi amaçladık.
Materyal ve metod: L929 fare fibroblast hücreleri enoksaparin sodyum 4000 IU, 2000 IU, 1000 IU, 500 IU ve 250 IU ile işleme tabi tutuldu. 24 saatte hücre morfolojisi değerlendirildi; hücre canlılığı metiltiyazol tetrazolyum deneyi ile analiz edildi ve propidium iodide/acridine orange boyamasıyla apoptozisin başlangıcını gösteren nükleer değişiklikler incelendi.
Bulgular: Enoxaparin sodyumun yüksek dozlarının (4000 IU, 2000 IU) sitotoksik etkiler yarattığı görüldü. Kontrol grubu ile karşılaştırıldığında, Dilüsyon III, IV ve V'de hücre canlılığında istatistiksel açıdan anlamlı bir fark bulunmadı.
Sonuç: Sonuçlarımıza göre, in vitro ortamda profilaktik doza rağmen, yüksek doz enoksaparin sitotoksik ve apoptotik etkiler göstermiştir. Uzun süreli yüksek doz enoksaparin sodyum, deri altı fibroblastların sayısını etkileyerek deri bütünlüğünü ve deri altı doku iyileşmesini bozabilir.

Anahtar Kelimeler: Enoksaparin sodyum, sitotoksisite, apoptoz, deri, fibroblast.

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INTRODUCTION

Low-molecular-weight heparins (LMWHs) are chemical or enzymatic depolymerization of unfractionated heparins (UFHs) with an average weight of 5,000 Da [1]. Unlike UFHs, short chains of LMWHs are less interacted with plasma proteins, directly inhibiting factor Xa [2]. In addition, as LMWHs exert less effects on platelets, the incidence of thrombocytopenia with LMWHs is relatively low than UFHs [2,3]. Since LMWHs do not contain long polysaccharide chains which inhibit factor IIa, they do not alter the partial thromboplastin with a very high absorption with subcutaneous administration, thereby, leading to a bioavailability ratio of up to 90% [2]. As the behavior of LMWHs following absorption is more predictable than UFHs, monitoring is not necessary. Although LMWHs can be administered intravenously similar to UFHs, high bioavailability ratio makes them suitable for subcutaneous administration, as well. In particular, enoxaparin sodium is an effective and safe first generation LMWH for the prevention and treatment of venous thromboembolism and for the prevention of mechanical heart valve thrombosis, as well as in specific patient populations including pregnant and cancer patients [4]. Alt-hough LMWHs indicated as safe drugs, LMWHs may lead to injection site problems such as irritation, pain, bruising, redness, and swelling. In addtion, severe skin reactions related to enoxaparin sodium can be found in the literature [5-7]. Delayed hypersensitivity reactions in the injection site are the most commonly reported reactions, although systemic reactions are rarely reported [6,7].

Although fibroblasts are overlooked due to their low amount in the subcutaneous tissue, they play a critical role in the continuity of intercellular connections, maintenance of tissue integrity, and forming a balanced fascial network, preserving the skin elasticity [8,9]. In addition, fibroblasts are essential for epithelial cell differentiation, inflammation processes, and wound healing, as they are mesenchymal-derived cells [10]. However, there is no study in the literature investigating the effect of subcutaneous enoxaparin injections on fibroblasts.

In the present study, we hypothesized that enoxaparin sodium, a LMWH, might exert toxic effects on the fibroblast cells in subcutaneous tissue. Therefore, we aimed to examine the cytotoxic effects of enoxaparin sodium on subcutaneous fibroblast cells, which are the first encountered cells in the subcutaneous tissue, in a cell culture model.

MATERIALS AND METHODS

There is no data in the literature which indicates the exact number of fibroblasts in subcutaneous tissue. However, this might be estimated according to histological preparations. In the present study, we created a cell culture model based on histology preparations [11]. There are approximately 16 fibroblast cells were counted in a 0.02 mm^2 subcutaneous section. It was estimated that there were 76,000 cells in 1 cm^2 area in vivo. In this study, 96-well plate (Greiner Bio-One, Germany) was used for fibroblast cell culture. Area of each well is 0.32 cm^2. Therefore, we found that we need to put 25000 fibroblast cells in each well in vitro.

Cell culture and treatment of cells with test material

L929 mouse fibroblast cells were cultured in a 96-well plate (Greiner Bio-One, Germany) and 25,000 cells/mL in each six replicate plates were seeded. The cells were, then, incubated in Dulbecco's Modi-fied Eagle’s Medium (DMEM)/Ham’s F12 (Biowest Inc., Nuaillé, France) containing 10% fetal bovine serum (FBS) (Biowest Inc., Nuaillé, France) at a humid environment and 95% air and -5% CO2 and 37°C for 12 hours. After incubation, the cells were treated with five dilutions of the test material [Clexane™ (enoxaparin sodium, 4,000 IU/0.4 mL). Dilutions were prepared in the medium as Dilution I: 4,000 IU; Dilution II: 2,000 IU; Dilution III: 1,000 IU; Dilution IV: 500 IU; and Dilution V: 250 IU. In the control group, the cells were incubated in the test material-free medium.

Assessment of cell morphology and viability

We described assessment of cell morphology and viability our previous studies [12,13]. Briefly, Altera-tions in the cell morphology were analyzed using an inverted microscope (IX70 Olympus, Japan). At 24 hours, all dilutions treated with the test material were compared with the control group. The cell viability was analyzed

through (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. At 24 hours of incubation, the media were removed and 12.5 µL MTT solution (Sigma-Aldrich, Germany) was added into 100 µL FBS-free DMEM/F12 for the each well. The cell culture plates were wrapped with aluminum foil and incubated for four hours. Then, MTT solution was removed and 100 µL isopropyl alcohol (Amresco Inc., USA) was added to discontinue reaction. The cell viability was measured through an ultraviolet (UV)-visible spectrophotometer (EZ Reac 400 Microplate Reader, Biochrom) at an absorbance of 560 nm wavelength.

In the in vitro setting, the cytotoxic effects of enoxaparin sodium were investigated. Baseline number of L929 cells was calculated as 25,000 in each well (25,000 cells/0.32 cm^2/96-well) (Corning Inc., NY, USA). The viability ratios of cells (%) were calculated according to the MTT results.

Acridine orange/propidium iodide staining was made for staining dead cells with degenerated nucleus to support cell viability [14]. At 24 hours of incubation, the media on the cells were removed and AO/PI (Sigma-Aldrich, Germany) was added without fixation at a v/v ratio of 1:1 and incubated for 20 sec. Subsequently, the cells were washed with phosphate buffer saline (PBS) (Sigma-Aldrich, Germany) for 10 sec and covered with a PBS: glycerol (v/v: 1:1) mounting medium. The cells were, then, examined under a florescence microscope. Dead cells were evaluated by counting red cells with fragmented nuclei. The AO/PI-stained cells were observed under a narrow band fluorescein (FITC) filter (520–560 nm) in green color, and PI-stained cells were observed under rhodamine filter (510–560 nm) as stained red.

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed in mean ± standard deviation (SD). The Kolmogorov-Smirnov test was used for the normality test. The analysis of variance (ANOVA) was used to compare the means of more than two groups. A post-hoc test (least significant difference [LSD] or Tamhane's test) was used to analyze significant differences among the groups. A p value less than 0.05 was considered statistically significant.

RESULTS

Assessment of cell morphology and viability

At 24 hours of incubation, there was a significant degeneration different from normal fibroblast morphology in the Dilutions I and II treated with enoxaparin sodium, compared to the control group. These cells were round shaped rather than normal fibroblast morphology (i.e., elongated cells) with dense nuclei (Fig 1A and B). When Dilution I was compared with Dilution II, there was a higher rate of degeneration in Dilution I. However, Dilutions III, IV, and V showed normal fibroblast morphology with elongated and spindle-shape patterns. Similar to the control group, the cells completely covered the culture dish and became confluent in all three Dilutions (Fig 1C, D, E, and F).

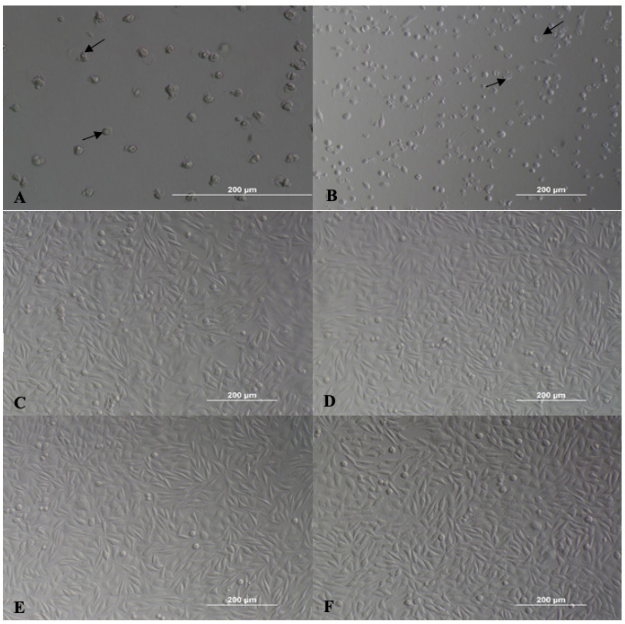

Figure 1. Morphological appearance of L929 mouse fibroblasts exposed to enoxaparin sodium Dilution I (A) (20×); Dilution II (B) (10×); Dilution III (C) (10×); Dilution IV (D) (10×); Dilution V (E) (10×); and Control (F) (10×) at 48 hours of incubation (black arrows indicate rounded and degenerated cells).

The results of the MTT assay are shown in Table 1. Compared to the control group, Dilution I showed the highest cytotoxic effect, followed by Dilution II (P=0,001, P=0,029). Compared to the control group, there was no significant difference in the cell viability in Dilutions III, IV, and V (P>0.05).

Accordingly, the cell viability decreased almost 50% in Dilutions 1 and 2 at 24 h of incubation with high-dose enoxaparin sodium. This finding indicated death of about half of subcutaneous tissue fibroblasts per 1 cm^2. (Table 2)

Table 1. MTT results of enoxaparin sodium at 24 hours in each dilution compared to control group

Time	Dilutions	Mean	sd	P
24h	D1	0,157	0,045	0,001
	D2	0,213	0,040	0,029
	D3	0,275	0,110	0,648
	D4	0,307	0,038	0,815
	D5	0,316	0,053	0,413
	Control	0,318	0,134	-

Mean: Absorbance (OD), sd: standart deviation, *P values compared to control group. Dilution I: 4000 IU; Dilution II: 2000 IU; Dilution III: 1000 IU; Dilution IV: 500 IU and Dilution V: 250 IU.

Table 2. Comparison of cell viability in in vitro and in vivo settings at 24 hours in each dilution

Dilutions	Cell Viability (%)	Percentage of rounded dead cells with AO/PI staining (%)
D1	42	51
D2	67	29
D3	87	18
D4	97	5
D5	99	2

Dilution I: 4000 IU; Dilution II: 2000 IU; Dilution III: 1000 IU; Dilution IV: 500 IU and Dilution V: 250 IU. Initial cell numbers: (25.000 cells/0.32 cm2/in vitro)

Based on AO/PI staining, the cells were assessed according to two main criteria: Normal fibroblast cells elongated with intact nuclei and were stained with green color, while dead cells were in red dye with round-shape morphology and fragmented nuclei. According to these criteria, the cytotoxic effect was prominent in Dilution I (Fig 2A). In Dilution II, there were rounded dead cells (Fig 2B). In Dilutions III, IV, and V, there was no significant alteration in the cell morphology, compared to the control group (Fig 2C, D, and E).

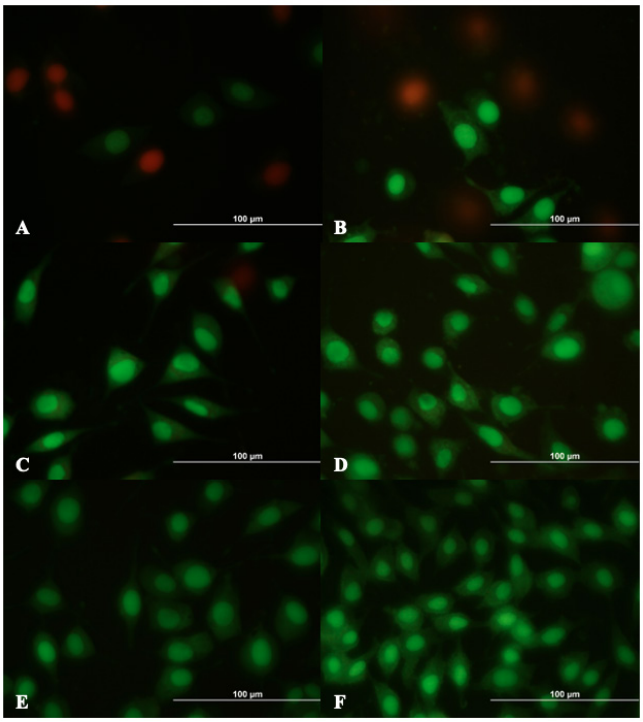

Figure 2. AO/PI staining of L929 mouse fibroblasts exposed to enoxaparin sodium. Red and round dead cells in Dilution I (A) (40×); Red and round dead cells and green healthy and normal cells in Dilution II (B) (40×); Green healthy and nor-mal cells in Dilution III (C) (40×), Dilution IV (D) (40×), Dilution V (E) (40×) and Control (F) (40×) at 24 hours of incubation.

DISCUSSION

The subcutaneous tissue is the innermost layer of the skin between the dermis and fascia.It is composed of subcutaneous fat, connective tissue, and lobules of fat. The thickness and components of the subcutaneous tissue vary depending on the site of the body and collagens and elastic fibers produced by the tissue fibroblasts play a key role in the maintenance of the integrity and shape of the cells and continuity of a balanced interaction between the cells and fascia and undermost muscle layers [8]. Fibroblasts are mesenchymal-derived cells with a spindle-shaped morphology and non-epithelial, endothelial, or immunological cells (in turn, cytokeratin; E-cadherine; 'CD31-' and 'CD45-') [15]. They are responsible for tissue homeostasis in normal physiological circumstances [16]. In addi-tion, fibroblasts release extracellular matrix (ECM) proteins, preserving the continuity of the matrix. In case of tissue injury, these cells are activated and differentiated into myofibroblasts which contract and participate in healing by reducing

the wound size and secreting ECM proteins [17]. As fibroblasts in the reticular dermis, hypodermal (subcutaneous) fibroblasts play a central role in wound healing [17]. Although fibroblasts are in a metabolic resting state in healthy tissues, they are activated and release chemokines, cytokines, and growth factors as well as ECM proteins, thereby, contributing to the healing process in case of tissue injury [15]. Although the number of fibroblasts is relatively low in the subcutaneous tissue than the other tissues, they are essential in wound healing. There is no data in the literature which indicates the exact number of fibroblasts in subcutaneous tissue. In our study, we evaluated healthy dermal histologic preparations and have created a cell culture model according to our evaluation results.

In case of any damage in the fibroblasts, cellular morphology and intercellular connections and signal conduction become impaired. Previous studies demonstrated that repetitive subcutaneous injections impaired morphology of fibroblasts and produced an elongated shape than a spindle shape [8]. In an acupuncture study, repetitive rotations of acupuncture needles led to a similar result, depending on the intensity of the application [9]. Of note, these studies discussed the mechanical or physical effect-induced alterations in the connective tissue fibroblasts. However, there is still no study investi-gating the direct effect of agent which is subcutaneously administered on fibroblasts. In an animal model, toxic effects of subcutaneous enoxaparin injection were evaluated using systemic parameters in mice and no direct cytotoxic effects on fibroblasts in the subcutaneous tissue were observed [18]. In the present study, we observed that cytotoxicity of enoxaparin sodium of the subcutaneous fibro-blast cells increased dependent with initial exposure doses. In addition, it was found that high dose of enoxaparin sodium was associated with high cell lost.

Enoxaparin sodium is subcutaneously administered generally through the anterolateral abdominal wall by altering the administration site in each attempt between the left and right anterolateral ab-dominal wall [8]. Several factors including injection site, duration and volume of the medication and the needle size play a role in the development of injection site reactions. The most common complications associated with LMWHs including enoxaparin sodium are delayed hypersensitivity reactions, eczema, and plaque formation in the injection site [19]. In particular, the most common delayed hypersensitivity reactions in the injection site include itchy erythema, infiltrated plaques, vesicular or bullous plaques, and necrotic plaques, while eczema, maculopapular exanthema or acute generalized exanthematous pustulosis can be secondarily seen [20].

The severity of skin reaction may vary depending on the age and sex of the patient, the presence of pregnancy or obesity. Obesity is an important factor for the dose adjustment of the medications used for prophylaxis or treatment of venous thromboembolism [21,22]. Although guidelines for the dose adjustment of enoxaparin sodium for overweight or obese patients have been published, the initial dose should be modified according to the body weight of an individual patient. Consequently, high dose enoxaparin is required in obese patients [23]. When the dose is uptitrated based on the body weight, the amount of enoxaparin exposure to the fibroblasts also increases, thereby, inducing cytotoxic effect of the drug. However, the cytotoxic effects of the medication itself have not been investigated in these studies. Our study results showed that high-dose enoxaparin sodium induced loss of nearly half of the cell viability. In the in vivo setting, this finding indicates death of about half of subcutaneous tissue fibroblasts per 1 cm^2. The doubling time of human fibroblasts is 24 h which is longer than the doubling time of L929 cells (16 h) in the in vitro model [24]. This suggests that regeneration is longer in the in vivo setting and repetitive drug administration may lead to irreversible subcutaneous tissue damage. The absorption of enoxaparin sodium is directly dose-dependent and is immediately and totally absorbed following the subcutaneous injection with a bioavailability of nearly 100% [25]. Accordingly, high-dose enoxaparin is needed to achieve an immediate bioavailability in the in vitro setting; however, it is associated with increased cytotoxic effects in the injection site (as shown in Dilution I).

Nonetheless, there are some limitations to this study. First, the L929 mouse fibroblasts were used

instead of human fibroblasts. However, these fibroblasts have been widely used in cytotoxicity studies, being a well-defined cell line. Their shorter doubling time than human fibroblasts is another advantage of these cells in the in vitro setting. Second, this study used a two-dimensional in vitro model. Further studies using three-dimensional models would contribute to the existing literature and support our findings.

Conclusion: In conclusion, despite prophylactic dose in the in vitro setting, high dose enoxaparin showed cytotoxic effects. Based on these results, long-term high dose enoxaparin sodium may affect the number of subcutaneous fibroblasts, impairing the skin integrity and subcutaneous tissue healing. However, further studies are needed to gain a better understanding of the clinical and subclinical effects of cytotoxicity of LMWH in subcutaneous fibroblasts.

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Note: The first two authors contributed equally to this study

Ethics Committee Approval: The authors declared that studies in non-primary vertebrate animal cell cultures are not subject to ethics committee approval as stated in the " in ninth item , 1e subitem " of dated-numbered "28.03.2016-80" the" Hacettepe University Animal Experiments Ethics Committee Directive". In addition, the stated situation was confirmed by the Hacettepe University Animal Experiments Ethics Committee meeting. (date: March 30, 2021; decision no:2021/03-24).

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RESEARCH ARTICLE

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Results of Conjunctival Autograft Transplantation Combined with Amniotic Membrane Transplantation Surgery in Recurrent Pterygium Cases

Tekrarlayan Pterjiyumlu Olgularda Konjonktival Otogreft Transplantasyonu ile Kombine Amniyotik Membran Transplantasyonu Cerrahisi Sonuçları

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ABSTRACT

Aim: To evaluate the results of conjunctival autograft transplantation (CAT) combined with amniotic membrane transplantation (AMT) surgery in recurrent pterygium cases.
Methods: This is a retrospective interventional case series involving patients admitted to our clinic with recurrent pterygium. Patients' examination and postoperative findings were taken through standard slit-lamp microscope examinations. Fourteen eyes of 14 patients were included in the study. All cases were treated with excision, followed by CAT combined with AMT. Corneal recurrence was evaluated as the main result. Fibrovascular proliferation greater than 1 mm on the cornea was accepted as recurrence after surgery.
Results: The mean age of patients was 56.4 ± 7.5 (range: 40 – 65) years. During the mean follow-up period of 24.9 ± 10.7 (range: 12 – 50) months, pterygium and symblepharon recurred in only one eye (7.1%) after 4 months.
Conclusion: In this study, it was seen that acceptable results were obtain and low recurrence rates could be achieved with the combination of CAT and AMT for the treatment of recurrent pterygium.

Key words: Pterygium, conjunctival diseases, amnion, cornea

ÖZ

Amaç: Tekrarlayan pterjiumlu olguların tedavisinde konjonktival otogreft transplantasyonu (KOT) ile kombine amniyotik membran transplantasyonu (AMT) sonuçlarını değerlendirmek
Metot: Bu çalışma kliniğimize tekrarlayan pterjium ile başvuran hastaları içeren retrospektif girişimsel bir vaka serisidir. Hastaların muayeneleri ve postoperatif bulgular standart yarık lamba mikroskopi ile yapıldı. Çalışmaya 14 hastanın 14 gözü alındı. Tüm olgularda pterjium eksizyonunu takiben KOT ile kombine AMT yapıldı. Ana sonuç olarak korneal nüks değerlendirildi. Kornea üzerinde 1 mm'den fazla fibrovasküler proliferasyon ameliyat sonrası rekürrens olarak kabul edildi.
Bulgular: Hastaların ortalama yaşı 56.4 ± 7.5 (aralık: 40- 65) yıldı. Ortalama 24.9 ± 10.7 (aralık: 12- 50) aylık takip süresi sonunda sadece bir gözde (% 7.1) 4 ay sonra pterjium ve symblepharon tekrarladı.
Sonuç: Bu çalışmada, tekrarlayan pterjium tedavisi için KOT ve AMT kombinasyonu ile kabul edilebilir sonuçlar alındığı ve düşük nüks oranları sağlanabileceği görüldü.

Anahtar kelimeler: Pterjium, konjonktival hastalıklar, amnion, cornea

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INTRODUCTION

A pterygium is a fibrovascular degeneration of the bulbar conjunctiva with progressive involvement of the cornea. The lesion occurs more frequently at the nasal limbus than the temporal and has a characteristic wing like appearance [1]. Over the years, several surgical techniques have been introduced to treat pterygium, but none have achieved complete success in efficacy and safety. Recurrence of the condition remains a major problem; there is still ongoing debate regarding the “ideal” pterygium surgery. Factors associated with recurrence include the age of the patient, the fleshiness of the pterygium, and the patient’s exposure to ultraviolet radiation [2-5].

Recurrent pterygium treatment is challenging. It is often resistant to conventional surgeries. Although various surgical approaches have been advocated, recurrence is still common, with an incidence ranging up to 55%. Treatment of recurrent pterygium is also more difficult than treatment of primary pterygium because recurrent pterygium is often accompanied by increased conjunctival inflammation and accelerated corneal involvement. Subconjunctival fibrosis is frequently seen in recurrent pterygium as well and sometimes results in restriction of eye movement or the formation of symblepharons [6]. Therefore, it is extremely important that the safest and most effective procedures for recurrent pterygium patients be determined at the beginning of treatment.

Surgical procedures for the treatment of recurrent pterygium included lamellar keratoplasty, conjunctival autograft, limbal autograft, amniotic membrane transplantation (AMT), and lamellar scleroplasty [7]. Of these treatment methods, free conjunctival autograft to cover the bare sclera reduces recurrence and has been accepted worldwide. AMT, however, has also become increasingly widespread in recent years as a treatment of pterygium. The amniotic membrane has antifibrotic and anti-inflammatory properties and reduces proliferation of subconjunctival fibrovascular tissues, thus reducing pterygium recurrence. In addition, amniotic membrane is easy to obtain, and does not cause any anatomical changes in the patient’s other tissues [8,9].

Drawing on previous research and literature, in this study we examined the surgical results of conjunctival autograft transplantation (CAT) combined with AMT surgery in patients with recurrent pterygium.

MATERIAL AND METHOD

Study Design and Participants

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and was approved by the ethics committee (25/01/2018, Istanbul Medipol University Ethics Committee, document no; 10840098-604.01.01-E-2703). A retrospective chart review was made. Fourteen eyes with recurrent pterygium were evaluated in 14 patients. All eyes were treated with excision, followed by CAT combined with AMT (Figures 1, 2, and 3). The AMT applied preserved human amniotic membrane taken from a tissue bank. Patients with follow-up times of less than 12 months, those with glaucoma, vitreoretinal disorders, pseudopterygium, connective tissue disorders, systemic vasculitis, diabetes mellitus, patients with primary pterygium and early recurrent pterygium (horizontal length from limbus < 4 mm), were excluded from the study. Routine ophthalmic examinations were performed before and after surgery. The horizontal length of pterygium invasion on the cornea was recorded using slit-lamp microscopy. The main outcome measurement was recurrence rate after surgery (fibrovascular proliferation greater than 1 mm on the cornea was accepted as pterygium recurrence). The number of previous surgeries, symblepharon formation, graft rejection and complications during the intraoperative period were noted for each patient. Graft rejection was defined as the occurrence of cellular infiltration and epithelial defect on the graft area, without an infectious focus.

Surgical Technique

All surgeries were performed by a single surgeon (CT) under general anesthesia. The pterygium borders to be expelled were first marked with tissue pellets. Next, the pterygium head was superficially incised with a crescent blade, starting from the intact epithelial border on the corneal surface. The pterygium and fibrovascular tissues were then excised using conjunctival scissors

and forceps. On the corneal surface where the pterygium was excised, a round low-rotation (average of 200–250 rpm) drill (diamond burr) with abundant irrigation was used, and smooth ocular surface formation was achieved. Next, the bare sclera was measured with a caliper, and a conjunctival autograft of the same size was harvested from the upper conjunctival region and attached to the sclera. The amniotic membrane was then cut to the appropriate size to cover the surgical zone and placed with the epithelial side up to cover the conjunctival autograft, also extending the area of cornea. The procedure was completed by suturing (8-0 vicryl) the amniotic membrane to the conjunctival fornixes.

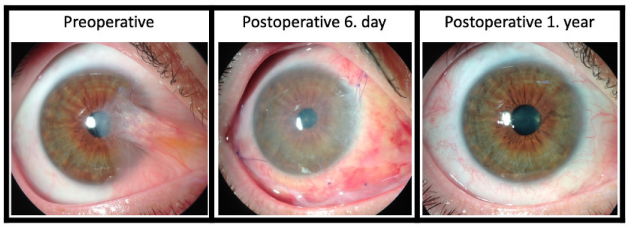

Figure 1. Preoperative right eye photograph of our 48-year-old female patient who had recurrence and had previously operated for pterygium four times in other centers. Significant movement limitation and symblepharon developed in the eye. It is also seen that the pterygium tissue covers the optic axis and pupillary area (left). On the 6th postoperative day, the entire surface is covered with amnion membrane and the conjunctival autograft under the amnion membrane is seen to be quite healthy (middle). Postoperative 1st year view of the eye (right).

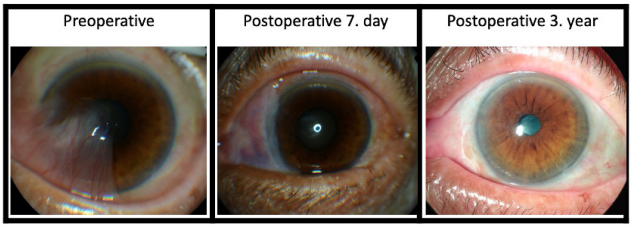

Figure 2. Preoperative left eye photograph of a 65-year-old female patient. The patient had been operated twice in another center due to pterygium and recurrence developed. There are two-headed pterygium, symblepharon and eye movement restriction, which cover the pupillary area and optic axis in the cornea (left). On the 7th postoperative day, the surgical surface is covered with the amniotic membrane and the conjunctival autograft under the amnion membrane is seen to be healthy (middle). Postoperative 3rd year view of the eye (right).

Postoperatively, all patients received netilmicin eye drops three times a day for 2 weeks, dexamethasone eye drops three times a day for 1 month, and artificial tear drops four times a day for 1 month. In cases with apparent inflammation after 1 month, a steroid regimen was continued based on sufficient disappearance of inflammation, as determined by slit-lamp findings. Sutures and amniotic membrane residues were removed at the postoperative follow-ups, when the amniotic membrane began to dissolve and peel off (mean 8.9 ± 1.3 days). Patients were followed up at 1st, 3rd, 7th, 15th days, and 1st month after surgery. After the 1st months visits were scheduled for every 3 months. All data here are means ± standard deviations.

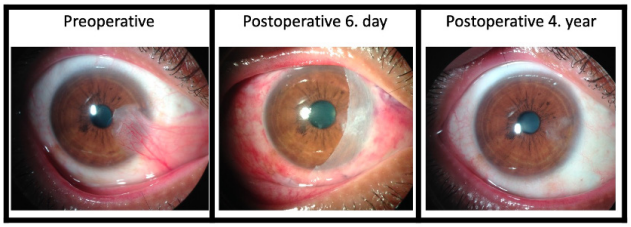

Figure 3. Preoperative right eye photograph of a 53-year-old male patient who had been operated five times for pterygium in other centers. There is a highly vascularized recurrent pterygium formation (left). On the 6th postoperative day, the surgical surface is covered with the amniotic membrane and the conjunctival autograft under the amnion membrane is seen to be healthy (middle). Postoperative 4th year view of the eye (right).

RESULTS

The mean age of patients was 56.4 ± 7.5 (range: 40 - 65) years. The cases included in the study consisted of 8 (57.1%) female and 6 (42.9%) male patients. The mean number of previous surgeries for pterygium was 2.1 ± 1.2 (range: 1– 5). The mean horizontal pterygium length was 4.5 ± 0.7 (range: 4 – 6) mm. In all eyes, the epithelial defects of the corneal and conjunctival areas had recovered within 2 weeks. Preoperative and postoperative slit-lamp views in representative eyes are shown in figures 1, 2, and 3. During the mean follow-up period of 24.9 ± 10.7 (range: 12 – 50) months, pterygium and symblepharon recurred in only one eye (7.1%) after 4 months. There were no intraoperative complications. Amniotic membrane rejection was not observed in any patient, nor were any serious side effects due to the medication. A mildly increased intraocular pressure (29 mm Hg) was observed on the 9th postoperative day in a 40-year-old male patient. In this case, dexamethasone eye drops were stopped and loteprednol etabonate eye drop was prescribed instead. Following this adjustment, intraocular pressure normalized. The patient continued with loteprednol etabonate for 1 months. The mean

Table 1. Demographic and clinical data of all patients.

Patient no	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gender	F	F	M	F	M	F	M	M	F	F	F	M	F	M
Age (years)	48	58	62	63	59	65	57	53	44	59	62	63	57	40
Previous surgeries number	4	3	2	1	3	2	1	5	1	3	2	1	1	1
Length of pterygium (mm)	6	5	4	4	5	6	4	5	4	5	4	4	4	4
Symblepharon	+	+	-	-	+	+	-	-	-	+	-	-	-	-
Restriction of movement	+	-	-	-	-	+	-	-	-	+	-	-	-	-
Follow-up period (month)	18	34	15	22	25	41	21	50	17	15	29	23	27	12
Epithelial healing (day)	7	6	6	7	7	8	5	6	5	7	5	5	6	7
Preoperative CDVA	0.1	0.3	0.4	0.2	0.4	0.1	0.6	0.4	0.5	0.1	0.7	0.5	0.4	0.9
Postoperative CDVA	0.4	0.5	0.5	0.4	0.5	0.4	0.7	0.6	0.6	0.1	1	0.7	0.6	0.9
AM peel off time (day)	10	7	8	9	7	9	7	10	11	10	9	8	9	11
Recurrence	-	-	-	-	-	-	-	-	-	+	-	-	-	-

CDVA; Corrected distance visual acuity (Snellen decimal system), AM; Amniotic membrane.

corrected distance visual acuity (CDVA) before surgery was 0.4 ± 0.23 (Snellen decimal system). Postoperative CDVA increased in 12 patients. Postoperative mean CDVA was 0.56 ± 0.22. Table 1 summarizes the data of all patients.

DISCUSSION

Over the years, several surgical techniques have been developed to treat pterygium, but none have achieved complete success in safety and efficiency. The ideal surgical treatment of pterygium is still discussed today. The technique of bare sclera excision has been abandoned due to unacceptably high recurrence rates (30–70%). For years, the most effective treatment method has been tried to be found. Within these treatment methods, the use of free conjunctival autograft to cover the bare sclera reduces recurrence and has accepted worldwide [8,9].

The clinical behaviour of primary and recurrent pterygium is quite different. Recurrent pterygium exhibits a more aggressive fibrovascular growth pattern than primary pterygium. Surgery is therefore more difficult in recurrent pterygium patients; symblepharon formation and motility restriction often occur due to conjunctival inflammation, as well as corneal scarring and limbal stem-cell deficiency. Surgery management strategies must include management of the recurrence itself, such as controlling inflammation and complications from previous surgeries [8].

Several studies in the literature discuss the management of recurrent pterygium. Miyai et al. [7] reported successful treatments with limbal allograft, preserved amniotic membrane transplantation, and intraoperative mitomycin C. In their study, 12 patients with recurrent pterygium had undergone intraoperative MMC after pterygium excision. Limbal allograft was then successfully applied to close the limbal tissue defect, and AMT to close the scleral tissue defect. Pterygium did not recur in any of the patients involved in this study. Symblepharon reappeared in only 3 eyes, while diplopia recurred in 2 eyes. Shimazaki et al. [6] reported 27 recurrent pterygium cases who were treated with AMT, combined with either limbal autograft transplantation (LAT, n = 15) or CAT (n = 12). They observed acceptable results, with pterygium recurring in only 4 patients. Ono et al. [10] evaluated the efficacy and safety of preserved limbal allograft transplantation, combined with AMT, on 84 eyes in 80 recurrent pterygium cases. They observed 10 recurrences (11.9%) in their series but did not observe any major complications or graft rejection. They posited that preserved limbal allograft, together with AMT, is a safe and effective procedure for recurrent pterygium treatment. Yao et al. [11] studied 7 multi-recurrent pterygium cases with symblepharon and motility restriction. After using CAT combined with AMT and intraoperative MMC 0.02% on the bare sclera for 5 minutes, they observed recurrence in only 1 patient, and less severe pterygium than the patient had exhibited before surgery. They concluded that this combination method could successfully restore ocular surface integrity and prevent recurrence in patients with multi-recurrent pterygium with symblepharon. Fallah et al. [12] also reported that CAT combined with AMT was an effective and safe surgical method for recurrent

pterygium treatment. Finally, Sangwan et al. [13] performed CAT, combined with AMT and intraoperative application of MMC (0.04% for 3 minutes), to treat 2 advanced recurrent pterygium cases. They observed acceptable results in their cases.

Although we used similar treatment combinations as other studies, our surgical method differed in several ways. In other studies, the amniotic membrane was usually transplanted under CAT or adjacent to the CAT to cover the exposed large bare sclera. On the other hand, we applied AMT to our patients in a way to cover the entire surgical area after CAT was applied to the area where the pterygium was excised. Our main theoretical reason for choosing AMT on CAT was that when AMT is applied under CAT, the amniotic membrane may act as a barrier between CAT and sclera and may impair graft feeding, resulting in graft necrosis and hypersensitivity to the amniotic membrane. It may also be more difficult to remove the AMT from under the CAT in rejection situations. In addition, when AMT is used over CAT, it can prevent symblepharon and conjunctival adhesions from occurring after surgery. Finally, AMT has an antifibrotic and anti-inflammatory effect on the ocular surface. It is also possible to reduce postoperative irritation and pain by closing the CAT sutures with AMT; we sutured the amniotic membrane into the loose fornix conjunctiva to decrease stinging and irritation. Theoretically, the main disadvantage of this method is that when the conjunctiva is covered with amniotic membrane may not have sufficient regulator effect on the fibroblasts under the conjunctiva. In our study, we did not use mitomycin-C as an adjuvant in the surgical treatment of our patients. This is because, although rare, we avoided possible complications of mitomycin-C that could threaten vision and ocular surface integrity.

In the surgical treatment of pterygium, CAT alone is often sufficient and is the first method that comes to mind. However, in our case series, there were usually patients with severe pterygium who had multiple operations. Therefore, we thought that the combination of CAT and AMT might be more effective in the surgical treatment of these cases.

The present study had several strengths, including following the patients for at least 12 months, which allowed a better perspective on long-term recurrence rates. Moreover, the same surgeon performed all treatment procedures, eliminating surgeon-related factors. We conclude that the results of combined CAT with AMT in patients with advanced recurrent pterygium may be satisfactory. In conclusion, recurrent pterygium patients in our study were concerned about having to undergo surgery again because they had already been operated on many times previously. Therefore, we think the treatment of recurrent advance pterygium should be explained well, and it would be beneficial to treat after this with suitable methods. However, further randomized prospective studies are necessary to determine the validity of this technique over other surgeries.

Limitations: Our study did encounter some limitations that should be noted; in particular, the small patient population, lack of control group and the retrospective nature of the study.

Conclusion: In this current study, a combined surgical treatment method in recurrent pterygium cases is discussed. In conclusion, we think that these cases should be evaluated carefully and all therapeutic alternatives with effective results should be considered. In this sense, we believe that CAT combined with AMT surgery is an effective and safe method in the treatment of recurrent pterygium.

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The relationship between procalcitonin, D-dimer, ferritin, troponin, and lactate levels with COVID-19

Prokalsitonin, D-dimer, ferritin, troponin ve laktat düzeylerinin COVID-19 ile ilişkisi

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ABSTRACT

Aim: Coronavirus disease-2019 patients may experience an increase in inflammation or clotting disorders. It is believed that some biomarkers can be decisive in decision to hospitalize. Our aim in this study was to investigate the relationship between serum procalcitonin, troponin, D-dimer, ferritin and lactate levels, and Coronavirus disease-2019.

Methods: Patients suspected of suffering from the Coronavirus disease-2019 and whose serum biochemistry and blood gas tests were performed, were included in the study. The relationships between the blood parameters of the patients with negative or positive Polymerase Chain Reaction test results, and those who were hospitalized or non-hospitalized, were examined.

Results: Out of a total of 452 patients, 244 (54%) were male and 208 (46%) were female. There was a statistically significant difference between polymerase chain reaction test positive and test negative groups in terms of procalcitonin, D-dimer, lactate and troponin levels (p□0.005). There was no statistically significant difference between hospitalized and non hospitalized patient in terms of ferritin (p=0.224). However, there was a significant difference in terms of procalcitonin, D-dimer and troponin levels (p□0.005).

Conclusion: Our results revealed that high serum procalcitonin, D-dimer, lactate and troponin levels are associated with the decision to hospitalize Coronavirus disease-2019 patients, whereas ferritin levels played no such role.

Keywords: Coronavirus disease-2019, Procalcitonin, D-dimer, Troponin, Lactate

ÖZ

Amaç: Koronavirüs hastalığı-2019 hastalarında enflamasyon artışı veya pıhtılaşma bozukluğu artışı görülebilmektedir. Bazı biyobelirteçlerin, hastaneye yatış yapılmasında belirleyici olabileceği düşünülmektedir. Bu çalışmadaki amacımız, serum prokalsitonin, troponin, D-dimer, ferritin ve laktat düzeyleri ile Koronavirüs hastalığı-2019 arasındaki ilişkiyi araştırmaktı.

Metod: Koronavirüs hastalığı-2019 şüphesi olan, kan biyokimya ve kan gazı tetkikleri çalışılan hastalar çalışmaya dahil edildi. Polimeraz zincir reaksiyonu test sonuçları negatif veya pozitif olan hastalar ve taburcu veya servise yatışı yapılan hastaların kan sonuçları arasındaki ilişkileri incelendi.

Bulgular: Toplam 452 hastanın, 244 (% 54)'ü erkek ve 208 (%46)'i kadın idi. Polimeraz zincir reaksiyonu testi pozitif olan grup ile negatif olan grup arasında prokalsitonin, D-dimer, laktat ve troponin düzeyleri istatistiksel olarak anlamlı bulundu (p□0.005). Yatış yapılan hastalar ile yapılmayan hastalar arasında ferritin seviyesinde istatistiksel olarak bir anlamlılık yoktu (p=0.224). Ancak prokalsitonin, D-dimer, troponin seviyelerinde anlamlı bir farklılık çıktı (p□0.005).

Sonuç: Elde ettiğimiz sonuçlarla yüksek serum prokalsitonin, D-dimer, laktat ve troponin düzeylerinin Koronavirüs hastalığı-2019 hastalarının hastaneye yatışı ile ilişkisinin olduğunu söyleyebiliriz. Ancak ferritin düzeylerinin hastaneye yatış endikasyonunda bir rolü yoktu.

Anahtar Kelimeler: Koronavirüs hastalığı-2019, Prokalsitonin, D-dimer, Troponin, Laktat

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INTRODUCTION

With reports emanating out of China of viral pneumonia cases with an unknown cause at the end of 2019, various studies were conducted and a new type of coronavirus was soon identified. The Coronavirus disease-2019 (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), quickly spread to 6 continents and more than 200 countries, and became the first ever pandemic caused by a coronavirus [1]. Initially considered to be a respiratory infection, data now suggests that COVID-19 is a systemic (cardiovascular, gastrointestinal, neurological, hematopoietic and immune system) disease. Patients usually present with fever, fatigue, dry cough, loss of appetite, myalgia, dyspnea, loss of smell and taste, nausea, vomiting, diarrhea, nasal discharge and conjunctivitis [2]. It has been found that older people or those with comorbidities have a higher risk of mortality, but fatal complications can also occur in young people without an underlying disease [3]. As of the time this article is written, the number of deaths from COVID-19 has surpassed 2,5 million worldwide, while nearly 28,000 people have lost their lives in Turkey [4] and there remains a great deal that is unknown regarding COVID-19. Some studies have investigated the relationship between blood levels and this coronavirus. For example, lymphopenia, prothrombin time/partial thromboplastin time length and high D-dimer were found to be precursors to severe disease [5]. One current study hypothesized that progressive increase in PCT levels may be positively associated with the severity of COVID-19 and predict a poor prognosis [6].

In light of the high prevalence of COVID-19 disease and limited hospital capacities, determining the profile of patients who needs be hospitalized has become an important goal. Therefore, our study focused on the changes in some blood parameters such as PCT, D-dimer, ferritin and troponin in the follow-up and treatment of patients diagnosed with COVID-19. Our objective was to compare polymerase chain reaction (PCR)-positive and PCR-negative patients and hospitalized versus non-hospitalized patients, based on these parameters.

MATERIAL AND METHODS

A total of 452 patients who came to the emergency medicine (ER) with clinical presentation of COVID-19 between April 1, 2020 and September 1, 2020, had their case studied retrospectively. According to the G-power analysis, it was planned to have at least 350 patients in the study, which received the approval of the Ethics Committee of the Faculty of Medicine of Ataturk University, designated B.30.2.ATA.0.01.00/416, on October 1, 2020. Age, gender, complaints upon presentation, real-time reverse transcription (RT)-PCR test results, PCT, D-dimer, ferritin and troponin levels, as well as hospitalization and non-hospitalization statuses of patients were examined. Laboratory and PCR-test results of patients were obtained from the hospital's database while vital signs and clinical information were gathered from patient files. In our hospital, an RT-PCR test is performed using the ELISA method on an AB Applied Biosystems GeneAmp PCR System 9700 device. D-dimer was tested using the immunoturbidimetric method on a AQT90 Flex device, whereas PCT, ferritin and troponin were tested using the ECLIA method with a Beckman Coluter Unicel Dxl 600 device.Patients who died, patients with incomplete blood parameters and those under 18 years of age, were excluded from the study. The blood levels parameters of the remaining patients who were either hospitalized or non-hospitalized, were thereby compared. Those with a poor clinical picture (breathing difficulty (≥ 30/min, oxygen saturation ≤%93 and/or PaO2/FiO2≤300 mmHg) [7], those who were PCR-positive or negative and had extensive ground-glass opacity showing in thorax computed tomography (CT) that is consistent with viral pneumonia, were accepted as the hospitalized group. High-resolution CT was performed in all patients using a Siemens Somatom Emotion 16 model device. Patients with negative RT-PCR results and normal thorax CTs were accepted as the non-hospitalized group.

Statistical Analysis

The data was provided as mean ± standard deviation (SD), median (range) or number (percentage). For continuous variables, the assumption of normal distribution was checked using the Shapiro-Wilk W-test when the sample size was <50, and

the Kolmogorov-Smirnov test when the sample size was ≥50. In the comparison between the two independent groups, independent samples t-test was conducted when the distribution was normal, and Mann-Whitney u test was used when data was not normally distributed. Categorical variables were compared using the Chi-square test. The receiver operating characteristic (ROC) curve analysis was conducted for PCT, troponin, D-dimer and lactate, to determine whether any of these variables affected the determination to hospitalize. The ROC curve analysis was also utilized to determine the cut-off values, the best of which were calculated using Youden's Index. The data was analyzed using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA) for Windows. For all values, p<.05 was considered statistically significant.

RESULTS

The mean age of patients was 54 ± 20 (range: 18-96). The number of male patients was 244 (54%), while the number of female patients was 208 (46%). According to the results of combined throat / nasal swab test performed in the ER, 67 (14.8%) patients were PCR-positive and 385 (85.2%) patients were PCR-negative.

Complaints of the patients with suspicious contact admitted to the ER were muscle pain (myalgia) (48.2%), fatigue (19.7%), fever (18%). The others were asymptomatic (13.7%) (Table 1).

Table 1. Demographic and clinic data of COVID-19 patients.

Gender (n,%)	Female	208 (46)
	Male	244 (54)
Presenting Complaint (n,%)	Myalgia	218 (48.2)
	Fatigue	89 (19.7)
	Asymptomatic	62 (13.7)
	Fever	83 (18.4)
RT-PCR (n,%)	Positive	67 (14.8)
	Negative	385 (85.2)
Hospitalization (n,%)	Hospitalized	108 (23.9)
		344 (76.1)

All blood parameters of the PCR-positive group were lower than those of the PCR-negative group. There was no statistically significant difference between the two groups in terms of serum ferritin levels (p=0.713). However, there was a statistically significant difference between PCT, D-dimer, troponin and lactate levels of two groups (p values were 0.001, 0.001, 0.001 and 0.001, respectively) (Table 2).

Table 2. Evaluation of blood parameters according to RT-PCR result

	RT-PCR positive (n:67)	RT-PCR negative (n:385)	P value
Age	50±20	55±20	0.032*
Ferritin	1188.8±1658	1223.6±1916.4	0.713*
PCT	3.33±13.7	5.45±43.43	0.001*
D-dimer	1222.8±3773.6	1923.9±6368.3	0.001*
Troponin	20.8±90.6	200.1±1844.3	0.001*
Lactate	1.6±1.0	2.1±1.6	0.001*

P*: Mann-Whitney U test, PCT: Procalcitonin

The number of patients hospitalized after ER admission was 108 (23.9%), while the number of discharged outpatients was 344 (76.1%) (Table 1). The mean age of hospitalized patients was higher than the mean age of non-hospitalized patient, however, this finding was determined not to be statistically significant (p=0.009). The blood parameters of hospitalized and non-hospitalized patient were also compared. There was no statistically significant difference in ferritin levels between these two groups (p=0.224), however there was a statistically significant difference between PCT, D-dimer, troponin and lactate levels of the two groups (p values were 0.001, 0.001, 0.004 and 0.001, respectively) (Table 3).

Table 3. Evaluation of blood parameters by hospitalization

	Hospitalized Patients (n:108)	Non-hospitalized Patients (n:344)	P value
Age	59 ± 20	53 ± 20	0.009**
Ferritin (ng / ml)	1602.5 ± 2268	1090.5 ± 1714.4	0.224**
PCT (ng/ml)	15.5 ± 79.6	1.6 ± 5.3	0.001**
D-dimer (ng/ml)	4468.8 ± 11575.3	937.1 ± 1188.1	0.001*
Troponin (ng/m)	589.1 ± 3374.1	35.1 ± 149.4	0.004**
Lactate (mmol/L)	2.7 ± 2.5	1.9 ± 1.1	0.001**

P*: Mann-Whitney U test, P**: Independent t-test, PCT: Procalcitonin

The area under the ROC curve (AUC) and statistic values of each study variable with a 95% confidence interval are specified in Table 4, to show which ones indicate the risk of hospitalization in COVID-19 patients. Based on the findings, a PCT higher than 0.16 ng/ml, D-dimer higher than 1430 ng/ml, troponin higher than 26.6 ng/m and lactate higher than 2.75 mmol/L, are associated

with a higher risk of hospitalization in COVID-19 patients (Table 4). Figure 1 presents the sensitivity and specificity of the PCT, D-dimer, troponin and lactate in predicting hospitalization of COVID-19 patients in the ROC curve analyze (Figure 1).

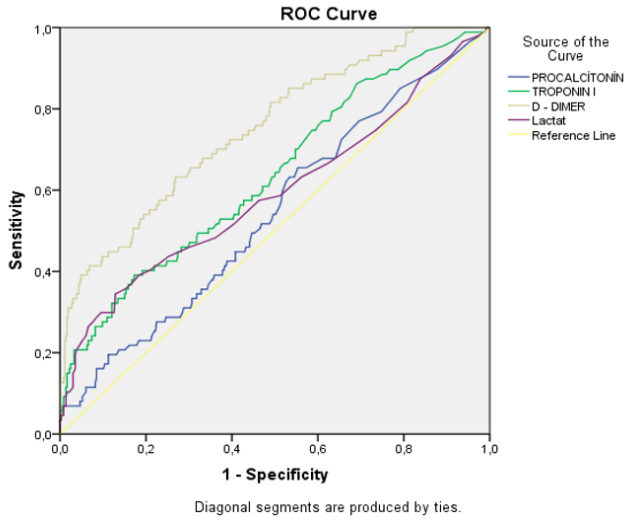

Figure 1. Presents the sensitivity and specificity of the PCT, D-dimer, troponin and lactate in predicting hospitalization of COVID-19 patients.

DISCUSSION

In this study, we examined whether there was any relationship between the levels of some biomarkers, hospitalization indication and RT-PCR test results in COVID-19 patients. We have found a relationship between high serum PCT, D-dimer, lactate and troponin levels and hospitalization of these patients.

As a result of the increase in the number of COVID-19 patients in recent months, the issue of which patients will be hospitalized becomes more important. Even though the RT-PCR test, which is still required for COVID-19 diagnosis, comes back negative, the diagnosis should be supported with laboratory and radiological findings [8]. RT-PCR positive patients are considered confirmed cases. In the early period, PCR negative patients should also undergo thorax CT as the radiological imaging method. However, thorax CT follow-up is not recommended for evaluating the response to treatment [9]. PCR test results are still the main focus for diagnosis, treatment and hospitalization, however given the low cost and rapid results, blood parameters can be used, without waiting for PCR test results.

The most frequent laboratory findings in seriously ill hospitalized patients are lymphopenia, hypoalbuminemia, and increased ferritin and D-dimer (10). Leukocyte levels and lymphocyte counts, which are indicators for inflammation, are normal in the early stage of the disease when there are non-specific symptoms [11]. However, clinical signs of the disease start to become apparent 7 to 14 days after symptom onset and this is the systematic increase of inflammatory markers and cytokines, which is also known as the cytokine storm [12]. It is believed that the severity of COVID-19, even its fatal outcomes, is a consequence of the cytokine storm syndrome [13].

Ferritin is an important mediator of immune system disorders. Hyperferritinemia contributes to cytokine storm through direct immunosuppressive and proinflammatory effects [14]. A study on patients who died of COVID-19 found that ferritin levels were high after hospitalization as well as during their hospital stay [15]. In their study, Wu et al. showed high serum ferritin to be associated with ARDS development; no significant association was found with survival [16]. Although ferritin levels were high in the hospitalized patient group in our study, no statistical significance was found between hospitalized and non-hospitalized patient groups.

Coagulation disorders appear to be more common in severe cases of COVID-19. D-dimer levels are associated with negative outcomes among community-acquired pneumonia patients, which may also reflect the severity of COVID-19 [17]. In a descriptive study by Chen et al, high D-dimer levels were measured in 36% of 99 patients [18]. Another study on 41 patients in China, showed that D-dimer levels were higher at the time of admission among patients requiring intensive care. The median D-dimer for intensive care patients was 2.4 mg/L and 0.5 mg/L for non-intensive care patients ($p = 0.004$) [19]. Our study reached conclusions similar to those in the literature: D-dimer levels were high in both PCR-negative and hospitalized groups.

PCT is released by thyroid parafollicular C cells, a 116-amino acid peptide precursor to the hormone calcitonin [20]. PCT has been widely researched

for its use as a biomarker in bacterial infections [21]. There have been some studies that proved PCT levels are positively correlated with the severity of COVID-19. In a study of Lippi et al. where they measured the PCT levels of 38 patients, 32 were discharged and 6 died in intensive care: PCT levels were low in non-hospitalized patients [22]. Our study found PCT levels to be high among hospitalized patients. Statistically significant results were obtained in PCR-positive and the hospitalized group.

Troponin is a marker of myocardial damage, mainly myocardial infarction or myocarditis. High troponin levels are quite common among COVID-19 patients and are associated with fatal outcomes. Along with the fact that it does not have a definitively proven mechanism, it is believed that it may be through viral myocarditis, cytokine-induced myocardial damage and microangiopathy [23]. High troponin levels were found to be common among hospitalized COVID-19 patients and the duration of stay at the hospital was also longer in patients with high troponin levels [24]. Our study found that the troponin level was higher among PCR-positive patients, compared to PCR-negative patients. We assume that this finding is independent of PCR tests and may be related to the patient's clinical status. The troponin levels of hospitalized patients were quite high compared to the non-hospitalized group.

Serum lactate is a byproduct of anaerobic metabolism. Lactate levels can show the severity of tissue hypoperfusion and hypoxia [25]. In tissue hypoxia, where there is no oxygen, pyruvate molecules cannot enter the mitochondria and are converted to lactate. Lactate accumulation is a result of increased production and/or decreased removal. In one study, higher lactate levels suggested that anaerobic glycolysis began early due to insufficient oxygen supply [26], therefore higher lactate levels may be considered an indicator for insufficient oxygen supply.

When we searched the literature, we found that there were few studies investigating the relationship between COVID-19 and lactate levels, it was therefore our hope that ours would guide future studies. We found lactate levels in the PCR-negative group were significantly higher than in the PCR-positive group and seem to be a rapid and reliable indicator for lung damage, and could even be used to better adapt the treatment of COVID-19 patients. Although there are an increasing number of studies being performed on this coronavirus, we hope the results of our study can be of use as it is the first to compare this quantity of blood parameters according to PCR test results, as well between hospitalized and non-hospitalized patients.

Limitations of the study

Our study has some limitations, One of which was that the hospital where the study was conducted is one of two large hospitals in the city and the other institution being used as the designated pandemic-response hospital. This situation resulted in a relatively low number of COVID-19 patients presenting. Another limitation is that the epidemiologist made the determination of whether a patient should be hospitalized or not, and that the hospitalization criteria depend on multiple factors. The presence of various diseases that might affect the patients' parameters and the fact that we did not evaluate other blood parameters, are both limitations. Finally, the retrospective design of this study may be considered an additional limitation.

In conclusion, patients can be hospitalized according to some blood parameters and RT-PCR results. In our study, we found that besides the clinic, serum PCT, D-dimer, lactate and troponin levels were higher in hospitalized patients. We are of the opinion that extensive studies are needed to investigate the relationship between these blood parameters and the severity of COVID-19.

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Seasonal variation of immune hemolytic anemia

İmmün Hemolitik Aneminin Mevsimsel Değişkenliği

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ABSTRACT

Aim: Immune hemolytic anemia is an autoimmune disease that is related to autoantibodies against erythrocytes. Such antibodies appear for a variety of reasons such as hematologic and oncologic malignancies, infections, and connective tissue diseases but in many cases, a true etiologic agent has not been discovered. Many hematologic as well as rheumatologic disorders have seasonal variations but there have not been many studies evaluating the possibility of seasonal variation of immune hemolytic anemia.
Methods: It was investigated whether the patients with immune hemolytic anemia who were diagnosed and followed in the hematology outpatient and inpatient clinic of Suleyman Demirel University from 2002 to 2018 had a significant seasonality. We also evaluated whether there was any seasonality relationship between gender and beginning of the hemolytic attacks.
Results: There was no significant difference when seasons were grouped as spring, summer, autumn and winter, according to gender (p = 0,122). The evaluation of seasons in two groups as autumn-winter and spring-Summer revealed that male patients tended to suffer immune hemolytic anemia in autumn-winter, whereas females, significantly, tend to contract the disease in spring-Summer (p=0,046).
Conclusion: Immune hemolytic anemia had significant seasonality pattern depending on gender. More prospective studies are needed to support these findings in this study.

Keywords: Hemolytic anemia, seasonality, gender

ÖZ

Amaç: İmmün hemolitik anemi, eritrositlerle ilişkili otoantikorlara bağlı otoimmün bir hastalıktır. Bu tür antikorlar, hematolojik ve onkolojik maligniteler, enfeksiyonlar ve bağ dokusu hastalıkları gibi çeşitli nedenlerle ortaya çıkar ancak, bazı durumlarda, etyolojik faktör tespit edilememektedir. Hematolojik ve romatolojik bozuklukların birçoğunun mevsimsel varyasyonları vardır, ancak immün hemolitik aneminin mevsimsel değişim olasılığını değerlendiren çok fazla çalışma yoktur.
Metod: 2002-2018 yılları arasında Süleyman Demirel Üniversitesi hematoloji polikliniğinde ve yatarak tedavi kliniğinde tanı ve takibi yapılan immün hemolitik anemili hastaların anlamlı mevsimselliğe sahip olup olmadığı araştırıldı. Ayrıca, immün hemolitik anemi atağının başlangıcı ile cinsiyetler arasında mevsimsellik ilişkisi olup olmadığı araştırıldı.
Bulgular: Cinsiyete göre mevsimler ilkbahar, yaz, sonbahar ve kış olarak gruplandırıldığında anlamlı fark yoktu (p = 0,122). Mevsimleri sonbahar-kış ve ilkbahar-yaz olarak iki grupta değerlendirdiğimizde, erkek hastaların sonbahar-kış aylarında immün hemolitik anemiye eğilimli olduklarını, kadınların ise ilkbahar-yaz aylarında önemli ölçüde hastalığa yakalanma eğiliminde olduklarını bulduk.
Sonuç: İmmün hemolitik aneminin, cinsiyete bağlı olarak, istatistiksel olarak anlamlı mevsimsellik paterni olduğunu gösterdik. Bu çalışmadaki bulguları desteklemek için daha fazla prospektif çalışmaya ihtiyaç vardır.

Anahtar Sözcükler: Hemolitik anemi, mevsimsellik, cinsiyet.

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INTRODUCTION

Immune hemolytic anemia is an autoimmune disease that progresses with the formation of autoantibodies against erythrocytes. It is a rare disease and its incidence is around one in 100 000 [1]. Depending on the types of antibodies, the disease can be defined as a warm antibody disease, cold antibody disease and paroxysmal cold hemoglobinuria. The most common type is warm antibody (70%) which causes IgG or complement mediated hemolysis at body temperature (37 oC) [2, 3]. Immune hemolytic anemia due to cold antibody is seen around 20% of cases and leads to hemolysis with IgM type autoantibody. This antibody binds to erythrocytes when the body temperature drops in a cold environment and causes hemolysis. While 8% of cases had both types of autoantibodies [4], 2% of cases presented with Donath-Landsteiner type autoantibodies: such antibodies bind to erythrocytes when the body temperature drops in the cold environment, but hemolysis occurs only when the body temperature returns to normal levels. These antibodies, which have historically been observed as a sign of tertiary syphilis, are now emerging after some viral infections [5]. The most common etiology of immune hemolytic anemia is idiopathic, however viral infections, connective tissue diseases, drugs, post-transplantation, hematologic and oncologic malignancies are other known causes [6]. Evaluation of the etiology is of great importance, especially in the choice of treatment; in hemolytic anemia due to malignancies, connective tissue and autoimmune disorders, the treatment of the primary disease that causes hemolysis may stop the hemolytic process, but these non-specific treatments without etiology research may suppress the underlying pathology, leading to serious delays in actual diagnosis and treatment [2, 3, 6]. Corticosteroids in warm antibody positive patients and Rituximab in cold antibody positive patients, are the treatments of choice [2, 3]. Immune hemolytic anemia is a chronic disease in adults; the response rate of first-line treatment is high, but so as the relapse rate and new treatments with different drugs are often needed [6].

The relationship of the seasons in the emergence of hematologic diseases was firstly noticed in clinical observations and later confirmed by various trials. Studies evaluating the relationship between seasons and acute lymphoblastic leukemia, acute myeloid leukemia, Hodgkin lymphoma and immune thrombocytopenic purpura showed significant difference in the seasonality of these diseases. We could only find one abstract evaluating the relationship between seasons and immune hemolytic anemia, and a search in Pubmed and other indexes did not reveal any studies from our country. Therefore, our goal was to investigate whether there is any relationship between the emergence of immune hemolytic anemia and the seasons.

MATERIAL and METHOD

This is a retrospective study evaluating patients diagnosed with immune hemolytic anemia who applied to the Hematology Department of the Suleyman Demirel University Medical Faculty Hospital from 2002 to 2018. Permission for the study with number 205 was obtained from Suleyman Demirel University Faculty of Medicine Ethics Committee in 19.06.2019. Patients above 18 years old and who were not pregnant were included in the study. Patients who had been diagnosed and treated prior to 2002 and after 2018, were excluded from the study.

In the definition of etiology for the study, autoimmune hemolytic anemia was related to idiopathic, connective tissue disorders, hematologic malignancies and oncologic malignancies, whereas drug usage was classified separately. Etiology was further re-evaluated as primary and secondary and additional disorders were grouped separately. Patients who had atherosclerotic disease or autoimmune disorders were also analyzed separately. While transfusion independence was accepted as a response criterion for treatment, each different pharmacological agent or splenectomy treatment received was considered as a separate treatment. Treatment groups were organized as steroid treatment on one hand and other treatments on the other. Response to each line of treatment was recorded. Seasons were divided into groups as winter (December, January, and February), spring (March, April, and May), Summer (June, July, and August) and autumn (September, October,

and November). The seasons were then re-grouped as autumn-winter and spring-Summer and further analysis was performed for seasonal variation, while analysis was carried out not only seasonally, but also by months. The demographic properties, the parameters described above and their seasonal variations were then analyzed for any significance.

Statistical analysis was made using SPSS version 22.0. The suitability of variables to normal distribution was examined using the Komogorov-Smirnov/Shapiro-Wilk tests. Descriptive statistics n (%) was presented with mean ± standard deviation and median ± interquartile range. To determine whether the research group was the same in every season, a single group chi-square test was performed. Frequency of immune hemolytic anemia according to seasons was given by using cross tables according to gender, etiology, additional disease and treatment status. Whether there had been a difference in these frequencies between groups was compared using the chi-square, or the Fisher test where the values observed in the cells did not provide chi-square test assumptions. The age variable was compared using the Kruskall-Wallis test between groups, since it was determined that the age continuous variable did not show normal distribution. P <0.05 was considered as statistically significant.

RESULTS

A total of 37 patients with immune hemolytic anemia were evaluated. While 67.6% of the study group were women, 89.2% of them were primary hemolytic anemia, 91.9% received steroid treatment. The average age of the group was 54.3 ± 16.6 years. The demographic and clinical features of the patients are shown in Table 1. To determine whether the ratio of patients with immune hemolytic anemia differ between seasons, a single group chi-square test was performed. There was no statistically significant difference between expected and observed values according to the seasons (Table 2; p = 0.184).

The distribution of the demographic data of the patients such as gender, etiology, additional disease, treatment and age by season was examined (Table 3). When seasonal differences were evaluated according to the gender of patients with immune hemolytic anemia, there was no significant difference between seasons (p = 0,122). The evaluation of seasons in two groups as autumn-winter and spring-Summer revealed that males tended to suffer hemolytic anemia in autumn-winter whereas females significantly tend to contract the disease in spring-Summer (Table 1; p = 0.046). When the seasonal distribution of the patients according to etiology, comorbidities, treatment preferences and age groups was examined, there was no statistically significant difference between them (Table 3). Furthermore, no statistical difference was found when data were evaluated by monthly distribution.

DISCUSSION

In this study, evaluation of the relationship between seasons and attacks of immune hemolytic anemia revealed no significant difference between seasons. When seasons were regrouped as autumn-winter and spring-Summer, it was shown that male patients significantly had more attack in autumn-winter, whereas female patients had significantly more attack in spring-Summer. There were several studies evaluating the seasonality of hematologic disorders. In their study, Karimi and Yarmohammadi found a significant increase of childhood acute lymphoblastic leukemia in the autumn and winter seasons [7]. In another study conducted in patients with adult acute myeloid leukemia, a significant increase in the diagnosis of disease was observed in adult men in December and January [8]. The authors suggested that infections can be associated with this condition. In our study, no relationship could be found between seasons and disease and a subgroup analysis of data evaluated by months failed to find any statistical significance. Borchman et al. evaluated the seasonal pattern of incidence and mortality of Hodgkin Lymphoma and found that incidence of Hodgkin Lymphoma was increased in March. The authors speculated that this might be related to vitamin D status of patients [9]. These retrospective studies yielded conflicting results of seasonality of the hematologic malignancies; therefore large prospective studies are needed to evaluate this subject.

There were also studies showing a possibility of relationship between the incidence of hematologic

Table 1: The demographic properties and seasonal-gender distribution of immune hemolytic anemia

				spring-summer		autumn-winter	
		N	%/ort ±SS	N	%	N	%
Gender	Male	12	32.4%	9	75.0%	3	25.0%
	Female	25	67.6%	10	40.0%	15	60.0%
Etiology	Primary	33	89.2%				
	Secondary	4	10.8%				
Atherosclerotic disease	-	20	54.1%				
	+	17	45.9%				
Autoimmune disorder	-	33	89.2%				
	+	4	10.8%				
Other chronic disorders	-	22	59.5%				
	+	15	40.5%				
Steroid treatment	-	3	8.1%				
	+	34	91.9%				
Secondary treatments	-	32	86.5%				
	+	5	13.5%				
Response to treatment	-	4	10.8%				
	+	33	89.2%				
Age		37	54.3+_16.6				

-: Negative +: Positive

Table 2: Hemolytic anemia attack per month (single group chi-square test)

		Observed value(N)	Expected Value(N)	P
Months	January	4	2.7	0.905
	February	3	2.7	
	March	1	2.7	
	April	2	2.7	
	May	2	2.7	
	June	2	2.7	
	July	4	2.7	
	August	5	2.7	
	September	3	2.7	
	October	2	2.7	
	November	2	2.7	
	December	2	2.7	

disorders other than malignancy and seasons. Raval et al. evaluated 73 patients with thrombotic thrombocytopenic purpura (TTP) and found no seasonal variation in TTP [10]. In contrast to previous study, Park et al. had found that, significantly more TTP attacks occurred in spring [11]. In a study from our country, the incidence of idiopathic thrombocytopenic purpura (ITP) increases significantly in the spring: the authors thought that this was related to pollen exposure in this season [12]. In another study one year later, it was shown that ITP, which lasted less than 3 months, was significantly higher in January and February, but there was no such monthly or seasonal change in chronic ITP [13]. The authors speculated that the peak seen in January and February was related to viral infections. Unlike previous studies, a retrospective trial with 69 181 patients in the United States did not detect any relationship between ITP-related hospitalizations and seasons [14]. These conflicting results showed that more studies are needed to investigate the relationship between seasons and non-malignant hematologic disorders.

We could only find one study about the relationship between autoimmune hemolytic anemia and seasons. Guntupalli et al. retrospectively evaluated 48 416 hospitalizations in United States from 2000

Table 3: The seasonal distribution of demographic and clinic characteristics of patients

		N	%	N	%	N	%	N	%	p
Gender	Male	3	25.0%	6	50.0%	2	16.7%	1	8.3%	0.122
	Female	4	16.0%	6	24.0%	3	12.0%	12	48.0%	
Etiology	Primary	7	21.2%	11	33.3%	4	12.1%	11	33.3%	0.649
	Secondary	0	0%	1	25.0%	1	25.0%	2	50.0%	
Atherosclerotic disease	-	4	20.0%	8	40.0%	2	10.0%	6	30.0%	0.678
	+	3	17.6%	4	23.5%	3	17.6%	7	41.2%	
Autoimmune disorders	-	7	21.2%	10	30.3%	5	15.2%	11	33.3%	0.539
	+	0	0%	2	50.0%	0	0%	2	0%	
Other additional disorders	-	5	22.7%	6	27.3%	3	13.6%	8	36.4%	0.829
	+	2	13.3%	6	40.4%	2	13.3%	5	33.3%	
Steroid treatment	-	1	33.3%	1	33.3%	0	0%	1	33.3%	0.849
	+	6	17.6%	11	32.4%	5	14.7%	12	35.3%	
Secondary treatments	-	6	18.8%	11	34.4%	5	15.6%	10	31.3%	0.556
	+	1	20.0%	1	20.0%	0	0%	3	60.0%	
Response to treatment	-	1	25.0%	2	50.0%	1	25.0%	0	0%	0.470
	+	6	18.2%	10	30.3%	4	12.1%	13	39.4%	
Age	Median±interquartile range		51.0±14.0		41.5±33.0		54.0±14.0		57.0±23.0	0.522

-: Negative +Positive

to 2012 and found a notable increase in winter and autumn, and a significant drop during summer months [15]. In our study, while there was no difference when seasons were grouped separately, a significant increase in frequency of hemolytic attacks was seen in men in autumn-winter and in women in spring-Summer seasons. This could be attributed to several reasons. Men tend to get diverse viral infections more easily compared to women and in autumn and winter, incidences of viral infections are also higher than other seasons, so this could explain the male predominance of hemolytic attacks in autumn-winter [16, 17]. Immune dysregulation, which was triggered by exposure to antigenic stimulus and derived by T helper 1 lymphocytes, was the major pathway for autoimmune disorders. Previous studies have shown that the number of circulating lymphocytes undergoes seasonal changes, reaching a peak in winter months [18]. So, this could also be a link to higher incidences of hemolytic attacks in males during winter period. In spring and autumn, pollen exposure is higher than the other seasons [19] and this led to more antibody production than the other seasons. In fact, some studies have shown that there could be a relationship between allergy and autoimmune disorders [20]. Duarte-García et al. found that arthritis and photosensitivity were more common in summer in women with systemic lupus erythematosus [21]. Hassan et al. also found significant SLE activity in sunny periods [22]. And lastly, Boctor et al. found a significant increase in the activity of lymphocytes in female patients, compared to males in summer periods when stimulated with Phytohemagglutinin and Concanavalin A [18]. So, both pollen stimulus and the increase in activity of lymphocytes, can lead to significant hemolytic attack activity in women. Additional unexplained factors may play a role in seasonal variation of immune hemolytic anemia, however additional prospective studies should be performed to evaluate this subject.

Limitations

Our study had some limitations. It was not a prospective study and the number of patients who participated in this study was not very large: a prospective study with a large number of patients is clearly needed. We could not reach the data regarding the antibody status of the patients. Etiology of warm and cold antibody-induced hemolytic anemia can be different, and it might have been interesting to see whether there was any significant seasonality between cold and warm antibody induced hemolytic attacks. Our study is however unique namely for being the first of its

kind in evaluating the subject in our country. Based on the inconsistent results from previous studies evaluating seasonality of hematologic disorders other than immune hemolytic anemia, repeated studies should be done to evaluate the seasonal variations. Our study highlights the subject for these reasons and can be a steppingstone for future research exploring hemolytic anemia.

Conclusion

In conclusion, male patients had suffered significantly more hemolytic attacks in the fall and winter periods compared to females, whereas females had a higher incidence of hemolysis in the spring and summer seasons. This could be attributable to antigenic stimulus, such as pollen exposure and viral infections or variations in activity of T lymphocytes to antigenic stimulus. Large, prospective studies are needed to explain the etiology of these variations.

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The Relationship Between Obesity, Depressive Symptoms and Sleep Duration in Children and Adolescents

Şişman Çocuk ve Ergenlerde Depresyonun Uyku Miktarı ve Şişmanlığın Derecesi ile İlişkisi

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ABSTRACT

Aim: In this study, we aimed to investigate the relationship between symptoms of depression and sleep duration with anthropometric measurements in obese children and adolescents.

Methods: Forty children and adolescents (25 girls) diagnosed with obesity were included. Sociodemographic and clinical data were obtained using the sociodemographic and clinical data form. Age, gender, height and weight values were recorded; body mass index (BMI), percentile and standard deviations (SDS) were calculated. Children's Depression Inventory (CDI) was used to assess depression. The degree of obesity was classified according to the BMI values of the cases, the cases in class 1 and 2 were grouped as "subgroup 1", and the cases in class 3 as "subgroup 2". Groups were compared in terms of sociodemographic features, depression and sleep duration.

Results: Mean age of the group was 12.8±2.6 years. There was a significant difference in patients with depression in terms of body weight, BMI, BMI-SDS and sleep duration compared to those without depression. CDI scores in subgroup 2 were significantly higher than subgroup 1. CDI scores were positively correlated with body weight, BMI and BMI-SDS and negatively correlated with sleep duration. Sleep duration was negatively correlated with body weight and BMI. **Conclusion:** Depressive symptoms and sleep duration may be associated with the severity of obesity in children. We consider that the assessment of depression and sleep disorders by a pediatric psychiatrist is important and useful in children with obesity.

Keywords: Obesity, depressive symptoms, body mass index, sleep

ÖZ

Amaç: Bu çalışmada amacımız şişman çocuk ve ergenlerde şişmanlık ile ilişkili antropometrik ölçümler ile depresyon ve uyku süresi arasındaki ilişkiyi araştırmaktır.

Yöntemler: Çalışmaya kırk şişman çocuk ve ergen dahil edildi. Vücut kitle indeksi yaş ve cinsiyete göre 95 persentil ve üstünde olanlar şişman olarak tanımlandı. Sosyodemografik ve klinik veriler yazarlar tarafından oluşturulan sosyodemografik ve klinik veri formu kullanılarak elde edildi. Çalışmaya katılan çocuk ve gençlere "Çocuklar için depresyon ölçeği (ÇDÖ)" doldurtuldu. Katılımcıların boy ve kilosu kaydedildi, vücut kitle indeksi (VKİ) ve vücut kitle indeksi standart sapması (VKİ-SDS) hesaplandı. Çalışmaya katılanlar VKİ değerine göre şişmanlık şiddeti açısından Sınıf 1 ve sınıf 2 olanlar 1.grup ve sınıf 3 olanlar 2. grup olarak gruplandırıldı. Gruplar sosyodemografik özellikler, depresyon ve uyku süresi açısından karşılaştırıldı.

Bulgular: Çalışmaya katılan 40 olgunun 25'i kızdı ve ortalama yaş 12.8±2.6 idi. Depresyonu olanlarda vücut ağırlığı, VKİ, VKİ-SDS ve uyku süresi depresyonu olmayanlara göre anlamlı olarak farklıydı. 2.grupta (Sınıf 3) ÇDÖ puanları anlamlı olarak daha yüksekti. ÇDÖ skorları vücut ağırlığı, VKİ, VKİ-SDS ile pozitif, uyku süresi ile negatif korelasyon göstermekteydi.

Sonuç: Şişman çocuk ve gençlerde yüksek depresyon skorları şişmanlık şiddeti ve azalmış uyku süresi ile ilişkili olabilir. Şişman çocuk ve ergenlerin depresyon ve uyku bozuklukları açısından bir çocuk ergen psikiyatrisi uzmanı ile birlikte takip edilmesinin önemli ve gerekli olduğu düşünülmektedir.

Anahtar Kelimeler: Şişmanlık, Depresif belirtiler, vücut kitle indeksi, uyku

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INTRODUCTION

Obesity has recently become an increasingly common public health problem with a rising prevalence among children and adolescents across the world.[1,2] So far, many different methods have been used for identifying and grading obesity in childhood. According to a concensus published in 2017, a child over 2 years of age with a body mass index (BMI) ≥ 95th percentile for age and sex is defined as "obese".[3] Severe obesity was found to be more common in girls prior to adolescence and following the onset of puberty in boys.[5] It has been suggested that children with a BMI value less than 120% of the 95th percentile value could be classified as "class 1", whereas those with a BMI value between 120% and 140% of the 95th percentile for age and sex defined as class 2, and those with a BMI value over 140% of the 95th percentile for age and sex classified as "class 3".[4]

It is well known that obesity leads to various metabolic problems such as atherosclerosis, diabetes and cardiovascular diseases [2], and is associated with psychosocial difficulties as well. Obese children, who have difficulties in relationships with peers due to their physical appearance may face bullying.[6] Moreover, obesity can negatively affect the development of self-esteem and affect quality of life by causing physical difficulties in children and adolescents. [7]

In addition to the physical and psychosocial difficulties, obesity in children and young adults can also provide a basis for psychiatric disorders. In a review and meta-analysis study, Luppiano et al. concluded that obesity increased the risk of depression and that depression increased the risk for obesity.[8] It has also been reported that depression and obesity might have common clinical features such as sleep disorders.[9] It has been suggested that obesity decreases sleep duration in children and as a result, the feeling of hunger increased, in addition to a decline in insulin sensitivity. Thus, a decrease in sleep time seems to be associated with the appearance or/ and the persistence of obesity.[9-11] Children under the age of 5 are recommended to sleep at least 11 hours a day, whereas those aged 5 to 10 years of age are recommended to sleep at least 10 hours a day and children over 10 years old are recommended to sleep at least 9 hours a day.[10] It has been observed that the presence of short sleep time (<10.5 hours) in children aged 3 years is associated with obesity at the age of 7 years. [12] Another report suggests that every single hour increase in sleep time decreases the risk of obesity by an average of 9%, and as a result, that children and adolescents should sleep longer to prevent excessive weight gain and obesity.[13] Similarly, sleep duration has been reported to be reduced in children and adolescents who were diagnosed with depression and that insomnia in depression, was found to be associated with an increased risk of suicide.[9] It was stated that untreated sleep problems could make it difficult to get rid of both depression and obesity and might increase the risk of suicide in obese children and adolescents with depression.[9] The treatment of sleep problems might thus be considered to be a target for the treatment of obesity and depression.

Taken together, evaluating obese children and adolescents in terms of depression and the amount of sleep they get, may be helpful to reduce complications and be a more effective treatment and follow-up process. To assess the impact of depression and sleep duration in the severity of obesity, we aimed to investigate the relationship between anthropometric measurements, such as body weight, BMI, BMI standard deviation (SDS), and percentiles that have been used in defining and grading obesity with depression and sleep duration in obese children and adolescents.

PATIENTS AND METHOD

Forty children and adolescents who applied to the outpatient pediatric endocrinology clinic of our hospital and were diagnosed with obesity, were included in our study. Patients with a BMI ≥ 95% based on age and gender, were defined as "obese".[14] The subjects with endocrinopathies such as hypothyroidism, hypercortisolism and syndromes leading to obesity, were excluded. The study group consisted of children and adolescents without a diagnosis of mental retardation, autism spectrum disorder or psychotic disorder. Parents of all patients gave their informed consent for their child to participate in the study after its protocols

had been fully explained. This study was approved by the Local Ethics Committee of the HSU, Zeynep Kamil Women and Children's Diseases Training and Research Hospital on 19.12.2018 (approval number:160) and adhered to the Declaration of Helsinki.

Clinical interviews were conducted with children and their families who agreed to participate in the study and the scales were applied. Sociodemographic and clinical data, including sleep duration, were obtained using the sociodemographic and clinical data form created by the authors. Sleep duration was calculated by the data provided by parents, which was based on the sleep and wake up time of the children. Psychical examination of the patients was performed by experienced pediatric endocrinologists (A.G and B.Ö) and rating scales were applied by a nurse in the clinic, just after the clinical evaluation. Age, gender, height and weight values were recorded and the body mass index (BMI) was calculated with the (BMI) = weight (kg) / height2 (m2) formula; percentile and standard deviations (SDS) were calculated by using the ÇEDD Çözüm software (TPEDS Metrics) and evaluated according to the age and gender-appropriate charts.[15] The degree of obesity was classified according to the BMI values of the cases. Obese children with a BMI value less than 120% of the 95th percentile value were classified as “class 1”, whereas those with a BMI value between 120% and 140% of the 95th percentile for age and sex were defined as class 2, and those with a BMI value over 140% of the 95th percentile for age and sex as “class 3”. Cases in class 1 and 2 were grouped as “subgroup 1”, and the cases in class 3 as “subgroup 2”.[4]

Socioeconomic level (SEL) was established using the Hollingshead-Redlich scale.[16] Five different socioeconomic levels are defined in the scale: the highest socioeconomic level is stated as 1 and the lowest as 5.

The Turkish version of the “Children’s Depression Inventory (CDI)” [17] was used to assess depression. This scale is a 27 item self-report scale consisting of 3 options, where each item is scored between 0-2. The highest score that can be obtained on this scale is 54 and 19 is the cut-off value for depression. Higher scores indicate greater severity of depression.

Obesity subgroups were compared in terms of sociodemographic features, depression and sleep duration. Children and adolescents with depression (CDI score ≥ 19) were compared to patients without depression (CDI <19) in terms of sociodemographic and clinical features, as well as sleep duration. Correlation analysis were performed in terms of potential relationships between the severity of depression, anthropometric measurements and sleep duration in obesity.

Statistics

All the analysis were performed using the Statistical Package for the Social Sciences 22. The Shapiro-Wilk method was used to determine whether the data was normally distributed. Descriptive statistics were presented as numbers and percentages for categorical variables, mean±standard deviation or median (interquartile range) for continuous variables depending on normal distribution. The Chi-square test was used to compare ratio of gender. The Student t test or Mann-Whitney test was used compare continuous variables according to CDI and sociodemographic status, depending on parametric test assumptions. The Spearman rank correlation coefficient was calculated for the relationships between CDI score, anthropometric measurements and sleep duration. For all tests, a p-value of less than 0.05 was accepted as statistically significant.

RESULTS

Of the 40 obese children and adolescents in our study, 25 were girls (62.5%) and 15 (37.5%) were boys. The mean age of the subjects was 12.8±2.6 years. When analyzed in terms of socioeconomic level (SEL), 14 cases (35%) were at the 4th socioeconomic level (SEL). The sociodemographic and clinical characteristics of the participants are summarized in Table 1.

The number of patients with a CDI score of ≥19 was eight. When the study group was divided into two groups as CDI score <19 and CDI score ≥19, there was no significant difference between the groups in terms of age, gender and SEL (Table 1). There was a significant difference between patients with depression in terms of body weight

(p = 0.006), body weight SDS (p <0.001), BMI (p <0.001), BMI-SDS (p <0.001) and sleep duration (p = 0.002), compared to the patients without depression.

Table 1: Sociodemographic and clinical features of the whole group and comparisons in terms of CDI

	Whole Group n=40 Means (SD)	CDI<19 n=32 (80%) Means (SD)	CDI≥19 n=8 (20%) Means (SD)	p
Mean age (years)	12.8±2.6	12.6±2.8	13.5±2	0.376
Gender				0.052
Female	25 (62.5%)	17 (42.5%)	8 (20%)	
Male	15 (37.5%)	14 (35%)	1 (2.5%)	
SEL				0.159
1	2 (5%)	1 (2.5%)	1 (2.5%)	
2	5 (12.5%)	4 (10%)	1 (2.5%)	
3	11 (27.5%)	7 (17.5%)	4 (10%)	
4	14 (35%)	14 (35%)	-	
5	8 (20%)	6 (15%)	2 (5%)	
BW (kg)	81.4±24.9	76.3±21.3	101.7±29.3	0.006
BW-SDS	3.22±1.34	2.87±1	4.6±1.68	<0.001
Height (cm)	157.2±12.7	155.4±12.8	164.6±9.7	0.267
Height-SDS	0.65±1.3	0.51±1.3	1.19±1.2	0.108
BMI (kg/m2)	31.23±5.9	31±4.6	37.1±8.1	<0.001
BMI-SDS	2.86±0.69	2.69±0.53	3.5±0.9	<0.001
BMI %	99.31±1	99.2±1.08	99.78+0.34	0.141
Sleep Duration (minutes)	509±79	527±61	436±105	0.002

SEL: Socioeconomic level, BW: Body Weight, BW-SDS: Body Weight Standard Deviations, BMI: Body Mass Index, BMI-SDS: Body Mass Index Standard Deviation, SD: Standard deviation, CDI: Children's depression inventory

When the obesity subgroup analysis were performed, there was no significant difference found between the groups in terms of age, gender and SEL. However, CDI scores in subgroup 2 (Class 3) were significantly higher than subgroup 1 (Class1+Class2) (p = 0.003). There were no significant differences found between subgroup 2 and subgroup 1 in terms of sleep duration (p = 0.251) (Table 2).

According to the correlation analyses, CDI scores were positively correlated with body weight (r=0,445; p=0.004), body weight SDS (r=0.475; p=0.002), BMI (r=0.497; p=0.001) and BMI-SDS (r=0.523; p=0.001) and negatively correlated with sleep duration (r=-0.363; p=0.021). Moreover, sleep duration was negatively correlated with body weight (r=-0.619, p<0.001) and BMI (r=-0.607, p<0.001).

Table 2: Sociodemographic and clinical features of Subgroups 1 and 2

	Subgroup 1 (Class1 + Class 2)	Subgroup 2 (Class 3)	p
Mean age (years)	12.5±2.67	13.3±2.6	0.372
Gender			
Female	19 (47.5%)	6 (15%)	0.522
Male	10 (25%)	5 (12.5%)	
SEL			
1	-	2 (5%)	0.205
2	4 (10 %)	1 (2.5%)	
3	9 (22.5%)	2 (5%)	
4	10 (25%)	4 (10%)	
5	6 (15%)	2 (5%)	
CDI score	10.6±6.7	18.6±8.5	0.003
Sleep Duration (minutes)	518±64	485±109	0.251

SEL: Socioeconomic level, CDI: Children's Depression Inventory

DISCUSSION

Our study investigated the relationship between the anthropometric features related to obesity, symptoms of depression and sleep duration, in children and adolescents who were diagnosed with exogenous obesity. The rate of depression according to the CDI in our group was 20%. In comparison, it has been reported that the prevalence of depression in children and adolescents was around 2% and 4-8%, respectively [18], whereas Sutaira et al. detected depression in 10.4% of the obese children and reported that the risk of depression was higher in obese children compared to non-obese children.[19] Obesity has been suggested to be a risk factor for the development of depression and similarly, depression might be a risk factor for the development of obesity.[8] The relationship between obesity and depression may be due to the influence of the shared environment, common physiological or genetic factors.[20] Depression in obese individuals has been thought to be associated with the chronic inflammatory process of obesity, dysregulation of the hypothalamic-pituitary adrenal axis, psychosocial effects, neuroendocrine changes and negative effects of insulin resistance on the brain. [8,21-25] Similarly, depression has been found to play a role in the development of obesity due to psychosocial and neuroendocrine changes, in addition to the unhealthy lifestyle features such as decreased physical exercise, poor eating

patterns and irregular sleep, observed in patients diagnosed with depression [8]. Furthermore, the same researchers reported that the relationship between overweight and depression was weaker than the relationship between severe obesity and depression. [8] This data is compatible with our results: in our cases, it was concluded that the severity of depression increased in line with the increasing severity of obesity. Physical strain, pain and disruption of the body image due to the increasing severity of obesity might affect the development and severity of depression. In clinical settings, it is important to evaluate children and adolescents with severe obesity in terms of comorbid depression. Accompanying depression in obese children may complicate the lifestyle arrangements and as a result, may affect the treatment response. In particular, physicians in pediatric clinics should keep in mind the diagnosis of depression in obese children and adolescents, and refer them for a child and adolescent psychiatry evaluation.

Studies that were focused on the relationship between sleep duration and obesity suggested that there might be a relationship between decreased sleep time and obesity, and that increasing sleep duration could prevent children from the development of obesity. In addition, it has been proposed that when sleep duration decreased in those with depression, that duration of sleep might therefore be a risk factor for both obesity and depression. [10] Reeves and colleagues [9] reported that treatment of depression became complicated, and the risk of suicide increased in children and adolescents who slept less. It was stated that reduced amount of sleep in children with obesity might make treatment of obesity difficult and that providing adequate sleep could be an important factor to prevent obesity in children.[10] In our study, it was concluded that the amount of sleep decreased in obese children and adolescents with depression, compared to those without depression. Moreover, it was observed that duration of sleep decreased as body weight and body mass index increased. Our study supports the view that the amount of sleep might be associated with the severity of obesity and symptoms of depression. Assessment of sleep duration during follow-up and, if inadequate, adjusting the amount of sleep accordingly, may increase the success of the treatment in obese children and adolescents. Providing adequate sleep may help to increase the quality of life and lead to a healthier life for obese children and adolescents, by improving the symptoms of obesity and depression.

Limitations: There were some limitations in our study, namely that the data stems from a cross-sectional evaluation and does not allow for the establishment of a causal relationship. In addition, since the patients included in our study were selected from a clinical sample, it makes it difficult to generalize our results; the absence of a control group might also be considered as a limitation. Additionally, diagnosis of depression was made by CDI, the data about sleep duration was primarily based on information from parents and there we had a greater number of female than male participants in our study. Finally, the low number of patients enrolled in the study and the low number of patients with depression and severe obesity may be another limitation, and as a result of this low number, the effect of adolescence itself on depression was not be investigated.

Conclusion: Our study revealed that the severity of depression and parameters of obesity were positively associated in children and adolescents. In addition, a reduced amount of sleep was associated with both depression and severity of obesity. Clinicians should keep in mind that assessment of depression and sleep are therefore important factors in the evaluation and the intervention of obesity, in children and adolescents. A comprehensive multidisciplinary approach, including a child and adolescent psychiatrist, may also be beneficial in these cases, and those with severe obesity in particular should be evaluated for depression. Recognition and treatment of accompanying depression and sleep deprivation are thought to be important for the effective treatment of obesity. Further prospective studies with larger groups of obese children from clinical and non-clinical samples are needed to investigate the association between sleep duration, psychiatric symptoms and features of obesity.

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Increased Cesarean-Section Birth Rates and Affecting Related Factors

Artmış Sezaryen-Doğum Oranları ve Etkileyen İlgili Faktörler

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ABSTRACT

Aim: The present study aims to evaluate the opinions of physicians on increased C-section delivery rates and their coping ways working at different institutions.
Method: This descriptive, cross-sectional study included a total of 200 obstetricians and gynecologists with an experience of at least 20 years chosen through simple random sampling among registered in the Republic of Turkey, Ministry of Health da-tabase. In the questionnaire, their opinions about test exams and their opinions about solutions to decrease C-section rates were investigated.
Results: The majority of the participants (89.0%) responded "Strongly Agree" to the following item: "Factors other than medical causes may affect the decision for C-sec-tion". Similarly, the majority of the physicians responded "Strongly Agree" to the item on the increase in C-section rates along with malpractices and social pressure put by the patient and her relatives(89.0% and 89.5%, respectively). For the majority of the participants (84.0% and 85.0%, respectively), the main suggestions to overcome the increased C-section delivery rates was to lower; social pressure put against the physicians by the patient and her relatives, and removal of malpractice penalties re-latedfor possible adverse outcomes during normal delivery. In this study, the majority of the physicians responded "Strongly Disagree" to the items related to the imposing penalties and granting bonuses (56.0% and 56.5%, respectively). Similarly, 81.0% of the physicians responded "Strongly Disagree" to the item stating that healthcare planners correctly interfere with the main cause of increased C-section rates. **Conclusions:** The most important reasons for the high cesarean rates are seen as the fear of malpractice and social pressure of physicians working in both public and private sectors.

Keywords: Cesarean-section rates, physician, reason, factors

ÖZ

Amaç: Bu çalışmada farklı kurumlarda çalışan hekimlerin sezaryen doğumlardaki artış nedenleri ve çözüm yolları ile ilişkili görüşlerinin değerlendirilmesi amaçlanmıştır.
Yöntem: Kesitsel tipte ve tanımlayıcı olarak yapılan bu araştırmada T.C. Sağlık Bakan-lığı sistemine kayıtlı ve en az 20 yıl mesleki tecrübesi olan 200 kadın hastalıkları ve doğum uzmanı hekime ulaşılarak anket uygulanmıştır. Ankette hekimlerin sezaryen oranları artışı nedenleri ile ilgili görüşleri ve sezaryen oranlarını azaltmak için çözüm yolları ile ilgili görüşleri araştırılmıştır.
Bulgular: Ankete katılan tüm hekim grubunun önemli bir kısmı (%89.0) 'Sezaryen kararı alırken tıbbi nedenler dışında faktörler sezaryen kararını etkiler' maddesine 'kesinlikle katılıyorum' yanıtını vermiştir. Benzer şekilde hekimlerin çoğunluğu 'mal-praktis uygulamaları' ve'hasta ve yakınlarının yarattığı baskının' sezaryen doğum kararını arttırdığı ilişkin görüşe kesinlikle katılmaktadır (sırasıyla %89.0 ve %89.5). Katılımcıların %85'i sezaryen oranlarındaki artışı önlemek için çözüm yolu olarak; va-jinal doğumda oluşabilecek olumsuz sonuçlara ilişkin malpraktis cezalarının kaldırıl-masını,%84'ü vajinal doğumdaki olası olumsuz sonuçlar için hasta ve yakınları tarafından hekime uygulanan sosyal baskıyı azaltmayı görmektedir. Bu çalışmada hekimlerin çoğunluğu çözüm yolu olarak görülen cezalandırma veya ücret yoluyla ödüllendirme ile ilgili önerilere (sırasıyla %56.0 ve %56.5) "kesinlikle katılmıyorum" cevabı vermişlerdir. Yine hekimlerin %81'i sağlık planlayıcılarının sezaryen oranların-daki artışa doğru şekilde müdahale edebildiği fikrine kesinlikle katılmamaktadır.
Sonuç: Sezaryen oranlarının yüksek seviyelerde olmasında; hem kamu hem de özel sektörde çalışan hekimler açısından malpraktis korkusu ve sosyal baskı en önemli nedenler olarak görülmektedir.

Anahtar Kelimeler:Sezaryen oranı, hekim, sebep, faktörler

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INTRODUCTION

Cesarean section (C-section) delivery is a common surgical procedure in the presence of maternal or fetal conditions preventing normal delivery [1]. The World Health Organization (WHO) Department of Reproductive Health and Research states that the effects of C-section rates on other health outcomes such as maternal and perinatal morbidity, newborn outcomes, and psychological and social well-being are still unclear and, thus, C-section procedure is ideal only when is there a medical indication [2]. The department also proposes that C-section rates higher than 10% at the population level are not associated with reductions in maternal and newborn mortality rates. That ideal C-section rates should be 10 to 15% [2]. In a large-scale study including data from 150 countries, C-section was performed with a rate of 6 to 27.2% worldwide, and there was an increase at a rate of 12.4% between 1990 and 2014 [3].In previous studies conducted in Turkey, the overall C-section rate was found to be 14.3% in 1998 and increased up to 51.9% in 2013[4]. In 2015, Turkey ranked first in C-section delivery in the world among the Organisation for Economic Co-operation and Development countries [5].

The possible causes of steady increases in C-section rates have been still investigated, and no single specific cause has been identified, yet. Studies conducted in Turkey have mostly addressed medical causes [6-11]. These include patient characteristics such as advanced maternal age, obesity, fear of being in labor for hours, opinion that it is safer for baby, decreased tolerance to any kind of complications, and medical causes such as the opinion of healthcare providers that C-section is safer for delivery. However, none of these provides a sufficient explanation for increased rates of C-section delivery.

In recent years, physicians-related factors have also been suggested to play a role in the increased rates of C-section. Obstetricians and gynecologists are more likely to be sued and convicted to higher indemnity costs [12]. In Turkey, malpractice cases encourage physicians to perform defensive medical practices. Cakmak et al. [13] reported that malpractice occurred in C-section deliveries, mostly by physicians (92.3%) in private hospitals (46.2%).However,to the best of our knowledge, there are no data available about in our country regarding physician opinions leading them to perform C-section, except for malpractices (long working hours, understaffed facility, working environment-related problems, or payment issues). In the present study, we aimed to evaluate the opinions of physicians on increased C-section delivery rates and their coping ways working at different institutions.

MATERIAL AND METHODS

Study Population

This descriptive, cross-sectional study included a total of 300 obstetricians and gynecologists with an experience of at least 20 years, 100 of whom were chosen through simple random sampling among 1,518 hospitals registered in the Republic of Turkey, 2019 Ministry of Health database between February 2019 and December 2019. As all the universe was aimed to be reached, the sample was not taken. Accordingly, a total of 200 physicians (66.7%) were able to be reached. All participants were informed about the nature of the study, and a written informed consent was obtained. The study protocol was approved by AlaaddinKeykubatUniversity,Clinical Research Ethics Committee (Date: 03.09.2019, No. 10-11). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Development of a Likert-type Scale

Independent variables of the study were considered as sociodemographic features of the physicians. In contrast, dependent variables were considered as their opinions regarding the increase in C-section delivery and their suggestions for a solution. The item pool was formed consisting of the opinions on the causes of the increase in C-section rates, other than medical indications, and reducing these rates. For the development of attitude items in the pool, relevant studies were screened, and 30 opinions, including positive or negative meaning, were noted. These opinions were scaled,ranging from “Strongly Disagree” (starting from 1) to “Strongly Agree” (5) on a five-point Likert-type scale. A literature lecturer provided counselling for grammar and language development. Content and psychological structure analysis was performed

by academicians of obstetrics and gynecology and psychiatry.

Based on relevant amendments according to suggestions, a scale consisting of 30 items regarding the opinions on the increased C-section rates and its possible solutions was administered to a pilot group of 30 participants. Items-total correlations of the data collected in the pilot-scale were calculated using the SPSS version 21.0 software (IBM Corp., Armonk, NY, USA). Items with an item-total correlation value lower than 0.40 were excluded from the scale by the researchers, considering that these items were inadequate for item measuring. Finally, 17 items of a 30-item scale were used.

Reliability of Scale

Reliability of the scale was analyzed with the test-retest reliability using the SPSS version 21.0 software (IBM Corp., Armonk, NY, USA). A Cronbach alpha ( ) value of 0.751 was established for internal consistency.The calculated reliability coefficient indicates a medium level ofinternal consistency among the items.

Statistical Analysis

Statistical analysis was performed using the SPSS version 21.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max), or number and percentage.The Kolmogorov-Smirnov test was used to evaluate whether the data showed a normal distribution or not. The Student's t-test and chi-square test were used to compare the variables. The Mann-Whitney U test was carried out to compare Likert-type question responses. A pvalue of <0.05 was considered statistically significant.

RESULTS

In thisstudy, the scale was responded by a total of 200 obstetricians. Among the physicians included in the study, 25.5% worked in private hospitals, while 74.5% worked in state hospitals. There was no significant difference in age (p:0.815), sex (p:0.242), and professional experience (p:0.297) between the physicians working in the state and private hospitals. Sociodemographic characteristics of the participants are shown in Table 1.

Table 1. Sociodemographic characteristics of participants

Variable	Public Service (n=149)	Private practice (n=51)	Test statistics	p value
Age	50.4 ± 2.1	50.7 ± 2.3	T:1.092	0.275
Sex (Female)	23.5%	15.7%	χ2:1.371	0.242
Professional experience (year)	20.1 ± 3.6	20.4 ± 2.1	T:1.322	0.297

Note:Data are given in mean ± standard deviation. Chi-square and Student'st-test for group comparisons.

According to the responses of the participants regarding the causes of increased C-section rates other than good medical practices, there was no significant difference between the responses of the physicians working in the state and private hospitals(81.2% and 82.3%, respectively, χ2:0.101, p:0.750).

The median value was found to be 5 (Strongly Agree) for the following items: "C-section rates have always increased throughout my career"; "Factors other than medical causes may affect the decision for C-section"; and "Increased malpractice penalties associated with normal delivery may increase the C-section preference".A comparison of the opinions of the participants regarding the causes of the increase in C-section rates other than good medical practices is presented in Table 2.

The coping ways and suggestions of the participants to reduce the C-section rates other than good medical practices were evaluated, and there was no significant difference between the physicians working in the state and private hospitals in all answers (Table 3). The median value was 5 (Strongly Agree) for the following items: "Excluding complications from the judicial punishment scope except for professional committees" and "Lowering negative social pressure put against the physicians by the patient and her relatives for possible negative outcomes during normal delivery".In addition, the median value was 1 (Strongly Disagree) for the following items: "Imposing penalties when C-section rates are not reduced to the desired level" and "Granting bonuses for normal delivery."

The majority of the physicians responded "Strongly

Table 2. A comparison of the opinions of the participants regarding the causes of the increase in C-section rates other than good medical practices

Item	Public service (n=149)			Private practice (n=51)			Test statistics	p value
	Mean	SD	Median	Mean	SD	Median		
C-section rates have always increased throughout my career.	4.89	0.47	5	4.90	0.36	5	0.235	0.815
Factors other than medical causes may affect the decision for C-section.	4.82	0.57	5	4.86	0.45	5	0.333	0.739
Increased malpractice penalties associated with normal delivery may increase the C-section preference.	4.80	0.64	5	4.88	0.39	5	0.287	0.774
Social pressure put by the patient and her relatives has an effect on increased C-section rates.	4.81	0.61	5	4.88	0.38	5	0.261	0.794
Hospital and delivery room conditions have an effect on increased C-section rates. ¬	2.66	0.90	2	2.94	0.78	5	0.090	0.929
Lacking midwife follow-up and assistance has an effect on increased C-section rates.	2.97	0.87	3	2.65	0.91	3	0.098	0.922
Social life planning due to long working hours has an effect on increased C-section rates.	1.10	0.30	1	1.09	0.27	1	0.466	0.641
Low financial gain of normal delivery for physicians has an effect on increased C-section rates.	2.58	0.96	2	2.57	1.02	2	0.140	0.889
Healthcare planners correctly interfere with the main cause of increased C-section rates.	1.19	0.39	1	1.20	0.40	1	0.128	0.898

Note:SD: Standard deviation. Mann-Whitney U test for group comparisons.

Table 3. Coping ways of the participants other than good medical practices

Item	Public service (n=149)			Private practice (n=51)			Test statistics	p value
	Mean	SD	Median	Mean	SD	Median		
Imposing penalties when C-section rates are higher than expected	1.39	0.58	1	1.45	0.67	1	0.353	0.724
Removal of malpractice penalties related to the negative consequences that may occur in vaginal delivery	4.74	0.65	5	4.84	0.41	5	0.704	0.482
Granting bonuses when C-section rates are reduced to desired level	1.50	0.61	1	1.49	0.64	1	0.259	0.796
Imposing penalties when C-section rates are not reduced to desired level	1.50	0.61	1	1.49	0.64	1	0.259	0.796
Enhancing the level of knowledge and communication capability of midwives and other allied healthcare providers who assist delivery	2.97	0.87	3	2.94	0.78	3	0.098	0.922
Improving the physical setting of delivery	2.66	0.91	2	2.65	0.91	2	0.090	0.929
Granting bonuses for normal delivery	2.58	0.96	2	2.57	1.02	2	0.140	0.889
Lowering social pressure put against the physicians by the patient and her relatives for possible negative outcomes during normal delivery	4.73	0.70	5	4.57	0.56	5	0.620	0.535

Note:SD: Standarddeviation. Mann-Whitney U test for group comparisons.

Agree" to the item on the increase in C-section rates along with malpractices and social pressure put by the patient and her relatives (89.0% and 89.5%, respectively). For the majority of the participants (84.0% and 85.0%, respectively), the main suggestions to overcome the increased C-section delivery rates was to lower social pressure put against the physicians by the patient and her relatives, andto remove malpractice penalties relatedfor possible adverse outcomes during normal delivery. In this study, the majority of the physicians responded "Strongly Disagree" to the items related to the imposing penalties and granting bonuses (56.0% and 56.5%, respectively). Similarly, 81.0% of the physicians responded "Strongly Disagree" to the item stating that healthcare planners correctly interfere with the main cause of the increased C-section rates.

DISCUSSION

In the current study, the opinions of physicians on increased C-section delivery rates and their coping ways working in state or private setting were evaluated. The study results showed that there was no significant difference in the opinions regarding the causes of increased C-section rates other than good medical practices between the physicians working in the state and private hospitals.

With the adoption of Medical Malpractice Law in 2005, the rate of C-section delivery has dramatically

increased in Turkey[4,5]. In the present study, our cohort is a representative sample as having minimum 20-year professional experience and working as a specialist both before and after the execution of Medical Malpractice Law, which allows them to evaluate the effect of the law on their own C-section rates. To illustrate, the rate of C-section delivery was lower before the execution of the law, while the rate significantly increased after the law was enacted [4,5]. This supports the opinion that the C-section delivery rate has been on a dramatic rise since the adoption of the Medical Malpractice Law for the majority of our study participants. In addition, the majority of the participants (89.0%) responded “Strongly Agree” to the following item: “Factors other than medical causes may affect the decision for C-section”. Similarly, the physicians (89.5%) responded “Strongly Agree” to the item on the increase in C-section rates along with malpractices.Italy has the highest rate of births through C-section in Europe, with 34% [3]. In recent studies, C-section delivery rates have increased in parallel with the increased malpractices and that penalties to be paid per case have also logarithmically increased, and that physicians are concern about malpractice laws [14]. Accordingly, it is stated that the increase in C-section rates is associated with malpractice laws in the United States of America, and this association is more evident in those who are undereducated and with a low socioeconomic status [15]. It is also considered that the implementation of malpractice law may decrease normal delivery rates following C-section [16]. In a study including 2,300 physicians, the cumulative C-section rates increased among physicians sued for malpractice over the years [17]. The Penalty Code negatively affects many physiological and emotional decision-making mechanisms [16-17]. Based on the current evidence, malpractices significantly and permanently affect medical decision-making mechanisms of physicians.

In the present study, the majority of the physicians working in the state and private hospitals responded “Strongly Agree” with a rate of 89.5% to the item suggesting that social pressure put by the patient and her relatives affects increased C-section rates. For 84.0% of the respondents, the main suggestion to overcome this concern is to lower social pressure put against the physicians by the patient and her relatives for possible negative outcomes during normal delivery. In particular, negative perception and physical violence against physicians appear to be an important problem in Turkey. In the literature, it has been reported that healthcare providers are the group with the highest risk of being subjected to violence, and there has been an increase in acts of violence in years [18]. It is also known that many healthcare providers are subjected to or witness verbal abuse, whether reported or not,and violence is not only committed by patients themselves, but also their relatives and the mass media [19]. The relationship between the physician and the patient is considered to be one of the main factors affecting the medical decision-making process [20]. It is thought that social pressure increases the stress level of healthcare providers, affects their general state of health, causes problems related to early retirement, and leads to low-quality service offered by professionals to the patients [21]. In recent years, the introduction of advanced technology and communication tools has increased the expectation of patients about visiting “error-free, beyond compare” physicians. They are expected to be target-oriented and reach the solution as soon as possible. The increasingacts of violence and all these expectations adversely affect the general states of health of physicians and the decision-making process of the delivery type.

The C-section delivery rate, which was approximately 7% in 1993, almost doubled after fiveyears. According tothe 1998 Turkey Demographic and Health Survey database, the overall birth rate with C-section delivery is 13.9%. In subsequent studies, this rate ranges between 21.2 and 36.7% in 2008, and is about 48.0% in 2013, although population-based studies would provide more insight into this issue [22,23]. According to the WHO report published in 2014 [2], C-section rates higher than 10% at the population level are not associated with reductions in maternal and newborn mortality rates, and C-section should be only medically applied, when necessary.

In recent years, the Republic of Turkey, Ministry of Health has employed financial penalties for exceeding individualspecificC-section rate; however, these precautions have fallen behind the expected level [22].This supports the opinion

of the majority of our participants that healthcare planners are unable to correctly interfere with the leadingcause of the increased C-section rates and granting bonuses. Imposing penalties are insufficient measures, when C-section rates are not reduced to the desired level. In this study, the majority of the physicians responded “Strongly Disagree” to the items related to the imposing penalties and granting bonuses (56.0% and 56.5%, respectively. Similarly, 81.0% of the physicians responded “Strongly Disagree” to the item stating that healthcare planners correctly interfere with the leadingcause of increased C-section rates.Bonus and penalty scheme is one of the oldest systems which is designated to motivate an individual to change his/her behavior [23], and penalty system is at the lowest level of Kohlberg’shierarchy of moral stages and commonly used [24]. Penalty system negatively affects the performance and productivity of the personnel and associated with increased illegal and unethical behaviors of the personnel [25]. Similarly, one of the most important criticisms on performance system related to bonus system is that it adopts a customer-oriented understanding rather than a patient-oriented mindset and puts financial gains to the forefront which creates a conflict between healthcare providers and patients [26]. Developing these organizational systems, restoring trust between patients and physicians, providing justice for physicians, and giving mutual responsibilities may be helpful to achieve the most optimal results.

Limitations: The study evaluated the opinion of senior obstetricians, although this result may not represent all population working as an obstetrician in Turkey, and that may be caused lack of population validity. Also,aLikert type-scale was used in this study to understand reasons for the underlying increase in C-section rates that can limit the scope of thestudy and, thus, the overall outcome. Further well-designed, qualitative studies are needed to gain a better understanding of clinical and patient’s decision for C-section.

Conclusion: In conclusion, the increased C-section rates in Turkey are one of the significanthealth issues. Interventions to eliminate possible causes seem to be insufficient, and malpractices and social pressure are among the most prominent factors which increase the C-section delivery for both physicians working in the state and private settings. Necessary precautions should be taken to reduce C-section rates in the practice of obstetrics and gynecology. However, further studies are needed to draw a definite conclusion on this topic.

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The Effect of Retrograde Intrarenal Surgery on Kidney Function in Renal Stone Treatment

Böbrek Taşı Tedavisinde Retrograd İntrarenal Cerrahinin Böbrek Fonksiyonuna Etkisi

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ABSTRACT

Aim: In this study, we aimed to evaluate the alteration in renal function by analyzing the estimated glomerular filtration rate after Retrograde Intrarenal Surgery, and present the factors which have an impact on alteration.
Methods: We analyzed 88 patients who underwent RIRS for renal stones between May 2018 and February 2019, prospectively. Estimated glomerular filtration rate was calculated by modification of diet in renal disease (MDRD) formula preoperatively, on the first postoperative day, on the first postoperative month and the third postoperative month. All data was recorded on the follow-up form. All procedures were performed under general anesthesia.
Results: The stone-free rate of the study was 79.5%. Preoperative eGFR was 99.86 mL/min/1.73m^2 for the study group. The eGFR was calculated 101.80 mL/min/1.73m^2 on the first postoperative day and 111.66 mL/min/1.73m^2 on the third-month follow-up. The change in eGFR was 1.94 mL/min/1.73m^2 in the early period and 11.8 mL/min/1.73m^2 in the long-term follow-up period. There was a statistically significant improvement in eGFR in the long-term follow-up period than the early period when compared to preoperative renal function.
Conclusion: On the contrary of former stone removal modalities, RIRS can stabilize postoperative kidney function. It may even help improve postoperative kidney function in patients with preoperative renal dysfunction. Urologists may keep in mind the RIRS option in patients with this condition.

Keywords: ureteroscopy, lithotripsy, kidney, function

ÖZ

Amaç: Bu çalışmada, Retrograd İntrarenal Cerrahi sonrasında eGFR'yi analiz ederek böbrek fonksiyonundaki değişikliği değerlendirmeyi ve değişikliği etkileyen faktörleri sunmayı amaçladık.
Yöntem: Mayıs 2018 ile Şubat 2019 arasında böbrek taşı nedeniyle RIRS yapılan 88 hastayı prospektif olarak analiz ettik. eGFR, ameliyat öncesi, ameliyat sonrası ilk gün, ameliyat sonrası ilk ay ve ameliyat sonrası üçüncü ayda MDRD formülü ile hesaplandı. Tüm veriler takip formuna kaydedildi. Tüm işlemler genel anestezi altında yapıldı.
Bulgular: Çalışmanın taşsızlık oranı %79,5 idi. Preoperatif eGFR 99,86 mL/dak/1.73m$_2$ idi. eGFR, postoperatif ilk günde 101.80 mL/dak/1,73m^2 ve üçüncü ay takibinde 111,66 mL/dak/1,73m^2 olarak hesaplandı. eGFR'deki değişiklik erken dönemde 1,94 mL/dak/1,73m^2 ve uzun dönem takip döneminde 11,8 mL/dak/1,73m^2 idi. Üçüncü ay takiplerinde preoperatif böbrek fonksiyonuna göre ve erken döneme göre eGFR'de istatistiksel olarak anlamlı bir iyileşme vardı.
Sonuç: Eski taş çıkarma yöntemlerinin aksine RIRS, postoperatif böbrek fonksiyonunda stabilizasyon sağlayabilmektedir. Hatta preoperatif böbrek fonksiyonu bozuk olan hastalarda operasyon sonrası böbrek fonksiyonlarında düzelmeye yardımcı olabilmektedir. Ürologlar, ameliyat sonrası böbrek fonksiyonlarında iyileşme olmasını istedikleri hastalarda RIRS seçeneğini akılda tutabilirler.

Anahtar Kelimeler: üreteroskopi, litotripsi, böbrek, fonksiyon

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INTRODUCTION

Shock wave lithotripsy (SWL), retrograde intrarenal surgery (RIRS) and percutaneous nephrolithotomy (PNL) are the recommended interventions for urinary system stones, depending on the localization and size of the stone. Beside the stone's characteristics, the anatomic features of the kidney influence the decisions regarding disease management [1]. The purpose of the treatment is to achieve high stone-free rates, avoiding surgical complications. Although SWL is a minimally invasive option, the need for multiple sessions and low success rates for the stones located in lower calyx, are the common disadvantages. Additionally, possible complications such as renal hematoma may lead to death [2].

The favourite modality for larger stones is PNL, which may lead to parenchymal damage when the surgeon has to make multiple punctures to access the collecting system during the procedure, and that may also result in a decrease in renal function. Furthermore, PNL may result in some severe surgical complications [3, 4]. As a result, RIRS has been accepted as a minimally invasive treatment for urinary system stones because of few surgical complications and the fact that these are low grade. It has therefore increased in popularity among urologists, even for larger stones, because of its high success rate and that it offers more comfort for the patients.

Several studies evaluated the glomerular filtration rate alteration after PNL or SWL. The results showed that renal functions did not deteriorate in the mid or long term after the intervention, echoing the data in the literature which revealed that resolving the obstruction in the kidney improved the renal function, when compared to preoperative status. However multiple punctures during PNL could have some adverse effects on renal functions [4-8].

Flexible ureteroscopes and laser fibers were designed for use under irrigation fluids. Ureteral access sheaths (UAS) were used to facilitate the manipulation of ureteroscopes and laser fibers, and also provide a stable fluid pressure in the renal collecting system [9]. However stone burden, residual stone fragments and hemorrhage during the procedure may reduce the discharge of the irrigation fluid, and result in higher intrarenal pressure than required. High pressure leads to renal calyceal distension which may cause renal functional deterioration [10]. The other possible reason for renal malfunction is the direct damage of the laser energy to the renal parenchyma when dusting the stone in calyces [11].

In this study, we aimed to evaluate the alteration in renal function by analyzing the estimated glomerular filtration rate (eGFR) after RIRS and present the factors which have an impact on alteration.

MATERIAL AND METHOD

After the approval from the institutional review board (Decision Number: 2017-KAEK-189_2018.05.30_13), we analyzed 88 patients who underwent RIRS for renal stones between May 2018 and February 2019 prospectively. Patients who have prior double J catheter (DJ), solitary kidney, a history of chronic renal failure, concomitant ureteral stone, ureteral stricture or urinary tract anomaly and missing data during follow-up, were excluded from the study. All patients were evaluated with non-contrast computerized tomography (CT) preoperatively and postoperatively. Patients with stones located in different sites of the kidney were investigated by complete blood count, urine analysis, urine culture, urea, creatinine and routine biochemical tests before the operation. The characteristics of the stone and the patient were recorded on the follow-up forms. The sum of all longest dimensions of all stones was recorded as the stone size in case of multiple stones. The stone density was assessed by the CT in Hounsfield Unit (HU). The time between starting endoscopy and end of DJ stent insertion was defined as operation time. Estimated glomerular filtration rate (eGFR) was calculated on the day before the surgery, by modification of diet in renal disease (MDRD) formula:

(eGFR $=175 \times (\text{Serum creatinine})^{-1.154} \times (\text{age})^{-0.203}$ x0.742 [if female]x1.212 [if Black]).

The calculation was repeated on the post-operative first day, on the post-operative first month and the post-operative third month. All data

were recorded on the follow-up form. The patients were treated with appropriate antibiotics when a urinary tract infection was diagnosed, and all interventions were performed after a sterile urine culture obtained.

Informed consent was obtained from all patients. Intravenous first-generation cephalosporin was administered 30 minutes before the surgery for the surgical prophylaxis. All procedures were performed under general anesthesia. First, the surgeon accessed the ureter by a 9.5 F ureteroscope (Karl Storz®, Tuttlingen, Germany) for a safe dilatation under the guidance of a guidewire. The 7.5 F ureteroscope was used to reach the stone in the RIRS procedure. Ureteral access sheath (Elite Flex®, Ankara, Turkey) was placed in the ureter in all RIRS cases. A 7.5 F flexible ureteroscope (Flex-X2®, Karl Storz, Tuttlingen, Germany) was used for RIRS. A 200 mm laser fiber (Ho YAG Laser; Dornier MedTech®; Munich, Germany / Dornier Med-Tech GmbH, Medilas H20 and HSolvo, Wessling, Germany) was used for laser lithotripsy. The energy of the laser was between 0,8 – 1,5 Joule and 8 – 15 Hz. At the end of the operation, a ureteral stent was placed in all patients. Operation time was defined from the beginning of cystoscopy to the end of ureteral stent placement. Intraoperative data were recorded and patients who had no complication were discharged on the postoperative first day.

All patients were checked with a complete blood count, urine analysis and biochemical tests perioperatively. On the first month follow-up, in cases of no residual stone fragments requiring auxiliary interventions, the DJ catheter was displaced after the patient was examined and checked with an X-Ray. All laboratory and screening findings were recorded in the follow-up form. The patients were monitored with CT and routine laboratory tests on the third-month follow-up. The follow-up period for all patients was a minimum of three months after surgery.

All analyses were carried out using the SPSS 25.0 statistical software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). The data distributions were evaluated using the Kolmogorov-Smirnov test. In case of discordance between the graphics and test results, skewness and kurtosis values were considered. Comparisons of preoperative eGFR and postoperative eGFR were performed by paired samples t-test. Other numerical data were analyzed using Student's t-test and for categorical data, the chi-square tests were used. The possible influencing factors (age, gender, hydronephrosis, obstruction, stone location, stone volume, stone density, preoperative creatinine level) on renal functional alterations, were analyzed with binominal regression analyses; $p<0.05$ was considered as statistically significant.

RESULTS

The study population consisted of 88 patients, 54 (61.4%) of whom were males and 34 (38.6%) were females. The mean age of the patients was 46.43 ± 14.768 years-old, and the mean body mass index (BMI) was 28.20 ± 5.64. Twenty-one patients had comorbid diseases such as hypertension, diabetes mellitus, coronary artery disease, chronic respiratory disease and others. The mean stone volume was 751.86 ± 689.00 mm3. The demographic data of the patients and stone characteristics are shown in Table 1.

The stone-free rate of the study was 79.5%. Totally 14 (15.9%) patients had surgical complications which were mostly low-grade. Only two patients required surgical intervention: one for DJ catheter migration and one for renal colic because of steinstrasse. The operation outcomes are shown in Table 1.

Preoperative mean eGFR was 99.86 ± 2.55 mL/min/1.73m^2 for the study group. Mean eGFR was calculated 101.80 ± 2.66 mL/min/1.73m^2 on the first postoperative day and 111.66 ± 2.34 mL/min/1.73m^2 on the third-month follow-up. Mean change in eGFR was 1.94 ± 15.66 mL/min/1.73m^2 in the early period and 11.8 ±14.58 mL/min/1.73m^2 in the long-term follow-up period. There was a statistically significant improvement in eGFR in the long-term follow-up period than the early period, when compared to preoperative renal function ($p=0.023$). Twenty patients had improvement and only three patients had a deterioration of their chronic kidney disease stage. The comparison of eGFR alteration and renal functional outcomes preoperative and postoperative periods are shown in Table 2 and 3.

Table 1. Characteristics Of Patients and Stones and Operation Outcomes

Parameters	n=88
Age (year)	46.43 ± 14.768
Gender (n,%) Male Female	 54 (61.4%) 34 (38.6%)
Comorbidity (n,%) Hypertension Diabetes Mellitus Coronary Artery Disease Chronic Respiratory Disease Other	 6 (6.8%) 7 (8.0%) 3 (3.4%) 1 (1.1%) 4 (4.5%)
Obstruction (+/-) (n,%)	47 (53.4%)/41 (46.6%)
Hydronephrosis (n,%) No Grade 1 Grade 2 Grade 3 Grade 4	 41 (46.6%) 13 (14.8%) 30 (34.1%) 4 (4.5%) 0 (0%)
Stone Localization (n,%) Upper Calyx Middle Calyx Lower Calyx Renal Pelvis Ureteropelvic Junction Multicaliceal	 6 (6.8%) 7 (8.0%) 18 (20.5%) 31 (35.2%) 17 (19.3%) 9 (10.2%)
Number Of Stones	1.50 ± 0.87
Stone Volume (mm^3)	751.86 ± 689.00
Operation Time (minute)	67.33 ± 34.82
Fluoroscopy time (second)	16.18 ± 12.62
Hospitalization (day)	1.44 ± 2.18
Stone-free (%)	79.5%
Complication (n,%) Grade I Grade II Grade III Grade IV Grade V	 8 (9.1%) 4 (4.5%) 2 (2.2%) 0 (0%) 0 (0%)
Mean Pre-operative eGFR (mL/min/1.73m^2)	99.86 ± 2.55
Mean Post-operative 1st Day eGFR (mL/min/1.73m^2)	101.80 ± 2.66
Mean Post-operative 3rd Month eGFR (mL/min/1.73m^2)	111.66 ± 2.34

BMI: Body Mass Index; eGFR: Estimated Glomerular Filtration Rate

The multinominal regression analysis revealed that the higher preoperative creatinine levels (p=0.001) and female gender (p=0.018) were predictive factors for improvement after RIRS. The absence of obstruction had a negative correlation with deterioration after RIRS (p=0.021).

Table 2. Comparison of eGFR Alteration During Follow-Up

	Follow-Up Period		eGFR Change	p
Mean eGFR (mL/min/1.73m^2)	Pre-operative	Postoperative 1st day		
	99.86 ± 2.55	101.80 ± 2.66	1.94 ± 15.66	0.247
	Preoperative	Postoperative 3rd month		
	99.86 ± 2.55	111.66± 2.34	11.8 ± 14.58	p=0.023

eGFR: Estimated Glomerular Filtration Rate

Table 3. Renal Function Analysis

	Preoperative	Postoperative 3rd month
Group (n,%) Stage I Stage II Stage III Stage IV Stage V	 61 (69.3%) 22 (25%) 4 (4.5%) 1 (1.1%) 0 (0%)	 74 (84.1%) 13 (14.7%) 1 (1.1%) 0 (0%) 0 (0%)
CKD Stage Improvement (n,%)		20 (22.7%)
CKD Stage Deterioration (n,%)		3 (3.4%)

CKD: Chronic Kidney Disease

DISCUSSION

There are several reasons specified for renal failure. In a meta-analysis, it is reported that urinary obstruction precipitates urinary tract infections. The harmful metabolites during infection result in damage to renal parenchyma and compromise renal function. The other point stated in the research are the effects of surgical treatment. Open surgery may directly damage the renal tissue, which deteriorates the kidney function. Endoscopic urinary system stone treatment may increase the intrarenal pressure and lead to pyelovenous reflux, which deteriorates the urine flow.

Increased intrarenal pressure may result in increased renal vascular resistance. In addition, age, gender and genetics have a role in kidney diseases [12, 13]. Urolithiasis is one of the factors leading to infection, obstruction and surgery; thus, numerous studies have evaluated the relationship between urinary system stone diseases and renal function. The other focus has been the alteration of renal function after stone treatment.

Resolving the obstruction enhances the urine flow, decreases the intrarenal pressure and improves renal function; in addition, removing the stone in the tract eliminates the obstruction and the triggered infection, thus ameliorating the kidney function [11, 14]. On the other hand, a recent study reported that there was no alteration at the molecular level after the operation [15]. One of the limited number of studies conducted for the evaluation of the relationship between flexible ureteroscopy and renal function, reported that acute kidney injury was observed in one patient (0.06%) and only 5 (3%) patients had deteriorated renal function on the first postoperative day [16]. A review about the impact of urinary stone removal on renal function reported that alteration in the early period was transient and usually negligible, however it was more evident when the patients were evaluated with molecular markers [11]. The outcomes of this study are compatible with the literature and showed no statistically significant alteration in renal function on the postoperative first day (p=0.247).

Renal functions may be more stable in the late-term evaluation because early-term evaluation may be influenced by many postoperative metabolic factors. Demirtas et al. revealed that the kidney functions decreased in the very early hours after surgery then recovered in the late-term follow-up [17]. Reeves et al. reported that kidney functions for all CKD group patients improve in long term follow-ups; however increase in CKD group II, IIIa and IIIb patients were statistically significant, but others were not [18]. Desai et al. stated that all patients in their study had a stable renal function at the three months follow-up [19]. Similar to the literature, we observed a statistically significant improvement in eGFR at the three-month follow-up, compared to preoperative levels in our study (p=0.023). Assessment of renal function after an intervention has been widely studied in the literature. Hoarau et al. stated that they encountered a change towards a better CKD stage in 23 patients (14.1%) and worse in 8 (4.9%) after stone removal. In another study, Lee et al. reported that only 5.9% of patients in their cohort had improved eGFR [20]. In this study, twenty patients (22.7%) improved eGFR and CKD stage at third month, while only three (3.4%) deteriorated and 65 (73.9%) remained stable. Although we observed three deteriorations, none of them needed dialysis and all of them were CKD stage I patients who had worsened into CKD stage II. Renal functions improvement in our study was higher than reported in the literature because previous researchers did not use UAS and place the DJ stent routinely; they also had patients with renal insufficiency and multiple procedures while we did not include these group of patients in our research.

The multinominal regression analysis revealed that preoperative renal failure and female gender were the predictive factors for improvement on renal function. On the other hand, the absence of urinary tract obstruction was negatively correlated with renal function deterioration. Piao et al. reported that female gender was a predictive factor of renal function improvement on the third month follow-up and explained this difference by stating that the female hormones had inhibitor roles during the inflammatory processes [21]. The other predictive factors reported in the literature were pre-existing chronic kidney disease, setting off multiple procedures [11, 16]. Reeves et al. stated that none of the factors such as age, preoperative eGFR, stone size and operative time were found to be significant predictors [18].

Limitations of the study: Although there is a lack of articles in the literature evaluating the renal function change in patients with urinary system stones after the intervention, we conducted this study to assess renal function. Nonetheless, our study has some limitations: single-center design of the study, a relatively small number of patients and using the only eGFR as the assessment tool of renal function. A large patient population should be evaluated with various assessment tools to

obtain more reliable results.

Conclusion: Retrograde intrarenal surgery and laser lithotripsy are safe and efficient treatment options in patients with renal stone. They allow high stone removal and low complication rates, and all patients are discharged the same day of the operation, when no complication is encountered. To the contrary of former stone removal modalities, RIRS can stabilize postoperative kidney function and it may even help improve postoperative kidney function in patients with preoperative renal dysfunction. Urologists may keep in mind the RIRS option in patients in this condition. However, multicenter, prospective randomized controlled trials with a large population are necessary to confirm the results of our study.

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The diagnostic value of parathormone washout in Tc-99m MIBI negative primary hyperparathyroidism cases

Tc-99m MIBI Negatif Primer Hiperparatiroidizm Olgularında Parathormon Yıkamanın Tanısal Değeri

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ABSTRACT

Aim: To determine the sensitivity and positive predictive value (PPV) of the measurement of parathormone (PTH) in fine needle aspiration (FNA) washout fluid, in the preoperative localization of hyperfunctional parathyroid lesions.
Methods: Medical records of patients diagnosed with primary hyperparathyroidism (PHPT) in our clinic between 2016-2020 were retrospectively evaluated. Thirty-six patients with PHPT who underwent preoperative FNA-PTH washout procedure were included in the study. FNA-PTH washout was only performed in patients with negative technetium-99m methoxy isobutyl isonitrile / single photon emission computed tomography (Tc-99m MIBI/SPECT) imaging. It was accepted to be higher than plasma PTH level as positive cut-off value for PTH washout in determining parathyroid lesions. Sensitivity, PPV, false positive, false negative and diagnostic accuracy values of PTH washout were calculated.
Results: PTH washout was false positive in 2 cases, false negative in 1 case and true positive in 33 cases. In the discrimination of true parathyroid lesions, the sensitivity of PTH washout was calculated as 97.05%, PPV 94.29% and diagnostic accuracy 91.67%. PTH washout levels correlated positively with plasma PTH and parathyroid lesion volüme (respectively, r=0.347, p=0.041 ve r=0.356, p=0.036). All patients tolerated the FNA-PTH flushing procedure well and no complications developed afterwards.
Conclusion: The FNA-PTH washout is a safe and useful method to localise parathyroid lesions in PHPT patients with negative Tc-99m MIBI/SPECT imaging.

Key words: Fine needle aspiration, MIBI/SPECT negative, primary hyperparathyroidism, washout

ÖZ

Amaç: Hiperfonksiyon gösteren paratiroid lezyonlarının preoperatif lokalizasyonun-da ince iğne aspirasyonu (İİA) yıkama sıvısında parathormon (PTH) ölçümünün du-yarlılık ve pozitif prediktif değer (PPD)'ini belirlemek.
Yöntem: 2016-2020 yılları arasında kliniğimizde primer hiperparatiroidi (PHPT) tanısı alan hastaların tıbbi kayıtları retrospektif olarak inceledik. Operasyon öncesi İİA-PTH yıkama prosedürü uygulanan 36 PHPT hastası çalışmaya alındı. İİA-PTH yıkama, sadece teknesyum-99m metoksi-isobutil-isonitril / tek foton emisyonlu bilgisayarlı to-mografi (Tc-99m MIBI/SPECT) negatif hastalarda yapıldı. Serum PTH seviyesinden daha yüksek PTH yıkama seviyeleri, pozitif kesme değeri olarak tanımlandı. İİA-PTH yıkama prosedürünün duyarlılık, PPD, yanlış pozitif, yanlış negatif ve tanısal doğruluk değerleri hesaplandı.
Bulgular: PTH yıkama 2 olguda yanlış pozitif, 1 olguda yanlış negatif ve 33 olguda gerçek pozitifti. Gerçek paratiroid lezyonların ayırt edilmesinde PTH yıkamanın du-yarlılığı %97.05, pozitif prediktif değeri %94.29 ve tanısal doğruluğu %91.67 olarak hesaplandı. PTH yıkanma seviyeleri, serum PTH ve paratiroid lezyon hacmi ile pozitif koreleydi (sırasıyla, r=0.347, p=0.041 ve r=0.356, p=0.036). Tüm hastalar İİA-PTH yıkama işlemini iyi tolere etti ve sonrasında herhangi bir komplikasyon gelişmedi.
Sonuç: İİA-PTH yıkaması, Tc-99m MIBI/SPECT negatif PHPT hastalarında paratiroid lezyonlarını lokalize etmek için güvenli ve kullanışlı bir yöntemdir.

Anahtar sözcükler: İnce iğne aspirasyonu; MIBI/SPECT negatif; primer hiperparatiroidizm; yıkama

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INTRODUCTION

Primary hyperparathyroidism (PHPT) is an endocrine disease characterized by hypercalcemia as a result of excessive parathormone (PTH) secretion of one or more parathyroid glands [1]. Although the traditional treatment of PHPT is bilateral neck exploration surgery, minimally invasive parathyroidectomy (MIP) has become the preferred method thanks to the development of tabpreoperative imaging methods. MIP has advantages, such as shorter operation time and hospital stay, smaller incision requirement, faster recovery and less pain. The selection of patients suitable for MIP and the success of surgery depend on the precise localization of the preoperative hyperfunctional parathyroid lesion [2, 3].

The most common imaging methods for the detection of hyperfunctional parathyroid lesions are parathyroid ultrasonography (US) and technetium-99m methoxy isobutyl isonitrile (Tc-99m MIBI) parathyroid scintigraphy [4, 5]. When Tc-99m MIBI is used together with single photon emission computed tomography (SPECT), its sensitivity in determining parathyroid lesion increases [6]. In a meta-analysis, the sensitivity and positive predictive value (PPV) of Tc-99m MIBI/SPECT imaging were 78.9% and 90.7%, respectively [6]. In PHPT cases that cannot be localized with parathyroid US and scintigraphic imaging, images such as SPECT / computerized tomography (CT), cervical magnetic resonance imaging and 18F-fluorocholine positron emission tomography (PET)/CT can also be used [8]. However, even when all imaging methods are performed, there are still patients whose parathyroid adenoma cannot be localized.

In PHPT cases with negative Tc-99m MIBI imaging, PTH measurement in the washout fluid of fine needle aspiration (FNA) of suspicious lesions identified by the US is an important procedure [9]. Although there are different recommendations for the positive cut-off value of the PTH wash, no consensus has yet been reached.

In this study, we aimed to present, together with our clinical experience, the cut-off value of FNA-PTH washout after US-guided FNA of suspected lesions identified by parathyroid US in PHPT cases with negative Tc-99m MIBI/SPECT imaging.

MATERIAL AND METHOD

The current study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki. All participants were informed and provided written informed consent.

Selection of Samples

The medical files of patients who were diagnosed with PHPT in our clinic and then underwent parathyroidectomy between 2016 and 2020, were scanned retrospectively. Thirty-six patients in whom in was not possible to detect a parathyroid lesion with Tc-99m MIBI/SPECT imaging and could undergo the US-guided FNA-PTH procedure, were included in the study [mean age 54.47±2.49 years, 27 females (75%), 9 males (25%)]. Demographic features, preoperative laboratory parameters (serum corrected calcium (cCa), phosphorus, magnesium, creatinine, albumin, PTH, 25-hydroxy vitamin D, 24-hour urine calcium excretion), clinical findings related to PHPT (osteoporosis, urolithiasis) and characteristics of suspicious lesions detected by preoperative US, were all recorded. Total serum calcium levels were corrected according to serum albumin levels [10].

The diagnosis of PHPT was made based on the presence of normal or high plasma PTH levels in case of hypercalcemia [1]. Parathyroid US and scintigraphy imaging were performed to determine preoperative localization.

Parathyroid US was performed with a high-resolution device with a 6-13 MHz linear transducer (Philips EPIQ 5, DEU). FNA-PTH washout was only performed in patients with negative Tc-99m MIBI/SPECT imaging.The volume of parathyroid adenoma was calculated by the ellipsoid model formula (length x thickness x width x 0.52)

FNA-PTH Washout Procedure

Neck skin was cleaned with 10% povidone iodine. After entering the suspicious lesion using a sterile 25 gauge needle and 10 mL syringe under US guidance, negative pressure was applied several times with a back-and-forth motion. After the aspirate material came to the injector, it was washed with 1 ml of 0.9% normal saline. Tubes were centrifuged and PTH was analyzed in

supernatant liquid.

PTH levels of both plasma and FNA washout fluid were measured by electro-chemiluminescence immunoassay (Elecsys PTH, Roche Diagnostics, Mannheim, Germany). It was determined that PTH washout was higher than plasma PTH level as a positive cut-off value in identifying parathyroid lesion. All patients with PHPT were operated with the minimally invasive parathyroidectomy method. Histopathological findings were evaluated in surgical specimens.

Biochemical analysis

Serum calcium, phosphorus, magnesium, alanine aminotransaminase (ALT), creatinine, albumin and urine calcium concentrations were measured using a Beckman Coulter AU 5800 chemistry analyzer (Beckman Coulter, Brea, CA, USA). The corrected calcium (cCa) was calculated using the following equation: cCa = [(4 - albumin) × 0.8] + Ca.

Serum 25-hydroxyvitamin D [25(OH)D] was measured by electro-chemiluminescence immunoassay (Elecsys Vitamin D; Roche Diagnostics, Mannheim, Germany).

Statistical Analysis

All statistical analysis were done using the SPSS 22.0. The Shapiro-Wilk test was performed to evaluate the distributions of variables. The data was given as mean ± standard deviation for the variables with normal distribution and was given as the median±interquartile range for those who did not have normal distribution. The sensitivity, PPV, negative predictive value (NPV) and accuracy of PTH washout in determining parathyroid lesions were calculated. True positive, false positive and false negative results of PTH washout were determined based on postoperative histology reports. Correlations between variables were evaluated with the Spearman and Pearson correlation tests. A result of $p < 0.05$ was determined to be statistically significant.

RESULTS

Demographic, clinical and preoperative laboratory features of the groups are given in Table 1. Twelve of the patients with PHPT (33.3%) had osteoporosis and 10 (27.8%) had urolithiasis.

Preoperative PTH washout levels and clinical characteristics of patients with PHPT are given in Table 2. After histopathological evaluation of the parathyroidectomy samples, parathyroid hyperplasia was found in 7 patients (19.4%), parathyroid carcinoma in 1 (2.8%), and parathyroid adenoma in 28 (77.8%). There was no significant difference in terms of PTH washout, plasma PTH and volume of parathyroid lesion between patients with parathyroid adenoma and parathyroid hyperplasia.

Table 1. Preoperative demographic and laboratory characteristics of patients with primary hyperparathyroidism

	Results
Age (years)*	54.47±12.49
Gender (female/male)	27/9
Plasma PTH (ng/L)	139±105
25(OH)D3 (ng/mL)	17.25±14.75
Creatinine (mg/dL)	0.85±0.3
ALT (U/L)	21±6
cCa (mg/dL)	11.16±0.81
Phosphorus (mg/dL)*	2.60±0.49
Magnesium (mg/dL)	2±0.28
Albumin (g/dL)*	4.28±0.41
Urine calcium excretion (mg/day)*	301.51±153.105

Datas expressed as median (±IQR) and *mean (±SD). Parathormone, PTH; 25-hydroxy vitamin D3, 25(OH)D3; alanine aminotransaminase, ALT; corrected calcium, cCa

Table 2. Preoperative PTH washout and clinical characteristics of patients with primary hyperparathyroidism

	Results
PTH washout (ng/L)	4750±15245
Volume of adenoma (cm3)	1.83±0.58
Urolithiasis (n, %)	10 (%27.8)
Osteoporosis (n, %)	12 (%33.3)

Datas expressed as median (±IQR)

PTH washout was false positive in 2 cases, false negative in 1 case and true positive in 33 cases. The sensitivity of PTH washout in determining true parathyroid lesions was 97.05%, PPV 94.29% and diagnostic accuracy 91.67%. No complications developed in any patient after the FNA-PTH washout procedure.

There was a positive correlation between plasma PTH and PTH washout levels ($r=0.347$, $p=0.041$). In addition, there was a positive correlation

between parathyroid lesion volume with plasma PTH and PTH washout levels (r=0.601, p=0.001 and r=0.356, p=0.036, respectively).

DISCUSSION

To our knowledge, this was the largest case study evaluating the diagnostic benefit of PTH washout in PHPT cases with negative Tc-99m MIBI/SPECT imaging. We support the fact that FNA-PTH washout is a safe and useful method for detect whether there is a hyperfunctional parathyroid lesion of suspicious lesions detected by parathyroid US.

Location of the parathyroid lesion is the most important factor affecting surgical success in primary hyperparathyroidism [11]. The most widely used first-line imaging technique for the localization is Tc-99m MIBI. However, there is false negativity in approximately 25% of cases [12]. In a previous study, we found the sensitivity to be 90.4% and the PPV to be 90.2% when we used the parathyroid US and scintigraphy images together [13]. It has been suggested that false negative parathyroid scintigraphy imaging may be related to the histological features and the small size of the lesions [14]. In addition, it has been suggested that parathyroid scintigraphy images can be more useful, especially in cases with hyperparathyroidism with higher serum PTH and calcium levels [15, 16]. In this study, PTH levels of the patients were moderately elevated (139±105 ng/L). In PHPT cases with negative Tc-99m MIBI imaging, FNA-PTH washout is a valuable procedure in determining whether suspicious images detected by US belong to a hyperfunctional parathyroid lesion. With the help of this method, parathyroid lesions can be distinguished from lymphoid and thyroid-derived lesions [9].

There are studies suggesting different positive cut-off values for PTH washout. Gokcay et al. found sensitivity at 90.3% and specificity at 88.9% in determining parathyroid lesion when they received 436.5 pg/mL as the positive cut-off value for PTH washout [17]. Kiblud et al. showed that a PTH washout result of more than 1 000 pg/mL had sensitivity of 75% and PPV of 74% in the verification of parathyroid tissue [18]. When aspiration materials of non-parathyroid lesions are diluted with saline, PTH washout levels are expected to be lower than plasma PTH [19]. There are some studies supporting this idea: in the studies where PTH washout/plasma PTH ratio was accepted as positive cut off above 1, they detected 100% of PPV and specificity of PTH washout [20-23]. In the current study, we also accepted PTH washout/plasma PTH ratio to be above 1 as a positive cut-off value, and we determined the sensitivity of PTH washout to be 97.05%, and PPV to be 94.29%. Gokçay et al. found that the PTH washout/plasma PTH ratio over 3.05 had a specificity of 89% [17]. This data suggests that when higher PTH washout / plasma PTH ratio is accepted as positive cut-off value, it may increase false negative results.

Studies have a shown positive correlations between parathyroid lesion volumes and PTH washout levels [20, 21]. In accordance with the literature, we also found a positive correlation between parathyroid lesion volume and the PTH washout level (r=0.356, p=0.036). Since the PTH washout level correlates between parathyroid lesion size, accepting a fixed number as a positive cut-off value for PTH washout may result in an increase in false negative or false positive results.

More than 90% of PHPT cases are due to parathyroid adenoma, while 6% are due to parathyroid hyperplasia. In this study, 19.4% of PHPT cases were parathyroid hyperplasia. The reason why these are higher than the literature data, may be related to the fact that FNA-PTH washout was only performed in patients with negative Tc-99m MIBI/SPECT imaging. Xue et al. showed that patients with parathyroid hyperplasia have a higher negative Tc-99m MIBI imaging rate [24].

There are some factors limiting the application of FNA-PTH washout. Firstly, it is necessary to identify a potential parathyroid lesion by parathyroid US. Secondly, sufficient aspiration should be performed with a fine needle from the determined lesion: insufficient sampling can cause false negative results. The volume of Tc-99m MIBI negative parathyroid lesions is usually small [25]. In accordance with the literature, parathyroid lesion volume was indeed found to be very low in the current study. In 50% of our cases, the largest size of the parathyroid lesion was less

than 1 cm. In addition, it should be noted that when the lesion is too deep, it may be difficult to reach it with a needle. In this study, only one patient had PTH washout level lower than plasma PTH level. Complications such as bleeding, hematoma, pain and hoarseness may develop as a result of the FNA procedure [9, 25]. However, our cases tolerated the procedure well and no complications developed after the procedure.

Limitations :Our study has some limitations. It is primarily a retrospective study. In addition, we could not comment on the specificity of the procedure, since patients with PTH washout levels lower than plasma PTH (except 1 patient) were not procedured. Studies involving a larger number of patients on which PTH washout negative cases are performed could better demonstrate the effectiveness of this procedure.

Conclusıon: US-guided FNA-PTH washout is an important method in detecting hyperfunctional parathyroid lesions with negative Tc-99m MIBI/SPECT imaging. We suggest that PTH washout is higher than plasma PTH as the positive cut off value during this procedure. However, further studies with a greater number of cases in different institutions are needed to confirm the positive cut-off value of PTH washout.

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RESEARCH ARTICLE

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Relationship between Whole Blood Viscosity and Lower Extremity Peripheral Artery Disease Anatomical Complexity and Symptom Severity

Tam Kan Viskozitesi ile Alt Ekstremite Periferik Arter Hastalığının Anatomik Kompleksliği ve Semptom Şiddeti Arasındaki İlişki

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ABSTRACT

Aim: Increased blood viscosity (BV) has good correlaton with lower extremity peripheral artery disease (LEAD). However, the relationship between BV and peripheral arterial disease (PAD) anatomical complexity and symptom severity have not been studied adequately so far. The aim of the present study was to assess the relationship between whole blood viscosity (WBV) and LEAD anatomical complexity and symptom severity.

Methods: The study included 240 consecutive patients with suspected PAD who had lower extremity peripheral angiography between March 2016 and March 2020. A Transatlantic İntersociety Consensus II (TASC II) A-B lesion was defined as anatomical simple LEAD, and a TASC II C-D lesion was defined as anatomical complex LEAD. Symptom severity of all patients were categorized from 0 to 6 according to Rutherford classification. WBV was assessed using a validated calculation formula derived from hematocrit and total plasma protein levels, both at low (LSR) and high (HSR) shear rate.

Results: TASC II C-D group presented significantly higher WBV values both at LSR (40.2 ± 9.5 vs. 46.5 ± 13.2; p < 0.001) and HSR (15.9 ± 0.5 vs. 16.5 ± 0,7; p < 0.001). In ROC analysis, a cut-off value of 16.1 WBV at HSR had 74.5% sensitivity and 68% specificity for predicting TASC II C-D (AUC: 76.2%, p < 0.001) and a cut-off value of 42.9 WBV at LSR had 73.4% sensitivity and 66.6% specificity for predicting TASC II C-D (AUC: 74.2%, p < 0.001). In multivariate analysis, both high WBV at LSR (OR: 2.121, 95% CI: 1.079 – 3.164, p < 0.001) and high WBV at HSR (OR: 2.737, 95% CI: 1.671 – 4.483, p < 0.001) were independent predictors for TASC II C-D. There was a significant positive correlation between WBV at LSR and Rutherford symptom category (0-6) (r = 0.412, p <0.001) and WBV at HSR and Rutherford symptom category (0-6) (r = 0.402, p <0.001).

Conclusion: Our data suggests that; increased WBV values may be associated with TASC II C-D lesions, which indicated more anatomically complex LEAD. Also WBV values positively correlated with Rutherford symptom severity.

Keywords: Blood viscosity, lower extremity, peripheral arterial disease

ÖZ

Amaç: Artmış kan viskozitesinin (KV), alt ekstremite periferik arter hastalığı (AEPAH) ile iyi korelasyonu vardır. Ancak, KV ve peripherik arter hastalığının (PAH) anatomik kompleksiliği ve semptom şiddeti arasındaki ilişki şu ana kadar yeterince çalışılmamıştır. Bu çalışmanın amacı, tam kan viskozitesi ve alt ekstremite periferik arter hastalığının anatomik kompleksliği ve semptom şiddeti arasındaki ilişkiyi değerlendirmektir.

Yöntemler: Çalışmaya Mart 2016 ile Mart 2020 tarihleri arasında alt ekstremite periferik anjiyografisi yapılan ve PAH şüphesi olan 240 ardışık hasta dahil edildi. Transatlantic Intersociety Consensus II (TASC II) A-B lezyonu anatomik olarak basit AEPAH olarak, TASC II C-D lezyonu anatomik olarak kompleks AEPAH olarak tanımlandı. Tüm hastaların semptom şiddeti Rutherford sınıflandırmasına göre 0 ile 6 arasında kategorize edildi. Tam kan viskositesi (TKV), hem düşük (DKH) hem de yüksek kayma hızında (YKH) hematokrit ve toplam plazma protein seviyelerinden türetilen doğrulanmış bir hesaplama formülü kullanılarak değerlendirildi.

Bulgular: TASC II C-D grubu hem DKH'de (40.2 ± 9.5'e karşı 46.5 ± 13.2; p <0.001) hem de YKH'de (15.9 ± 0.5'e karşı 16.5 ± 0,7; p <0.001) anlamlı olarak daha yüksek TKV değerleri gösterdi. ROC analizinde, YKH'deki 16,1'lik TKV kesme değeri TASC II C-D'yi tahmin etmek için % 74,5 duyarlılık ve % 68 özgüllük (p <0,001) gösterdi. DKH'deki 42,9'luk TKV kesme değeri TASC II C-D'yi tahmin etmek için %73,4 duyarlılık ve %66.6 özgüllük (p <0.001) gösterdi. Çok değişkenli analizde, hem DKH'de yüksek TKV (OR: 2.121, % 95 CI: 1.079 - 3.164, p <0.001) hem de YKH'de yüksek TKV (OR: 2.737, % 95 CI: 1.671 - 4.483, p <0.001) TASC II C-D için bağımsız predictor olarak saptandı. DKH'deki TKV ile YKH'deki TKV değerleri Rutherford semptom kategorisi ile (0-6) anlamlı pozitif korelasyon gösterdi (r = 0.412, p <0.001; r = 0.402, p <0.001, sırasıyla).

Sonuç: Verilerimiz gösteriyor ki; artmış TKV değerleri, daha anatomik olarak kompleks AEPAH'ı gösteren TASC II C-D lezyonları ile ilişkili olabilir. Ayrıca TKV değerleri, Rutherford semptom şiddeti ile pozitif korelasyon gösterdi.

Anahtar Kelimeler: Kan viskozitesi, alt ekstremite, periferal arteriyel hastalık

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INTRODUCTION

From mild plaque formation to chronic total occlusion lower extremity peripheral arterial disease (PAD) is a spectrum of atherosclerotic disease. There can be asymptomatic patients in the peak of the disease, however, symptoms may occur such as pain in the leg in any form (resting or active), gangrene and intermittent claudication within the process of the disease progression. Clinical representation of the disease is often draw parallel with the austerity of atherosclerosis [1]. TASC II sorting is the unity of ideas that is utilized for the evaluation of lower extremity peripheral arterial disease (LEAD) in consonance with the anatomic distribution and number and nature of lesions (stenosis, occlusion). The suggested classification for grading the symptom severity of PAD is the Rutherford classification [2]. TASC II classification, indicates the anatomical complexity of LEAD. Rutherford classification, indicates the symptom severity of LEAD.

A common cardiovascular disease that occur by the inflammation and endothelial dysfunction of which result in occlusive plaque formation within the arterial wall is atherosclerosis. Thus, endotelial shear stress (ESS) and haematological factors have a crucial role in the pathophysiology of the atherosclerotic process. The most crucial predictive factor of ESS is blood viscosity (BV) [3] whose major predictive factor is hematocrit, is a primary property of blood correlated to its internal erosion that causes blood to withstand flow. In consonance with the data, whole blood viscosity (WBV) has been proven as the predictor in several cardiovascular diseases. Furthermore, up-to-date studies have indicated that BV was in correlation with the increased prevelence of PAD [4,5]. Relationship between WBV and severity of LEAD has not been taken into consideration yet. In this study, we aim to evaluate the relationship between WBV and LEAD and anatomical complexity and sypmtom severity

MATERIAL AND METHODS

Patient selection

This cross-sectional retrospective study enrolled 240 patients with LEAD who underwent peripheral lower extremity angiography at Samsun Training and Research Hospital cardiology and cardiovascular surgery clinics due to suspected PAD in noninvasive tests between March 2016 and March 2020. Demographical data, baseline cardiovascular risk factors, medical history, antiagregan and anticoagulan drug used, echocardiographic data, laboratory values, ankle-brachial index (ABI) and peripheral angiography recordings were obtained using the hospital's medical database. Patiens who had former peripheral revascularization, anticoagulant drug utilization for each and every reason, acute coronary syndromes, non-atherosclerotic stenosis, patients with baseline anemia, history of blood transfusion within the last three months, active infection, chronic inflammatory diseases, malignancy, decompensated heart failure and missing data were not included in the study. The research procedures were revised and approved by the local hospital's ethics committee, according to the ethical considerations stipulated in the Helsinki Declaration.

Evaluation of LEAD complexity and symptom severity

Peripheral angiography was executed with a 6-French pigtail catheter by the utilization of an automatic pump injector. In order to do the assessment of lower extremities, the tip of the catheter was placed on top of the aorto iliac bifurcation. LEAD anatomical complexity was evaluated with TASC II classification. The Trans-Atlantic Inter Society Consensus (TASC) II score analysis (Table 1) was performed on bilateral aorto-iliac and femoro-popliteal arterial segments [6]. The patients' angiographic data were taken into consideration from the records of catheter laboratory by two interventional cardiologists and the TASC II grade was noted for each patient. Interobserver variations for measurements were less than 5%. A TASC II A-B lesion was defined as simple LEAD, and a TASC II C-D lesion was defined as complex LEAD.

Anamnesis, physical examination and ABI of all patients were recorded from the hospital database. The severity of their symptoms was categorized according to Rutherford's classification (symptom category, 0-6 score) [7] (Table 2).

Echocardiography

Table 1. TASC II classification for the assessment of anatomical complexity of peripheral arterial diseases [6]

	AORTO-ILIAC LESIONS	FEMORO-POPLITEAL LESIONS
TASC II-A	Single stenosis (<3 cm in length) in the CIA or EIA (unilateral / bilateral)	Single stenosis (<3 cm in length) in the superficial femoral artery or popliteal artery
TASC II-B	1. Single stenosis (3–10 cm in length) not extending into the CFA 2. Heavily calcified stenosis up to 3 cm in length 3. Unilateral CIA occlusion	1. Single stenosis (3–10 cm in length) not involving distal popliteal artery 2. Heavily calcified stenosis up to 3 cm in length 3. Multiple lesions, each <3 cm in length (stenoses or occlusions) 4. Single or multiple lesions in the absence of continuous tibial runo_ to improve inflow for distal surgical bypass
TASC II-C	1. Bilateral stenosis (5–10 cm in length) in the CIA and / or EIA, not extending into the CFA 2. Multiple stenoses or occlusions (each 3–5 cm in length) 2. Unilateral EIA occlusion not extending into the CFA with or without heavy calcification 3. Unilateral EIA stenosis extending into the CFA 4. Bilateral CIA occlusion	1. Single stenosis or occlusion >5 cm in length 2. Unilateral EIA occlusion not extending into the CFA with or without heavy calcification
TASC II-D	1. Diffuse, multiple unilateral stenosis involving the CIA, EIA, and CFA (usually >10 cm in length) 2. Unilateral occlusion involving both the CIA and EIA 3. Bilateral EIA occlusions 4. Di_use disease involving the aorta and both iliac arteries 5. Iliac stenosis in a patient with abdominal aortic aneurysm or other lesions requiring aortic or iliac surgery	Complete CFA or superficial femoral artery occlusion or complete popliteal and proximal trifurcation occlusions

TASC-II: Trans-Atlantic Inter-Society Consensus-2; CIA: Common iliac artery; CFA: Common femoral artery; EIA: External iliac artery

Table 2. Rutherford classification in patients with lower extremity artery disease [7]

Grade	Category	Symptoms
0	0	Asymptomatic.
I	1	Mild claudication
I	2	Moderate claudication
I	3	Severe claudication.
II	4	Ischemic rest pain
III	5	Ischaemic ulceration not exceeding ulcers of the digits of the foot (Minor tissue loss)
III	6	Severe ischaemic ulcers or frank gangrene (Major tissue loss)

Echocardiographic assessment was executed by the utilization of a VIVID 9-dimensional cardiovascular ultrasound system (Vingmed-General Electric, Horten, Norway) with a 3.5 MHz transducer. Echocardiographic examination were executed in left lateral decubitus position. Parasternal long and short axis views and apical views were used as standard imaging windows. The left ventricular ejection fraction (LVEF) was determined from the apical window by the utilization of the modified Simpson method.

Blood sampling

Biochemistry tubes without anticoagulants were used for biochemical tests and EDTA tubes for hematological tests.

WBV was calculated from hematocrit (HCT) and plasma total protein (TP) at a low shear rate (LSR; 0.5 s-1) and a high shear rate (HSR; 208 s-1) by the previously validated formula of de Simone et al. [8] :

For HSR, the WBV (208/sec-1) formula: (0.12 × HCT) + 0.17 (TP − 2.07)

For LSR, the WBV (0.5/sec-1) formula: (1.89 ×

HCT) + 3.76 (TP − 78.42).

The two of the WBV values were determined by taking into consideration Nwose and Richards' suggestions in the series on 'Whole Blood Viscosity Assessment Issues' that extrapolation of WBV from HCT in % and TP in g/L is the most applicable approach, specifically for estimating WBV in LSR [9,10]. Overall data were measured up between the TASC II A-B (simple LEAD) and TASC II C-D (complex LEAD) groups. Two different logistic regression models were established for WBV at a LSR and WBV at a HSR (models 1 and 2) to determine the risk factors affecting LEAD anatomical complexity. Separate risk elements of which are affecting LEAD austerity were measured up between each other by using these models.

Statistical Analysis

Data were analyzed using the IBM Statistical Package for the Social Sciences 22.0 (SPSS Inc., Chicago, IL, USA) program. In order to test normality of distribution Kolmogorov–Smirnov test was used. Quantitative variables with a normal distribution were specified as the mean ± standard deviation. Categorical variables were shown as number and percentage values. The independent samples t-test was used to compare normally distributed quantitative data and the Mann–Whitney U-test was used to analyze data that did not follow a normal distribution. Categorical data were analyzed using the chi-square test. The individual effects of all variables were examined in a univariate binary logistic regression analysis. Two different logistic regression models were established separately for LSR and HSR (Model 1 and 2); independent risk factors affecting outcome were calculated using multivariate logistic regression analysis, and the comparative results are presented. To determine the best predictive WBV for both shear rate for LEAD anatomical complexity, we used ROC curve analysis, and area under the curve, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy values corresponding to each cut-off value are presented. Odds ratios and 95% confidence intervals were calculated, and sensitivity and specificity values were generated for outcome classification. The correlation of WBV values at low shear rates with WBV values at high shear rates was assessed with Spearman's correlation coefficient. In addition, spearman correlation analysis was performed to examine the relationship between WBV at HSR, WBV at LSR and Rutherford symptom severity category (0-6). A p value of <0.05 was accepted as statistically significant.

RESULTS

Demographic, clinic and laboratory data of the study groups are summarized in Table 3. A total of 135 patients (44.1%) were male, and the mean age of the study population was 70.4 ± 10.5 years old. There were no significant differences between the two groups in terms of baseline demographic characteristics, medical history and medical treatment. Smoking was significantly higher in the TASC II C-D group. Hemoglobin (13.3 ± 1.2 g/dl vs. 13.9 ± 1.0 g/dl; p = 0.002), hematocrit (40.0% ± 3.6 vs. 42.3% ± 3.5; p < 0.001), total protein (6.8 ± 2.5 mg/dl vs. 7.3 ± 2.7 mg/dl; p = 0.012), low density lipoprotein cholesterol (97.9 ± 23 mg/dl vs. 112.8 ± 240 mg/dl; p = 0.005) levels, ABI (0.63 ± 0.12 vs 0.45 ± 0.22) and WBV values were significantly higher both for HSR (15.9 ± 0.5 vs. 16.5 ± 0,7; p < 0.001) and for LSR (40.2 ± 9.5 vs. 46.5 ± 13.2; p < 0.001) were significantly higher in the TASC II C-D group than the TASC II A-B group. The ROC curve analysis explored the discriminatory capability of WBV values at both shear rates for complex PAD. Using a cut-off value 42.9 of WBV at LSR (area under the curve = 0.742, p<0.001) had a 73.4% sensitivity and 66.6% specificity, and a cut-off value of 16.1 WBV at HSR (area under the curve = 0.762, p < 0.001) had a 74.5% sensitivity and 68.0% specificity (Figure 1). The associations of possible risk factors with anatomically complex LEAD were evaluated in univariate and multivariate logistic regression analysis. Univariate regression analyses showed that smoking, age, hypertension, diabetes mellitus, WBV at HSR and WBV at LSR were significantly associated with the occurrence of anatomically complex LEAD (for all, p < 0.05). We constituted two different models to further analyze the predictiveness of WBV levels for each shear rate at multivariate analysis (Model 1 and 2). In multivariate models adjusted with smoking, age, hypertension, diabetes mellitus and low density lipoprotein cholesterol; having a high WBV at LSR (OR: 2.121, 95% CI: 1.079 – 3.164,

p < 0.001) and having a high WBV at HSR (OR: 2.737, 95% CI: 1.671 – 4.483, p < 0.001) were found as independent predictors of anatomically complex LEAD (Table 4). In spearmen correlation analysis, WBV at HSR was significantly correlated with WBV at LSR (rs = 0.906; p < 0.001). There was a significant positive correlation between LSR at WBV and Rutherford categories (0-6) (r = 0.412, p < 0.001) and HSR at WBV and Rutherford categories (0-6) (r = 0.402, p < 0.001) (Figure 2A-B, respectively).

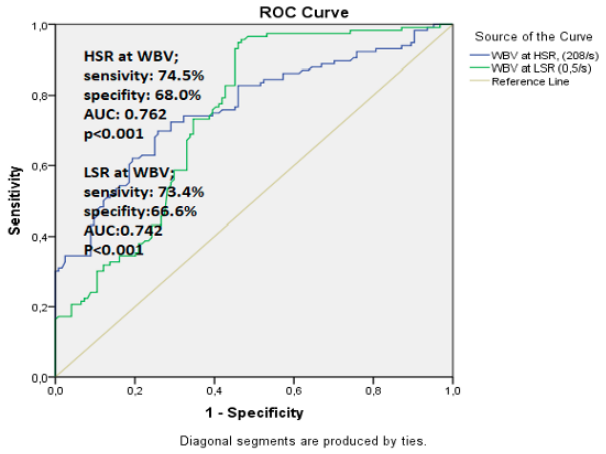

Figure 1. ROC curve analysis showing the predictive cut-off value of WBV at a high shear rate (blue line) and low shear rate (green line) for anatomically complex LEAD. LEAD, lower extremity peripheral artery disease; AUC, area under the curve; HSR, high shear rate; LSR, low shear rate; ROC, receiver operating characteristic; WBV, whole blood viscosity.

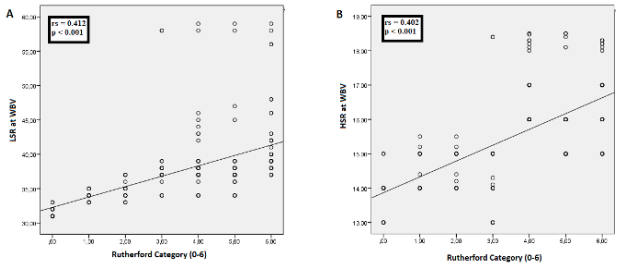

Figure 2. (A) Correlation between WBV at LSR and Rutherford categories, (B) Correlation between WBV at HSR and Rutherford categories.

DISCUSSION

This study demonstrates for the first time in the literature that the increased WBV is may be independent risk factor for the anatomical complexity of LEAD. Increased WBV levels may be associated with TASC II C-D lesions, which indicated a more anatomically complex LEAD. Besides, increased WBV values in LSR and HSR correlated positively with symptom severity assessed by Rutherford categorization.

Blood viscosity is the intrinsic resistance of blood flow in vessels. Its major determinants are: red blood cells, plasma viscosity, red cell deformation and aggregation. It has been hypothesized that increasing levels of blood viscosity within the general population may promote cardiovascular events through its potential theoretical effects on atherogenesis, thrombogenesis, or ischaemia [5]. Recent epidemiological studies demonstrated that association between blood viscosity and the conventional risk factors such as male sex, smoking, blood pressure and plasma lipids. But, in routine clinical practice, evaluation of WBV is limited. As a major component of Virchow's triad, WBV was rarely studied because of the requirement for various parameters during its evaluation. In their study De Simone et al. evaluated WBV with a simple, costless equation using total protein and HCT levels at different shear rates [8]. The prognostic value of estimated WBV calculated by this formula has been demonstrated in many studies for cardiovascular diseases [11-15]. In our study, we also preferred to use this formula to evaluate WBV.

Atherosclerosis is a leading cause of vascular disease around the worldwide. Its major clinical manifestations include ischemic heart disease, ischemic stroke, and PAD. The process of atherosclerosis is complex and multifactorial. Regular vascular physiology includes prevention of pathological conditions such as inflammation, proliferation, thrombosis and atherosclerosis. Endothelium is the most important structure that provides this protective effect. Shear stress created by blood flow on the endothelial surface plays an important role in the atherosclerotic process. Endothelial shear stress (ESS) is the most important component of blood viscosity [16]). Changes in blood viscosity affect the atherosclerotic process by direct and indirect mechanisms. Systemic vascular factors such as smoking, hyperlipidemia, hyperglycemia and hypertension, with increased blood viscosity causes endothelial dysfunction and promotes chronic fibro-inflammatory response. This response is associated with a disarming of the

Table 3. Demographic, procedural and clinical data for the study group.

Variables	TASC II A-B	TASC II C-D	p-value
	(n=124)	(n=116)	
Age, years	69.1 ± 10.9	71.7± 11.1	0.070
Gender, male n,(%)	67 (54)	68 (58.6)	0.471
Hypertension n,(%)	92 (74.1)	95 (81.8)	0.102
Diabetes Mellitus n,(%)	44 (35.4)	46 (39.7)	0.073
Hyperlipidemia n,(%)	46 (37.1)	42 (36.2)	0.880
Coronary Artery Disease n,(%)	40 (32.2)	47 (31.9)	0.090
Smoking n,(%)	49 (39.5)	61 (52.6)	0.042
eGFR, ml/min/1.73 m2	14.5 ± 0.2	15 ± 0.3	0.850
LVEF, %	53.6 ± 8.0	53.2 ± 8.7	0.740
Ankle-brachial index	0.63 ± 0.12	0.45 ± 0.22	0.001
Hemoglobin, g/Dl	13.3 ± 1.2	13.9 ± 1.0	0.002
Hematocrit, %	40.0 ± 3.6	42.3 ± 3.5	<0.001
White Blood Cell, 10^3/µl	10.2 ± 2.8	10.3 ± 2.9	0.690
Platelet, 10^3/µl	241 ± 73	244 ± 71	0.570
Total Protein, g/L	68.8 ± 2.5	73.4 ± 2.7	0.012
Albumin, g/Dl	3.6 ± 0.3	3.9 ± 0.4	0.071
Glucose, mg/dl	105 ± 17	107 ± 14	0.120
Total cholesterol, mg/dL	172 ± 35	169 ± 37	0.770
LDL cholesterol, mg/dl	97.9 ± 23	112.8 ± 240	0.005
HDL cholesterol, mg/dl	39 ± 10	36 ± 11	0.880
Triglycerides, mg/dl	118 ± 51	123 ± 56	0.470
Antidiabetic n,(%)	44 (35.4)	46 (39.6)	0.262
Statin n,(%)	50 (40.3)	54 (46.5)	0.183
ACEi/ARB, n (%)	75 (60.4)	78 (67.2)	0.691
CCB n,(%)	23 (18.5)	20 (17.2)	0.380
Beta blocker n,(%)	50 (81)	47 (78)	0.320
Pentoxifylline n,(%)	60 (48.3)	63 (50.8)	0.260
Cilastazol n,(%)	50 (40.3)	53 (42.7)	0.165
Acetylsalicylic acid n,(%)	40 (32.2)	44 (37.9)	0.196
Clopidogrel n,(%)	19 (15.3)	20 (17.2)	0.234
WBV at LSR (0,5/s-1)	40.2 ± 9.5	46.5 ± 13.2	<0.001
WBV at HSR (208/s-1)	15.9 ± 0.5	16.5 ± 0.7	<0.001

Quantitative variables with a normal distribution were specified as the mean ± standard deviation. Categorical variables were shown as number and percentage values . LVEF, Left ventricular ejection fraction; eGFR, estimated Glomerular filtration rate; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; ACE-i/ARB, Angiotensin converting enzyme inhibitor/angiotensin receptor blocker; CCB, Calcium channel blocker; WBV at HSR, Whole blood viscosity at high shear rate; WBV at LSR, Whole blood viscosity at low shear rate.

Table 4. The effects of variables on anatomically complex LEAD in univariate and multivariate logistic regression analysis

Variables	Odds Ratio (OR)	95% CI	p-value
Univariate logistic regression analysis			
Age	1.022	(0.998– 1.047)	0.030
Hypertension	1.869	(0.879– 3.975)	0.021
Diabetes Mellitus	1.739	(1.011 – 2.992)	0.011
eGFR	0.962	(0.562 – 1.628)	0.885
Coronary artery disease	1.347	(0.769 – 2.359)	0.292
Smoking	1.698	(1.017– 2.833)	0.045
LDL cholesterol, mg/dL	1.008	(0.998– 1.019)	0.021
Triglycerides, mg/dl	1.002	(0.997 – 1006)	0.470
White Blood Cell (10^3 µl)	1.017	(0.934 – 1.108)	0.690
Platelet (10^3 µl)	1.001	(0.997– 1.004)	0.681
WBV at LSR (0,5/s-1)	1.047	(1.022 – 1.074)	<0.001
WBV at HSR (208/s-1)	2.547	(1.541 – 3.917)	<0.001
Multivariate logistic regression analysis			
MODEL-1			
LDL cholesterol, mg/dL	1.010	(0.997– 1.023)	0.128
Age	0.531	(0.157 – 1.797)	0.309
Hypertension	1.939	(0.798– 4.712)	0.055
Diabetes Mellitus	1.254	(1.209 – 1.303)	0.078
Smoking	1.911	(1.108– 3.652)	0.052
WBV at LSR (0,5/s-1)	2.121	(1.079 – 3.164)	<0.001
MODEL-2			
LDL cholesterol, mg/dL	1.008	(0.997 – 1.020)	0.172
Age	1.023	(0.998 – 1.050)	0.079
Hypertension	1.914	(0.849 – 4.314)	0.067
Diabetes Mellitus	1.290	(1.112 – 1.562)	0.062
Smoking	1.914	(1.100 – 3.032)	0.058
WBV at HSR (208/s-1)	2.737	(1.671 – 4.483)	<0.001

WBV at HSR: Whole blood viscosity at high shear rate, WBV at LSR: Whole blood viscosity at low shear rate. LDL, low-density lipoprotein. eGFR, estimated Glomerular filtration rate

atheroprotective defenses encouraged by laminar blood flow. The consequences of this dynamic interaction between shear stress and systemic risk factors not only promotes atherosclerosis but also influences the disease progression and clinical outcomes. [17]. Recent studies have shown that WBV is associated with carotid thickening, coronary artery disease and PAD suggesting that rheologic factors are involved in the subclinical phase of atherosclerosis [18]. Futhermore, several large prospective studies have shown the important link between WBV and the risk of cardiovascular events [19,20]. In our study, we have found that WBV, LDL cholesterol

were higher and smoking was more prevalent in patients with anatomical complex LEAD (TASC II C-D) when compared to patients with anatomical simple LEAD (TASC II A-B). Accordingly, we have thought that these parameters act concordantly in the pathogenesis of atherosclerosis progression and may be closely related with the anatomical complexity of LEAD.

PAD is an atherosclerotic disease and can vary from small plaque formation to chronic totally occlusion of the arteries. Therefore, mild forms of the disease may be asymptomatic, and critical leg ischemia may occur as the condition progresses. The clinical presentation correlates with the involved artery segment. Numerous schemes have been developed to classify patients for clinical or prognostic features. Classification schemes are based on the symptoms of the patients, the anatomical distribution of the disease, or a combination of clinical factors such as the presence of limb ischemia and/or infection. Two systems are most commonly used to classify PAD; Rutherford classification [7] and TASC II classification [2]. LEAD symptom severity can be evaluated with the Rutherford classification. TASC II classification reflects the anatomical complexity of LEAD. TASC II C and TASC II D groups indicate a more complex and more diffused atherosclerotic involvement. Interventional treatment of the TASC II C-D lesions are also challenged when compared to TASC II A-B [6]. Therefore, in our study, we evaluated the patients as a simple (TASC II A-B) and complex (TASC II C-D) group in order to obtain more accurate statistical results.

Blood rheological factors are associated with peripheral arterial disese in the general population. Any change in hemorheological factors affects atherosclerotic process and microvascular circulation. The relationship of many hematological parameters with PAD has been evaluated [21,22]. WBV among hematological parameters plays an important role in peripheral artery disease. Increased WBV can contribute to the progression of atherosclerosis by changing ESS. Also, increased stasis, thrombosis and increased peripheral vascular resistance are thought to cause exacerbation of peripheral artery disease. Edinburgh Artery Study, involving 1581 adults assessed for peripheral artery disease, showed that blood viscosity independently associated with peripheral arterial occlusion and severity of the disease [23]. In the West of Scotland Coronary Prevention Study that enrolled 6595 patients, it was shown that WBV were associated with an peripheral atherosclerosis [4]. In our study, higher WBV values were found in the TASC II C-D group, in which the anatomical complexity of PAD was evaluated and accepted as an indicator of atherosclerotic burden, compared to the TASC II A-B group. In addition, WBV has been shown to be independently associated with the anatomical complexity of LEAD. These results support the close relationship between anatomical complexity of LEAD and WBV.

Blood rheological factors elevations may further contribute to the aggravation of symptoms by further reducing blood flow in patients with PAD. In Edinburgh study, the relationship between increased blood viscosity and symptom severity of PAD was shown [23]. Dormandy et al. found that there was a significant relationship between the worsening of peripheral circulatory disorders and baseline blood viscosity levels [24]. A second study by the same group reported that blood viscosity was higher in PAD patients with resting pain than in those with intermittent pain [25]. A third study by Dormandy et al., compared intermittent claudication with controls gruops and found blood viscosity was significantly higher in claudication group [26]. The researchers suggested that hyperviscosity may be the determining cause of claudication in LEAD patients. The Rutherford classification is used to evaluate lower extremity arterial ischemia according to the presence of ischemic signs and symptoms. Requirement of perfusion is determined according to the Rutherford category. In our study, we found that the symptom severity of LEAD evaluated by the Rutherford category was positive correlated with WBV. This result demonstrated that the positive correlation of increased WBV with increased symptom severity in LEAD. These results suggest that blood viscosity plays a critical role in the pathogenesis of lower-limb ischemia in the general population.

Smoking and blood viscosity are important risk factors of atherosclerosis. The close relationship between smoking and blood viscosity has been

also shown in many studies. Shimada et al. found that high blood viscosity was closely related to smoking and significantly decreased when smoking was quitted [27]. Increased hematocrit levels in smokers may also increase the blood viscosity which is calculated according to the formula used in our study. We have found that both smoking history and whole blood viscosity were significantly higher in anatomically complex LEAD when compared to simple LEAD. However, in our study, we have also shown that there is an independent relationship between whole blood viscosity and anatomical complexity of LEAD after the multivariate analysis which includes smoking as a separate parameter.

Limitations: Our study has several limitations. The other clinical classifications representing the severity of LEAD was not performed. The comments were done according to angiographic TASC II classification. The modest sample size, retrospective, non-randomized and single-center study are important methodological shortcomings. WBV was not validated by accurate measurement of viscosity using a viscometer. The extrapolation formula that we used in our study has been validated and utilized in several other studies but direct comparison of estimated and directly measured WBV in this patient population may strengthen our results and serve precision. In addition, other hemorheological factors that may affect blood viscosity such as platelet and erythrocyte aggregability and rigidity were not evaluated.

Conclusıon: To the best of our knowledge, this is the first study to evaluate the relationship between WBV and LEAD anatomical complexity and symptom severity. The results of this study demonstrate that the increased WBV values at LSR and HSR were an independent risk factor for TASC II C-D lesions, which indicated a more anatomically complex LEAD. Besides, WBV values in LSR and HSR positively correlated with symptom severity. Multicentre and prospective studies are needed in large populations to better characterize the relationship between anatomical complexity and symptom severity of LEAD and WBV.

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Relationship between Treatment Age and Poor Outcome in Slipped Capital Femoral Epiphysis

Femur Başı Epifiz Kaymasında Tedavi Yaşının Kötü Sonuç ile İlişkisi

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ABSTRACT

Aim; Slipped capital femoral epiphysis(SCFE) is an adolescent disease characterized by the slippage of the femoral head epiphysis from the femoral neck. The aim of this study is evaluate the factors affecting clinical outcome the surgical treatment of patients diagnosed with SCFE.

Methods; Patients who were admitted to the orthopedic outpatient clinic with the complaint of gait disturbance and underwent surgery with a diagnosis of SCFE and were followed for minimum one year were screened between January 2015 and December 2019. A total of 11 patients, 7 boys and 4 girls, included in the study, were followed mean 27 months (12-47). The degree of deviation was measured with Southwick's method. Both preoperative and postoperative hip clinical evaluation was performed using Heyman and Herndon Classification.

Results; The mean age of the patients was 12.45 (10-14). In the perioperative period, the mean BMI of the patients was measured as 26.15 (20.45-32.34) kg/cm2. Lateral radiographs of the affected hips were taken and measured using the Southwick method, and the mean angle was 45,490 (24,30-65,70). Heyman and Herndon classification was evaluated in two main groups clinically. There was no statistically significant difference between the first group and the second group in terms of gender, side, BMI, Southwick value, time between the first symptom and presentation, and the time between presentation and diagnosis. However, the mean age of the first group, whose postoperative Heyman and Herndon classification was evaluated as excellent and good, was statistically lower than the second group ($p = 0.016$). In addition, while no negative change was observed in the clinical score in any of the treated patients, improvement was observed in 6 patients and it was found that the preoperative score was significantly increased compared to the postoperative evaluation ($p = 0.014$).

Conclusion; In situ fixation with a single cannulated screw and early surgical treatment are two important factors in achieving good clinical results in patients with SCFE. It should be kept in mind that patients diagnosed with SCFE at an older age may also be associated with poor clinical results.

Keywords: Slipped Capital Femoral Epiphyses, treatment outcome, Heyman and Herndon Classification, single screw.

ÖZ

Amaç; Femur başı epifiz kayması (FBEK), femur başı epifizinin femur boynundan kayması ile karakterize bir adolesan dönem hastalığıdır. Bu çalışmada amaç; FBEK tanısı almış hastaların cerrahi tedavi sonuçlarını klinik ve radyolojik olarak inceleyerek klinik sonuca etki eden faktörleri değerlendirmektir.

Yöntem; 2015-2019 tarihleri arasında polikliniğe başvuran, FBEK tanısı ile cerrahi tedavi uygulanan ve en az takip süresi bir yıl olan hastalar çalışmaya alındı. 7 erkek ve 4 kız toplam 11 hasta, ortalama 27 ay (12-47) takip edildi. Southwick yöntemi ile kayma miktarı ölçüldü. Preoperatif dönemde ve postoperatif dönemde kalça klinik değerlendirmesi Heyman ve Herndon Sınıflaması kullanılarak yapıldı.

Bulgular; Hastaların yaş ortalaması 12,45(10-14) idi. Peroperatif dönemde hastaların VKİ ortalaması 26,15(20,45-32,34) kg/cm2 olarak ölçüldü. Etkilenen kalçaların yan grafileri çekildi ve Southwick değerleri ortalama 45,490(24,30-65,70) olarak ölçüldü. Klinik olarak Heyman ve Herndon sınıflaması iki grupta değerlendirildi. Birinci grup ile ikinci grup arasında cinsiyet, taraf, VKİ, Southwick değeri, ilk semptom ile başvuru arası süre ve de başvuru ile tanı arasında geçen süre ile ilişkili anlamlı bir fark tespit edilmedi. Postoperatif Heyman ve Herndon sınıflaması mükemmel ve iyi olarak değerlendirilen birinci grubun yaş ortalaması, orta ve kötü olan ikinci gruba göre istatistiksel olarak daha küçüktü ($p=0.016$). Ayrıca tedavi edilen hiçbir hastada klinik skor olumsuz yönde değişim gözlenmezken 6 hastada iyileşme gözlendi ve preoperatif skor postoperatif değerlendirmeye göre anlamlı olarak artmış olduğu tespit edildi ($p=0.014$).

Sonuç; FBEK olan hastalarda tek kanüllü vida ile olduğu pozisyonda tespit ve erken cerrahi tedavi iyi klinik sonuçların elde edilmesinde önemli iki faktördür. Daha büyük yaşta FBEK tanısı konan hastaların da kötü klinik sonuçlar ile birlikte olabileceği öngörülebilir.

Anahtar kelimeler: Femur başı epifiz kayması, tedavi sonuçlar, Heyman-Herndon sınıflaması, tek vida

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INTRODUCTION

Slipped capital femoral epiphysis (SCFE) is an adolescent disease characterized by the displacement of the capital femoral epiphysis from the femoral neck on the physeal plate level [1]. It is common before Tanner Stage 4 in boys and before menarche in girls. It is more common in boys than in girls, in the left hip than in the right, and in Blacks compared to Caucasians [2,3]. It is most frequently seen at the age of 10 to 16 years, and it is bilateral in 20 to 40% of cases [4,5]. Obesity is a significant risk factor. The risk increases, particularly in children whose percentile curves are ≥60 [3]. Apart from mechanical stress in the hip caused by obesity, renal failure, hormonal disorders such as hypothyroidism and growth hormone disorders, and some genetic diseases are considered risk factors in the etiology of SCFE [1]. The most common symptoms at the time of admission are hip pain and claudication. Hip pain can radiate to the groin, medial of the patella, and femur. Knee pain is also one of the initial symptoms [6]. There are four types depending on the formation type and duration of the clinical presentation as pre-slip, acute slip, chronic slip, and acute-on-chronic slip [7]. In addition, patients who are able to walk with the aid of crutches are considered stable, and patients with restricted pain who are unable to walk are considered unstable SCFE cases [8].

The main goal of SCFE treatment, which should be treated with appropriate methods as early as possible following the diagnosis, is to prevent slippage and early fusion of the epiphysis. Although there are numbers of treatment options, single-screw fixation in situ is frequently included in the current literature and is widely used [9,10]. However, there are numbers of factors affecting clinical success and surgery-related morbidity.

In the present retrospective study, we aimed to evaluate clinical and radiographic outcomes, to investigate the factors affecting the surgical treatment outcomes, and to examine the possible relationship between delay in diagnosis and morbidity in patients with SCFE.

MATERIAL AND METHOD

This single-center, retrospective study was conducted at Antalya Training and Research Hospital between January 2015 and December 2019. Patients who were admitted to the orthopedic and traumatology outpatient clinic with the complaint of gait disturbance and underwent surgery with a diagnosis of SCFE and were followed for minimum one year were screened. Those who had missing follow-up data and who underwent surgical procedures in external centers were excluded from the study. A total of 11 patients (7 boys and 4 girls) who met the inclusion criteria were included in the study. A written informed consent was obtained from each parent and/or legal guardian. The study protocol was approved by the Antalya Training and Research Hospital Ethics Committee (Ethics Committee number: 2020-219) The study was conducted in accordance with the principles of the Declaration of Helsinki.

The affected side, body weight, height, and body mass index (BMI) values of the patients were retrieved from the hospital database. The initial symptoms of the patients, the first admission to the outpatient clinic, date of first diagnosis, and date of surgery were recorded. All patients were followed for minimum one year. Endocrinological examination of the patients was made before the treatment. All patients were fixed with cannulated screws of 6.5 mm in diameter and 16-mm thread in length. The range of motion (ROM) of the hip and radiographic images were evaluated before and after surgery. The degree of deviation was measured using the Southwick's angle on preoperative lateral hip radiographs [11]. Clinical evaluation of the hip both preoperatively and postoperatively was performed using the Heyman and Herndon classification [12] (Table 1). The patients were further divided into two subgroups according to the Heyman and Herndon classification as follows: the first group with excellent and good results with close quantitative evaluation, and the second group with moderate and poor results along with at least 1-cm shortness and severe limitation of movement.

In addition, the length measurement and gait evaluation of the affected limb were compared with the unaffected limb. Postoperative clinical and radiographic evaluation was performed at 6, 12, and 24 weeks and at one year and in the final control visit. The patients were partially mobilized

at six weeks and completely mobilized by weight bearing at 12 weeks.

Table 1. Heyman – Herndon Classification.

Group	Degree	Definition
Group 1	Excellent	Excellent result has a normal range of hip movement, no limp, no pain, and leg shortening.
	Good	Good result had slight limitation of internal rotation, occasional pain, and leg shortening of < 1 cm.
Group 2	Fair	Fair result had persistent mild pain, loss of internal rotation, abduction, and leg shortening of > 1 cm.
	Poor	Poor result did not meet any of the above criteria

Surgical technique

All patients were operated by a single experienced surgeon under general anesthesia. A plan for screw position was made based on the computed tomography (CT) or magnetic resonance imaging (MRI) scans in the preoperative period. All patients were placed in the supine position. Acute reduction was not performed in any of the patients and fixation was performed in situ. The hip region was entered with a lateral straight incision of 2 to 3 cm. First, the lateral femoral cortex and the appropriate location for the femoral head were visualized by fluoroscopy using a Kirschner wire (K-wire). Drilling was performed on the K-wire in the appropriate position and fixation was, then, performed with a cannulated screw with a diameter of 6.5 mm and a thread length of 16 mm accordingly. Fixation was done with a single screw in all patients. Positional suitability and length of the screw were evaluated on anteroposterior and lateral radiographs after screw insertion(Figure 1).

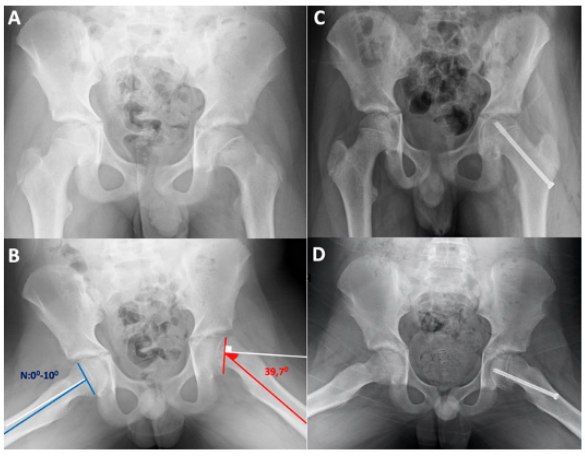

Figure 1. Slipped capital femoral epiphysis on the left hip in a 14 years old girl. A; Preoperative Anterior/Posterior pelvis X-ray. B; Preoperative lateral hip x-rays. Left side Southwick angle measurement is 39,70 degree. Right side is normal. C; Postoperative 6th month Anterior/Posterior x-ray view and D; lateral view.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). Whether the distributions of continuous variables were normally or not being determined Shapiro-Wilk test. Descriptive statistics for continuous variables were expressed as mean ± SD and number of cases with (%) were used for categorical data. While the mean differences between groups were compared Student's t test, otherwise the Fisher's exact test was applied for the comparison of categorical variables. Whether the differences in hip movement status between pre- and post-op was statistically significant or not was evaluated Wilcoxon Sign Rank test. A p value less than 0.05 was considered as statistically significant.

RESULTS

The median age of the patients was 12.45 (range, 10 to 14) years. The median follow-up was 27 (range, 12 to 47) months. The right side was affected in three patients and the left side was affected in eight patients. The median preoperative BMI was 26.15 (range, 20.45 to 32.34) kg/m2. Lateral radiographs of the affected hips were measured by the Southwick's angle and the median value was measured as 45.49° (range, 24.3° to 65.7°). The median time from the onset of the first symptom to the first admission to the outpatient clinic was 2.45 (range, 0 to 4) months, the median time from the admission to the outpatient clinic to diagnosis was 5.63 (range, 0 to 13) months, and the median time from the diagnosis to surgery was 0.5 (range, 0 to 2) months. One patient had a history of hypothyroidism requiring treatment, and one patient had a history of obesity under the control of pediatric endocrinology. According to the Heyman and Herndon classification before and after 12 months, none of the patients had worse results compared to baseline. The shortening was 1 cm in three patients and 2 cm in two patients. One of the patients having a 2-cm shortening had a clinical symptom(Table 2). The patients were

Table 2. Southwick angle measurement and demographic data of patients.

Patinets (n)	Age	Gender	Side	BMI* (Kg/m2)	Southwick Angle Degree (0)	Interval between first symptom to application (month)	Interval between application to diagnosis (month)	Endocrinopathy
1	10	F	Right	29.33	45.5	1,0	10,0	--
2	14	F	Left	32.34	39.7	3,0	7,0	--
3	12	M	Left	22.48	24,3	4,0	0,0	--
4	12	F	Left	20.45	54,8	2,0	5,0	--
5	13	M	Left	28.34	50,1	3,0	9,0	--
6	14	M	Left	28.91	65,7	4,0	5,0	Hypothyroidism
7	11	M	Left	24.78	44,9	4,0	0,0	Obesity
8	13	M	Left	23,46	46,2	0,0	10,0	--
9	14	M	Right	26,78	48,7	3,0	13,0	--
10	12	M	Left	26,3	34,4	1,0	0,0	--
11	12	F	Right	24,56	46,1	2,0	3,0	--

BMI: Body Mass Index

further divided into two subgroups according to the Heyman and Herndon classification as follows: the first group with excellent and good results with close quantitative evaluation, and the second group with moderate and poor results along with at least 1-cm shortness and severe limitation of movement. There was no statistically significant difference in terms of sex, affected side, BMI, Southwick's angle, and median time from the onset of the first symptom to the first admission to the outpatient clinic and from admission to diagnosis. However, the median age of the first group with excellent and good results according to the postoperative Heyman and Herndon classification was statistically lower than the second group (p=0.016). In addition, no worsening in the clinical scores was observed in any of the patients. An improvement was observed in six patients and the postoperative scores significantly improved compared to baseline (p=0.014).

DISCUSSION

Although SCFE is a rare disease in adolescents; it can lead to severe morbidity in case of possible delay in treatment. In its etiology, autosomal-dominant inherited genetic disorders, as well as structural and environmental factors have been proposed to play a role [13-15]. In our study, the male:female ratio was found to be 1.75. The median BMI was 26.15 kg/m2, consistent with the literature [1,14]. Although BMI is considered a predisposing factor, it showed no clinically significant effect on the results in this study. Hypothyroidism was detected in one patient and this patient was the one with the highest Southwick's angle measurement.

Treatment options vary both chronologically and depending on the clinical and radiographic findings. These options include fixation with plaster, closed internal fixation in situ, open epiphysiodesis, proximal femur osteotomies, and secure dislocation of the hip and epiphysis fixation. Plaster fixation is an earlier treatment method and has been abandoned in the current practice due to the long-term immobilization and high chondrolysis rates [16,17]. Closed fixation in situ is the most commonly used method in the current literature [9,10,18]. Except for studies suggesting the use of two threaded Steinmann nails due to the fact that they can be removed easily as described in the study of Nonweiler[19], the accepted method is fixation with a partially threaded, 6.5-mm cannulated screw [20]. In clinically and radiographically more advanced cases, epiphysiodesis or proximal femoral osteotomy is another treatment option [21,22]. In recent studies conducted in Turkey, the main fixation method is closed fixation in situ [6,23,24]. In all our patients, we used 6.5-mm screws in the supine position under the guidance of fluoroscopy and avoided the use of special traction tables.

In addition to the genetic causes in the etiology and the systemic effects of concomitant hormonal diseases, the fact that 20 to 40% of the cases are bilateral brings to mind the contralateral prophylactic fixation. The presence of accompanying hormonal causes and the chronological age of the patients are the main determinants of the possibility of slipping in

the contralateral hip [25]. Some authors have advocated that prophylactic surgery should be preferred in high-risk patients, while some others have claimed that a second surgery is associated with higher risks and more frequent clinical and radiographic follow-up alone is sufficient in these patients [25-27]. Currently, there is no consensus on this issue in the literature yet. In our study, prophylactic surgery was not performed in one patient with obesity and another with hypothyroidism. During follow-up, no slip was detected in any of the patients. In addition, no significant endocrinological factor affecting the clinical results was observed.

Loder Classification is one of the classification system used in SCFE(28). Patients are divided into two group as stable and unstable in this classification. The stable group was defined as patients who could be mobilized with or without crutches. And the unstable group was defined as patients who could not be mobilized not even with crutches. Loder showed in his study that the risk of developing avascular necrosis was observed in the unstable group. In other words, Loder Classifications provides prognostic information for complication of femoral head osteonecrosis. In our study, instead of the Loder classification, we used the Heyman Herndon Classification, which provides more clinical information. Considering the clinical evaluation, we quantitatively divided the patients into two subgroups based on the Heyman and Herndon classification. The first group had excellent and good results with no or mild limitation and no shortness. The second group had moderate and poor results with at least 1-cm shortening and moderate to severe clinical limitations. Accordingly, the median age of the first group with good postoperative clinical results was significantly lower than the second group with poor results. In a study evaluating BMI-for-age percentile, a significant correlation was found between bilateral SCFE and high BMI-for-age percentile [28]. However, there is no study in which a significant correlation was found between postoperative clinical results and age. According to our study, SCFE disease, which occurs at a late age has a worse prognosis than the group that occurs at an early age. In other words, development of SCFE in late age could be seen as a poor prognostic factor. To the best of our knowledge, therefore, our study is the first showing a significant correlation between the increase in age and poor clinical results of the treatment.

Besides the age of the patient, early diagnosis is an important factor for the clinical outcomes of treatment [1]. There are many publications in the literature showing that early diagnosis is associated with favorable clinical results [1,4,5]. In our study, it is a spectacular finding that all patients who were treated surgically within the first three months of diagnosis showed better clinical results. However, some of the patients who were operated after four months of diagnosis showed no improvement in the clinical outcomes. Nonetheless, no statistically significant difference was found between the patient groups (p=0.208). This can be attributed to the small sample size of the study.

Furthermore, fixation with a single screw yielded favorable results in our study, indicating statistical significance. Compared to the preoperative clinical evaluation, the clinical outcomes remained unchanged in five of 11 patients without any worsening, and a favorable progression was observed in six patients in the postoperative period. These findings confirm that fixation with cannulated screws, which is the treatment option that is at the forefront in the literature in recent years, seems to be a useful method.

Limitations of study: Nonetheless, there are some limitations to this study. First, it has a retrospective design with a small sample size. However, review of the literature on SCFE reveals studies using sample sizes similar to our study, except for those conducted in pediatric orthopedic centers [6,15,24]. The main strength of our study is its relatively long follow-up (median: 27 months) and the investigation of factors affecting the clinical outcomes, which have been very rarely examined in the literature. Long-term follow-up is valuable to obtain more accurate clinical results.

Conclusion: In conclusion, fixation in situ with a single cannulated screw and early surgical treatment are the main factors to achieve favorable and satisfactory clinical results in patients with SCFE. It should be also kept in mind that patients diagnosed with SCFE at an older age may experience poor clinical results.

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Comparison of psychopathologies of children of Parents with Chronic Psychiatric Disease

Kronik Psikiyatrik Bozukluğu olan Ebeveynlerin Çocuklarının Psikopatolojilerinin Karşılaştırılması

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ABSTRACT

Aim: In this study, children whose parents were diagnosed with schizophrenia and bipolar I disorder (BID), were compared with each other, and compared with those who did not have any mental disorders in their parents in terms of presence and types of psychopathology.
Methods: This study was carried out on a group of children aged 6 to 17 years. The parents of 11 children were diagnosed with schizophrenia, the parents of 36 children were diagnosed with bipolar disorder and 47 children whose parents did not have any mental disorders, were included in the control group. All children and adolescents filled in the State-Trait Anxiety Inventory for Children (STAI-CH); the parents completed a socio-demographic data form, Conners' Parent Rating Scale-Short form (CPRS-SF), DSM IV-based Screening and Assessment Scale for Behavioural Disorders in Children and Adolescents (T-DSM-IV) and Children's Sleep Habits Questionnaire (CSHQ)-Abbreviated Form.
Results: As a result of the assessments, Oppositional defiant disorder (ODD) symptoms were found to be higher in children who had parents diagnosed with the BID (BID-c) when compared with both children who had parents diagnosed with schizophrenia (SZ-c) and the control group (p=0,08). Learning problems were found to be higher in the SZ-c group when compared with both the (BID-c) and the control group (p=0,08). State anxiety was found to be higher in the SZ-c group when compared with the control group (p=0,020). No difference was found between the groups in terms of conduct disorder (CD), attention deficit (AD), hyperactivity, psychosomatic complaint, sleep disorder and trait anxiety (respectively; p=0,112, p=0,590, p=0,098, p= 0,776, p=0,741, p=0, 924).
Conclusion: The results of the study suggested that the symptoms of ODD may be more common in the BID-c group, and the learning problem might be more common in the SZ-c group. Care should be taken in terms of ODD in children of parents diagnosed with bipolar I disorder, and in terms of learning problems in children of parents diagnosed with schizophrenia.

Key Words: Schizophrenia, bipolar disorder, child, parents, psychopathology

ÖZ

Amaç: Bu araştırmada ebeveynlerinde şizofreni veya bipolar I bozukluk (BIB) tanısı bulunan çocuklar, ebeveynlerinde herhangi bir ruhsal bozukluk bulunmayan çocuklar ile ve birbirleri ile psikopatoloji varlığı ve türleri açısından karşılaştırıldı.

Yöntemler: Çalışmaya 6-17 yaş aralığında, ebeveyni şizofreni tanılı 11 çocuk, ebeveyni BIB tanılı 36 çocuk ve kontrol grubu olarak ebeveyninde herhangi bir ruhsal bozukluğu olmayan 47 çocuk dahil edilmiştir. Araştırmaya alınan tüm çocuk ve ergen-lere çocuklar için durumluk-sürekli kaygı envanteri (ÇDSKE) doldurtulmuştur, ebev-eynlerine de sosyodemografik veri formu, conners ana baba derecelendirme ölçeği kısa formu, (CADÖ-KF) çocuk ve ergenlerde davranım bozuklukları için DSM IV e dayalı tarama ve değerlendirme ölçeği (T-DSM-IV), çocuk uyku alışkanlıkları anketi kısa formu (ÇUAA) doldurtulmuştur.

Bulgular: Değerlendirmeler sonucunda, Karşıt olma karşıt gelme bozukluğu (KOKGB) belirtileri; BIB tanılı ebeveyne sahip çocuklarda (BIB-ç) hem şizofreni tanılı ebeveyne sahip çocuk (ŞZ-ç) grubuna göre hem de kontrol grubuna göre daha yüksek bulunmuştur (p=0,08). Öğrenme sorunu ise ŞZ-ç grubunda hem (BIB-ç) grubuna hem de kontrol grubuna göre daha yüksek bulunmuştur (p=0,025). Durumluk kaygı ŞZ-ç grubunda kontrol grubuna göre daha yüksek bulunmuştur (p=0,020). Davranım bozukluğu (DB), dikkat eksikliği (DE), hiperaktivite, psikosomatik yakınma, uyku bozukluğu ve sürekli kaygı açısından gruplar arası farklılık belirlenememiştir (sırasıyla; p=0,112, p=0,590, p=0,098, p= 0,776, p=0,741, p=0, 924) .

Sonuç: Araştırmanın sonuçları, BIB-ç grubunda KOKGB belirtilerinin daha sık olabileceğini, ŞZ-ç grubunda öğrenme sorununun daha sık olabileceğini düşündürtmüştür. Bipolar I Bozukluk tanılı ebeveynlerin çocuklarında KOKGB açısından, şizofreni tanılı ebeveynlerin çocuklarında ise öğrenme sorunu açısından dikkatli olunmalıdır.

Anahtar Kelimeler: Şizofreni, bipolar bozukluk, çocuk, ebeveynler, psikopatoloji

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INTRODUCTION

Schizophrenia and BID are psychiatric disorders characterized by high genetic transmission, with the highest risk factor being positive family history [1]. Children of parents with mood disorder and psychotic disorder are under great risk for the development of behavioral and emotional problems in childhood and serious mental disorders in later years [2]. According to the results of a recent study, the prevalence of psychopathology and comorbidity was higher in children whose parents were diagnosed with schizophrenia and bipolar I disorder (BID) than those who did not have any mental disorders in their parents, at baseline and at follow-up [3]. Because of this high risk, it is very important to understand the presence and types of the psychopathology of children of parents diagnosed with schizophrenia and BID. This information can provide early psychosocial intervention to these children.

Understandably, patients diagnosed with schizophrenia and BID wonder whether the same disorder or another psychiatric disorder, will develop in their children in the future. A correct risk assessment is the most important factor of the communication with the patients and their families. In this study, we hypothesized that we would find more psychopathology in the BID-c group and the SZ-c group than in the control group. Our aim was to find out the presence and types of psychopathologies in children of adults diagnosed with schizophrenia and BID, then to compare the groups with each other and with the control group. Knowing the common psychopathologies in children whose parents are diagnosed with BID and schizophrenia will provide early diagnosis and intervention, so that these children will experience less difficulty in their later years.

MATERIAL AND METHOD

Sample

As the study group, 36 children (between the ages of 6-17) of 36 patients (15 female, 11 male) diagnosed with Bipolar I, and 11 children (between the ages of 6-17) of 11 patients (5 female, 6 male) diagnosed with schizophrenia who referred to psychiatry polyclinic of mood and psychosis of a university hospital between the years 2017 and 2018, were included in the study. As the control group, 47 children between the ages of 6 and 17 who agreed to participate in the study and who referred to the pediatric polyclinic of a university hospital for a physical complaint, and whose parents did not have any mental disorders, were also included. A written informed consent form was received from 94 children who volunteered to participate in the study and from their parents. Approval was sought and received from the Ethical Board Committee for the study on 05.10.2017, bearing decision number 2017/279.

Data Collection Instruments

Socio-demographic Data Form: The information form designed by the researchers to collect the socio-demographic data of the cases.

Conners' Parent Rating Scale-Short form (CPRS-SF): The scale which includes 48 items. There are 6 items in Learning Problems (LP) factor, 6 items in Hyperactivity (HA) factor, 5 items in opposition (O) factor, 12 items in Conduct Problem (CP), 8 items in anxiety problem factor and 5 items in psychosomatic complaint factor. It was developed by Goyette et al. in 1978 [4]. CPRS-SF was adapted into Turkish by Dereboy et al. [5].

DSM IV-based Screening and Assessment Scale for Behavioral Disorders in Children and Adolescents - Turgay (T -DSM- IV): This scale was developed by Turgay based on DSM-IV diagnostic criteria [6]. It has 41 items. There are 9 items on hyperactivity and impulsivity (HI), 9 items on attention deficit (AD), 8 items on ODD and 15 items on CD.

Children's Sleep Habits Questionnaire (CSHQ)-Abbreviated Form: It was developed by Owens et al. in 2000 in order to analyze children's sleep habits and their problems related with sleep and it consists of a total of 33 items [7]. Validity and reliability study of this scale was conducted by Perdahlı Fiş et al. [8].

State trait anxiety inventory for children (STAIC-CH): STAIC-CH trait anxiety scale consists of 20 items and the child is asked to evaluate how s/he feels in "general". In STAIC-CH state anxiety, the child is asked to evaluate how s/he feels in "that moment" and it consists of 20 items. Validity and

reliability study of this scale was conducted by H. Şeniz Özusta [9,10].

Statistical Analysis

STATISTICA Version 13.3 was used for the statistical analysis of the data. Continuous data was summarized with average, standard deviation, minimum, maximum, median, 25. and 75. percentage values. Categorical variables were summarized as number and percentage (%) values. The medians of more than two independent groups were compared with the non-parametric method Kruskall-Wallis test. A Chi-square analysis was performed for the associations between patient and study groups and Conners' Parent Rating Scale-Short form and DSM IV-based Screening and Assessment Scale for Behavioral Disorders in Children and Adolescents, for conduct disorders in children and adolescents. The statistical significance level was accepted as (p) 0.05 for all comparisons and $p<0.05$ was considered as statistically significant.

RESULTS

The sample groups consisted of a total of 94 children between the ages of 6 and 17: 47 children, 76,6% (n=36) whose parents were diagnosed with BID and 23,4% (n=11) whose parents were diagnosed with schizophrenia as the case group and 47 children whose parents did not have any mental disorder. The average age of the children in the case group was 12.6±3.0 years, while the average age of the children in the control group was 12.1±3.0 years. 42.6% (n=20) of the case group were female and 57.4% (n=27) were male. 68.1% (n=32) of the control group were female and 31.9% (n=15) were male. The rate of females in the case group was statistically significantly lower than the rate of males (p=0.013). The average age of the mothers in the case group was 39.6±4.6 and the average age of the fathers in the case group was 42.4±8.9. The average age of the mothers in the control group was 39.4±5.3 and the average age of the fathers in the control group was 43.4±6.3. The average time of breastfeeding was 12.6±10.4 months in the case group and 13.5±7.4 months in the control group. No statistically significant differences were found between the case and the control group in terms of the average age of the children, the average age of parents, the average time of breastfeeding and the parents' level of education.

Conners' Parent Rating Scale-Short form (CPRS-SF)

The differences between study groups' CPRS-SF, CP, O, HA, anxiety and psychosomatic sub-dimension scores, were not statistically significant. However, the differences between LP sub-scale medians of SZ c and both the BID-c group and the control group, were statistically significant (p=0.025) (Table 1).

DSM IV-based Screening and Assessment Scale for Behavioural Disorders in Children and Adolescents - Turgay (T -DSM- IV)

According to the T-DSM-IV scale, there were 11 children with symptoms of AD, 45.5% (5) of these were in the BID-c group, 18.2% (2) of these were in the SZ-c group and 36.4% (4) were in the control group. The number of children with HI symptoms was 8, 37.5% (3) of these were in the BID-c group, 37.5% (3) of these were in the SZ-c group and 25.0% (2) were in the control group. The number of children with CD symptoms was 1 and that single child was in the SZ-c group. There were no statistically significant differences between the groups in terms of AD, HI and CD symptom distribution (respectively; p=0.112, p=0.590, p=0.098, p= 0.776, p=0.741, p=0.924).

A statistically significant difference was nevertheless found between the groups in terms of ODD symptom distribution ($p<0.008$). In children with ODD symptoms, the BID-c rate was higher than both the SZ-c rate and the control rate (Table 2).

Children's Sleep Habits Questionnaire (CSHQ)-Abbreviated Form

In the study, the rate of children with clinically significant level of sleep disorder was 72.2% (26) in the BID-c group, as 81.8% (9) in the SZ- c group and 70.2% (33) in the control group. There were no statistically significant differences between sleep disorder rates of the groups (p=0.741).

State trait anxiety inventory for children (STAIC-CH)

Table1. CPRS-SF subscale score comparison between groups

	BID-c group (n=36)		SZ-c group (n=11)		Control Group (n=47)		
	Av± SD	Median	Av± SD	Median	Av± SD	Median	p
CPRS-SF CP	6.75±5.74	5.00	7.00±6.24	4.00	5.02±3.96	4.00	0.479
CPRS-SF O	2.25±2.37	1.50	2.91±2.46	3.00	1.68±2.21	1.00	0.124
CPRS-SF HA	3.89±3.10	3.00	5.00±3.19	6.00	3.55±2.46	3.00	0.411
CPRS-SF LP	3.56±3.03	3.50	6.55±4.98	6.00	2.91±2.85	2.00	0.025
CPRS-SF Anxiety	5.75±3.63	5.00	5.09±2.30	5.00	5.45±3.83	5.00	0.851
CPRS-SF Psycho somatic	1.56±2.04	1.00	1.73±2.28	1.00	1.21±1.42	1.00	0.776

Kruskal-Wallis; CPRS-SF, Conners' Parent Rating Scale-Short form; CD, Conduct problem; O, Opposition; LP, Learning problem; BID-c, children who had parents diagnosed with Bipolar I Disorder; SZ-c, children who had parents diagnosed with schizophrenia; Avt±sd, Average ± standard deviation; p<0.05 statistically significant

Table 2. Comparison of T-DSM-IV Oppositional Defiant Symptom presence rates between groups

		T-DSM IV ODD No	T-DSM-IV ODD Yes	Total	P
BID-c group	Count	26	10	36	0.008
	% of those with symptom presence	32.9%	66.7%	38.3%	
SZ-c group	Count	8	3	11	
	% of those with symptom presence	10.1%	20.0%	11.7%	
Control group	Count	45	2	47	
		57%	13.3%	50.0%	

square; T-DSM-IV, DSM IV-based Screening and Assessment Scale for Behavioural Disorders in Children and Adolescents - Turgay; ODD, Oppositional Defiant Disorder; BID-c, children who had parents diagnosed with Bipolar I Disorder; SZ-c, children who had parents diagnosed with schizophrenia; p<0.05 statistically significant

The difference between STAIC-CH Trait anxiety scores of the groups in the study was not statistically significant. However, the differences between state anxiety scale score medians of the control group and the SZ-c group, were statistically significant (p=0.020) (Table 3).

Table 3. Comparison of the STAIC-CH scores between the groups

		Trait Anxiety	State Anxiety
BID-c group	Av± SD	35.34±6.202	34.60±6.950
	Median	35.00	36.00
SZ-c group	Av± SD	36.64±6.874	38.82±8.023
	Median	37.00	37.00
Control group	Av± SD	35.96±7.799	32.49±8.097
	Median	35.00	30.00
	p	0.924	0.020

Kruskal-Wallis; STAIC-CH, State trait anxiety inventory for children; BID-c, children who had parents diagnosed with Bipolar I Disorder; SZ-c, children who had parents diagnosed with schizophrenia; Avt±sd, Average ± standard deviation; p<0.05 statistically significant

DISCUSSION

Children of parents diagnosed with schizophrenia and BID have an increased risk of mental disorder [1,11]. In our study, although psychopathology frequency was found to be higher in the study groups in general, a statistically significant difference was ODD symptoms being higher in the BID-c group when compared with the control group, and LP and state anxiety being higher in the SZ-c group, when compared with the control group.

In our study, the differences between groups in terms of the CPRS-SF O sub-dimension scores were not found to be statistically significant. However, according to the T-DSM-IV scale, the rate of patients with ODD symptoms in the BID-c group was higher than both the SZ-c and the control group (Table 2). This result is in parallel with the results of recently conducted studies that show that all three sub-dimensions of ODD can predict the CD diagnosis, but in particular, the "angry/easily getting angry" mood sub-dimension can predict the mood disorders that can occur in the future [12,13]. However, in our study, ODD sub-dimensions were not examined separately. At the same time, in a recently conducted study comparing children who have parents with schizophrenia, BID and major depressive disorder diagnosis, the highest ODD level was found in the

group with BID diagnosed parents [14].

Although we found a statistically significant difference only for ODD symptoms for the BID-c in our study, different results were obtained in previous studies. In a controlled study conducted in our country, children who had parents diagnosed with BID had significantly higher rates of having both psychopathology and coexistence of these pathologies for life, when compared with children in the control group [15]. In a recently conducted meta-analysis, at least one mental disorder was found in 55% of children who had parents diagnosed with BID and major depression, and very little difference was found between the groups [16].

In our study, the CPRS-SF LP sub-dimension scores of the SZ-c group were found to be statistically significantly higher when compared with both the BID-c group and the control group (Table 1). Neuropsychological decline is a key feature of schizophrenia and many studies have shown the association between the prodromal phase and increased cognitive impairment, in high-risk adolescents [17]. A comparative meta-analysis of neurocognition in first-degree relatives of patients with schizophrenia and bipolar disorder, found that deficiencies in general intellectual ability, verbal learning, planning and working memory, may be more specifically associated with the risk of schizophrenia [18].

In our study, the result that LP scores were statistically significantly higher in the SZ-c group when compared with the BID-c group was in parallel with previously conducted studies. A large number of studies have found that cognitive problems were more specific in children who had parents diagnosed with schizophrenia, when compared with children who had parents diagnosed with BID [19,20].

In our study, no statistically significant differences were found between groups in terms of CP, AD and HI, according to the CPRS-SF and the T-DSM-IV scale. The association between diagnosis of schizophrenia and BID in parents and the risk of disruptive behavioral disorders, including conduct disorders (CD) in the offspring, has been demonstrated by previous epidemiologic studies [21]. According to the results of previous reports, an increased risk of attention deficit and hyperactivity disorder (ADHD) was observed in children whose parents were diagnosed with schizophrenia or BID, just like disruptive behavioral disorders [22,23]. This inconsistency with the previous literature can be due to our insufficient number of samples.

There were no statistically significant differences between the groups in terms of sleep disorders. Although there is no clear information about sleep disorders in children who have parents diagnosed with schizophrenia, sleep disorders were found to be BID prodromal syndrome in prospective studies that examined the children of parents diagnosed with bipolar disorder [24].

Although the difference between the STAIC-CH trait anxiety scores of study groups was not significant, the trait anxiety scores of the SZ-c and the BID-c groups were higher than the control group. State anxiety scores of the BID-c and the SZ-c groups were higher than those of the control group; however, only the difference between the state anxiety scale scores of the control group, and the SZ-c group was statistically significant (Table 3). Similar to the results of our study, previous studies found higher anxiety disorders in the children of parents diagnosed with schizophrenia and BID [23,25].

Limitations: The low number of participants and their lack of homogeneity, the unavailability of objective neuropsychiatric assessments, as well as subjective scales for evaluating cognitive abilities, such as learning problems and attention deficit, are among the limitations of our study. Other limitations of our study include the cross-sectional nature of the research and the structure of the control group, which consisted of children admitted to the hospital due to physical complaints, as there may be a higher risk for mental problems associated with physical problems.

Conclusion: The relationship of ODD and learning difficulty with the BID-c and the SZ-c is a complex process. Findings from this study demonstrate that ODD is more related to the BID-c group, whereas learning difficulty is more related to the SZ-c group. In the light of these results, there is a need for large scale follow-up studies and objective tests, to be able to definitively conclude that ODD is a BID-c specific psychopathology and learning

difficulty is a SZ-c specific psychopathology.

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RESEARCH ARTICLE

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Comparison of Volumetric and Cosmetic Scoring Changes After Treatment of Benign Thyroid Nodules Using Microwave Ablation and Ethanol Ablation Therapy

Mikrodalga Ablasyon ve Etanol Ablasyon Tedavisi Uygulanan Benign Tiroid Nodüllerinin Tedaviden Sonra Hacimsel ve Kozmetik Skorlama Değişikliklerinin Karşılaştırması

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ABSTRACT

Aim: This study retrospectively evaluated the effects of treatment of benign mixed-type and spongy-type thyroid nodules with microwave ablation (MWA) and ethanol ablation (EA) therapies. The changes in volume obtained by ultrasonography and cosmetic scores were examined. The efficiency of both treatment methods was also compared by statistical analyses.
Methods: Between July 2015 and July 2020, archive scanning was performed for patients who underwent MWA and EA in the radiology clinic. As a result, 57 MWA and 55 EA patients were included in the study. Nodule volumes from before the treatment, 3 months, and 6 months after ablation treatment were noted for each case. Cosmetic scores for all patients were also examined.
Results: A statistically significant difference was found in the mean nodule volume at 6 months between MWA and EA, with MWA being more successful ($p<0.05$). The MWA technique also resulted in significantly higher mean cosmetic score reduction ($p<0.05$).
Conclusion: Minimally invasive approaches are increasingly adopted in the treatment of benign thyroid nodules, and both MWA and EA are effective and safe treatments techniques. The results show that MWA treatment leads to better cosmetic scores and nodule volume changes in patients with benign mixed-type and spongy-type thyroid nodules.

Keywords: Thyroid nodule, microwave ablation, ethanol ablation, ultrasound, cosmetic score

ÖZ

Amaç: Bu çalışma geriye dönük olarak benign mikst-tip ve süngerimsi-tip tiroid nodüllerinin mikrodalga ablasyon (MWA) ve etanol ablasyon (EA) terapileri ve tedavi etkinliğini değerlendirmiştir. Ultrasonografi ve kozmetik skorlarla elde edilen hacim değişiklikleri incelendi. Her iki tedavi yönteminin etkinliği de istatistiksel analizlerle karşılaştırıldı.
Yöntem: Temmuz 2015 ile Temmuz 2020 tarihleri arasında radyoloji kliniğinde MWA ve EA yapılan hastalara arşiv taraması yapıldı. Sonuç olarak 57 MWA ve 55 EA hastası çalışmaya dahil edildi. Her vaka için tedavi öncesi, ablasyon tedavisinden 3 ay ve 6 ay sonraki nodül hacimleri not edildi. Tüm hastalar için kozmetik skorlar da incelendi. **Bulgular:** MWA ile EA arasında 6. ayda ortalama nodül hacminde istatistiksel olarak anlamlı bir fark bulunmuş olup, MWA daha başarılıydı ($p<0.05$). Ayrıca MWA tekniği, anlamlı olarak daha yüksek ortalama kozmetik skor azalmasıyla sonuçlanmıştır ($p<0.05$).
Sonuç: Minimal invaziv yaklaşımlar, benign tiroid nodüllerinin tedavisinde gider-ek daha fazla benimsenmektedir ve hem MWA hem de EA etkili ve güvenli teda-vi teknikleridir. Sonuçlar, MWA tedavisinin benign mikst-tip ve süngerimsi-tip tiroid nodülleri olan hastalarda daha iyi kozmetik skorlara ve nodül hacim değişikliğine yol açtığını göstermektedir.

Anahtar kelimeler: Tiroid nodülü, mikrodalga ablasyon, etanol ablasyon, ultrason, kozmetik skor

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INTRODUCTION

Thyroid nodules are mostly benign lesions that are quite common in a routine examination. Clinically, palpable nodules are found in 5-10% of the population, and there is a lifetime possibility of developing new thyroid nodules in 10% of patients [1,2]. The possibility of detecting nodules in the thyroid gland increases with age, and they are more common in females than males [3]. Nodules that cannot be palpated by physical examination are more likely to be detected by ultrasonography (USG), and the probability of encountering nodules in ultrasound examinations performed for screening purposes may exceed 50%. The widespread use of ultrasound imaging in daily practice has led to more detection of asymptomatic nodules [4,5].

Treatment is required for nodules that cause pressure on the respiratory tract and esophagus and cause a feeling of tension [6,7]. Although surgery is the main treatment method, aspiration can be applied for cystic thyroid nodules [8,9]. Minimally invasive approaches have been used more frequently in the treatment of benign mixed-type and spongy-type nodules in recent years, and ethanol ablation (EA) therapy can be easily performed [10,11]. Alternatively, microwave ablation (MWA) therapy is a relatively new minimally invasive treatment method that can significantly reduce the volumes of nodules [12]. MWA is widely used in the treatment of lung, liver, and kidney tumors, and its most important advantage is that it is less painful [13,14].

Mixed-type solid-component cystic nodules and spongy-type thyroid nodules are proven to be benign by fine-needle aspiration biopsies (FNAB). Ethanol is injected into the cyst, and ablation is performed on the nodule in these cases (Figure 1) [15-18]. In MWA therapy, a thin linear probe is inserted into the nodule with to reduce the nodule size and volume by using microwave energy [19]. In the follow-up of benign thyroid nodules, the volume changes are monitored with ultrasound, and the simple and very useful WHO cosmetic scoring system is applied before and after ablation treatment. The score's range is from 1 to 4 (1, no palpable mass; 2, no cosmetic problems, but palpable mass; 3, the mass only causes swallowing difficulties and is visible at close range; 4, the mass is easily visible). Accordingly, for scores ≥ 2, the choice of ablation treatment can be made by evaluating other accompanying symptoms [19,20].

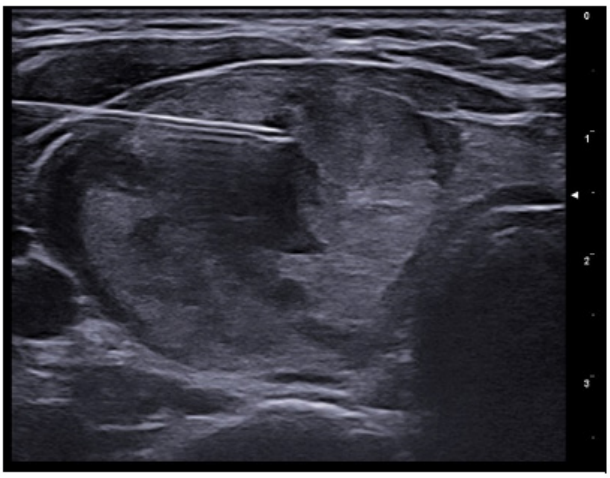

Figure 1. Ultrasound image showing a fine-needle inserted at the beginning of ethanol ablation treatment in a spongy-type nodule located in the right lobe of the thyroid gland.

With both ablation methods, the duration of hospital stay and the complications that may arise from surgery can be minimized [20,21]. In this study, the volumes obtained and changes in cosmetic scores after MWA and EA were evaluated retrospectively. In addition, a statistical analysis of the results was done to compare the efficiency of both treatment methods.

MATERIAL AND METHOD

This retrospective study was approved by the University of Health Sciences institutional review board (IRB protocol number: 20/347). Treatment methods were carried out according to approved guidelines. Written informed consent was obtained from all patients before biopsy and ablation procedures. Between July 2015 and July 2020, archive scanning was performed for patients who were proven to have benign with FNAB and underwent MWA and EA in the radiology clinic. The exclusion criteria were as follows: a history of chronic thyroiditis and thyroid gland surgery, a history of malignancy, the presence of congenital anomalies and pure cystic nodules, missing ultrasonographic data, BMI ≥35, and age under 18 years.

As a result, 57 MWA and 55 EA patients who

satisfied the eligibility criteria were identified and included in the study. Thyroid FNAB was performed before ablation therapies on the participants, and cosmetic scoring and USG data were also used to evaluate volumetric changes at the 3rd month and 6th month after treatment. In addition, the efficiency of MWA and EA was also compared.

B-mode ultrasound measurements of volume were performed by a radiologist who is experienced in USG and interventional radiology, and cosmetic scoring was performed for each patient in the same session. The volumetric measurements were performed using ultrasound images obtained in axial and sagittal planes by a 4–15-Mhz linear array probe (MyLab 9 eXP, Genova, Italy). EA and MWA procedures were applied in a single session.

No medication or contrast agents were administered during the ultrasound procedures at the 3rd and 6th month follow-up after the treatment. EA and MWA were performed on an outpatient basis, and patients were placed in a supine position with slight neck extension. They were followed-up throughout the day after the procedures were performed. Before the procedure, the location of the nodule was checked with ultrasound, and local anesthesia was applied under the skin with lidocaine. Ablation was performed under ultrasound guidance.

Statistical analysis

Statistical analysis of the data was performed using SPSS software (IBM SPSS ver. 22.0, IBM, Armonk, NY, USA). Descriptive statistics (median, frequency, percentage, minimum, maximum, mean, lowest, and highest) were used to express the central tendencies. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the normality of the quantitative data distributions. Correlations between parameters were tested using the Spearman correlation test. Pearson's chi-squared test was used to compare categorical variables. The Mann-Whitney U test was used to compare the differences between genders and laterality for data that were not normally distributed. The Kruskal-Wallis test was used to compare nodule volumes and cosmetic scores before the treatment and at the 3rd and 6th months. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 112 patients participated in this study, including 36 males (32.1%) and 76 females (67.9%). The youngest case was a 20-year-old female, the oldest case was a 78-year-old male, and the median age was 49.00 years. Table 1 shows the detailed distribution of the cases by gender, mean age, and median age.

Table 1: Demographics

Gender	Male	Female	Total
n	36	76	112
Minimum age	33	20	20
Maximum age	78	74	78
Mean age	52.54	46.46	48.10
Median age	52.00	46.00	49.00
Std. deviation (±)	9.74	12.63	12.17

All patients had a single nodule, 57 patients (50.9%) received MWA, and 55 patients (49.1%) received EA in a single session. There were mixed-type nodules in 61 cases and spongy-type nodules in 51 cases. No statistically significant difference was found between genders and nodule types according to the chi-squared test of independence ($p>0.05$). There was no statistically significant difference between genders and ablation types. Similarly, when the nodule type and ablation procedures were compared, no statistically significant difference was found ($p>0.05$). The smallest subgroup was 5 males with spongy nodules, and the largest group was 21 females with mixed-type nodules, and both underwent EA. The distribution of nodule types by gender and ablation procedure is shown in Table 2.

Table 2: Distribution of nodule types by gender and ablation procedure

			Ablation procedure		Total
			EA (n)	MWA (n)	
Male	Nodule type	Mixed-type nodule (n)	11	11	22
		Spongious nodule (n)	5	9	14
	Total		16	20	36
Female	Nodule type	Mixed-type nodule (n)	21	18	39
		Spongious nodule (n)	18	19	37
	Total		39	37	76

In all patients who underwent MWA and EA, the mean nodule volume was 21.12 ml before

treatment, 5.96 ml at the 3rd month, and 3.78 ml at the 6th month. In cases with MWA, the mean nodule volume was 21.43 ml before treatment, 5.55 ml at the 3rd month, and 2.76 ml at the 6th month ($p<0.05$). In the EA group, the mean nodule volume was 20.86 ml at pretreatment, 6.34 ml at the 3rd month, and 4.71 ml at the 6th month ($p<0.05$).

After MWA, there was a reduction in the mean nodule volume of 74.1% at the 3rd month and 87.2% at the 6th month. After EA, there was a 69.6% reduction in mean nodule volume at the 3rd month and 77.5% at the 6th month. A statistically significant difference was found in mean nodule volume between MWA and EA groups at the 6th month ($p<0.05$). All patients who underwent MWA and EA had a decrease in mean nodule volume at the end of the 6th month, and no recurrence was detected.

Before ablation therapies, the lowest cosmetic score was 2, and the highest score was 4 among all patients. After ablation procedures, the lowest score was 1, and the highest score was 4 at the 3rd month, while at the 6th month, the lowest score was 1, and the highest score was 3. The mean cosmetic score at the 3rd month after MWA was 1.46±0.62, that after EA was 2.20±0.75, and the difference was statistically significant ($p<0.05$). Similarly, the mean cosmetic score was 1.20±0.40 at the 6th month for MWA and 1.64±0.66 at the 6th month after EA, and the difference was statistically significant ($p<0.05$). Table 3 shows the nodule volumes at pretreatment, the 3rd month, and the 6th month according to the ablation type, as well as the cosmetic scores. There was no statistically significant difference between genders, post-treatment cosmetic scores, and nodule volume changes. ($p>0.05$).

Transient partial loss of voice developed in 4 patients who underwent EA and 5 patients who underwent MWA, but it resolved spontaneously by the end of the second week. Pain and tingling radiating to the teeth and ears were present in 5 patients who underwent EA and 6 patients who underwent MWA, but they disappeared completely by the end of the 5th day. No skin burns, discoloration, subcutaneous infection, hematoma, or scarring developed after MWA or EA.

DISCUSSION

Benign thyroid nodules are lesions frequently encountered in clinical practice during a physical examination and various imaging procedures (mainly ultrasound). USG is an important non-invasive, inexpensive, easily accessible imaging method that is widely used in the detection and evaluation of thyroid nodules. It provides information about the nodule size and structure, as well as changes in the thyroid gland parenchyma [2-4]. Surgery is used as the main treatment method for benign thyroid nodules when compression occurs due to the mass effect of the nodule on the trachea and esophagus or when there is a significant feeling of tension in the skin [5-7]. Complications that may occur after surgery have led physicians to seek different methods for the treatment and management of thyroid nodules, and minimally invasive approaches are more frequently being used [9,16].

EA has been increasingly used in the treatment of benign thyroid nodules (mainly pure cystic nodules). In thyroid nodules that are proven to be benign with FNAB, ethanol is injected into the nodule, ablation is performed, and the sizes and volume of the nodule are reduced [6-11]. MWA is a relatively new minimally invasive treatment method that can be used effectively and safely on benign thyroid nodules. A reduction in size and volume is achieved by introducing microwave energy to the nodule through an ultrasound-guided thin linear probe during the procedure [12,13]. Both ablation techniques are very useful in minimizing the complications that may arise from surgery and make great contributions to shortening the length of hospital stay [19-21].

Kim et al. examined 20 cystic and 22 solid thyroid nodules, and ultrasounds were performed at 1 and 6 months after EA. At the 6th month, a 65% decrease in cystic nodule volume and 38.3% decrease in solid nodule volume were achieved, but the decrease in solid nodule volume was much less than in our study [10]. In our study, there was a 77.5% decrease in the mean volume of the mixed-type and spongy-type thyroid nodules in the 6th month ultrasound after EA.

Iñiguez-Ariza et al. examined EA in pure cystic and mixed-type nodules, and a reduction of more

Table 3: Nodule volumes and cosmetic scores before and after ablation therapies

Ablation procedure		n	Minimum	Maximum	Median	Mean	Std. deviation (±)
MWA	Pre volume	57	0.70	172.00	14.05	21.43	31.46
	3rd vol	57	0.05	23.00	3.75	5.55	5.38
	6th vol	57	0.01	13.10	1.70	2.76	2.99
	Pre cos	57	2	4	3	3.09	0.78
	3rd cos	57	1	3	1	1.46	0.62
	6th cos	57	1	2	1	1.20	0.40
EA	Pre volume	55	0.49	185.73	11.02	20.86	29.38
	3rd vol	55	0.2	44.56	3.24	6.34	8.62
	6th vol	55	0.07	16.58	2.90	4.71	4,39
	Pre cos	55	2	4	3	3.12	0.79
	3rd cos	55	1	4	2	2.20	0.75
	6th cos	55	1	3	2	1.64	0.66
Total	Pre volume	112	0.49	185.73	11.94	21.12	30.23
	3rd vol	112	0.2	44.56	3.67	5.96	7.23
	6th vol	112	0.01	16.58	2.12	3.78	3.89
	Pre cos	112	2	4	3	3.10	0.79
	3rd cos	112	1	4	2	1.84	0.78
		112	1	3	1	1.43	0.54

*Pre volume: Nodule volume before ablation, 3rd vol: 3rd month volume, 6th vol: 6th month volume

*Pre cos: Cosmetic score before ablation, 3rd cos: 3rd month score, 6th cos: 6th month score

than 50% was observed in both nodule types during the 2-year follow-up. They showed that EA can be a good alternative to surgery in mixed-type thyroid nodules [20]. Korkusuz et al. applied MWA to 18 solid thyroid nodules and achieved a reduction of more than 50% in the mean nodule volume by the 3-month follow-up [12]. In another study, they evaluated the efficacy of the treatment by functional scoring before and after MWA and obtained data supporting the effective use of MWA in the treatment of benign thyroid nodules [13].

Feng et al. performed MWA therapy on 11 patients using linear probes of different thicknesses and lengths and detected a nearly 50% reduction in benign thyroid nodule volumes at the 9th month. As a result, they stated that MWA can be a feasible method of treatment using linear microwave probes of different sizes and thickness according to the size and location of the nodule [14]. Yue et al. applied MWA to 222 patients and 477 benign thyroid nodules, which is the largest related case series known in the literature. The mean nodule volume was reduced by more than 50% in the 6th month after ablation, and they stated that MWA therapy can be used safely and effectively on benign thyroid nodules [19].

Although EA is routinely applied to cystic nodules, it was applied to spongy-type and mixed-type thyroid nodules in our study, unlike many other studies [8,11]. We could not find any study in the literature comparing the efficiency of MWA and EA performed on mixed-type and spongy-type thyroid nodules. Thus, the comparison of the effectiveness of these methods in this study stands out as an innovation. This study has one of the largest case series in the literature in terms of the total number of cases and the number of cases of MWA and EA in a single session [6,7,19]. When cosmetic scoring and volumetric changes were evaluated, there was a significant difference between MWA and EA, and MWA was more successful in the treatment of mixed-type and spongy-type thyroid nodules.

Limitations: One limitation in this study could be that the ablation procedure, post-treatment ultrasounds, and cosmetic scoring were performed by a single radiologist. As another limitation, there was relatively low number of male cases of spongy-type nodules undergoing EA compared to females, and follow-up for more than 6 months after ablation may also be conceivable [5]. In addition, there is a lack of studies comparing both ablation methods in mixed-type and spongy-type thyroid nodules to compare our data, which is another limitation. Our study shows that EA

can also be used in mixed-type and spongy-type nodules, but there is still a need for new studies with larger case series to compare both methods.

Conclusion: Minimally invasive approaches are increasingly being adopted in the treatment of benign thyroid nodules. Both MWA and EA are effective and safe minimally invasive treatment techniques for these nodules and are highly effective in reducing the length of hospital stay. Our study showed that MWA therapy is slightly more successful in improving the cosmetic score and nodule volume in these cases, but EA can also be used safely. With new research conducted with larger case series, the effectiveness of these techniques in such thyroid nodules can be confirmed, and indications for use will become more widespread.

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RESEARCH ARTICLE

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A rare anatomical variation detected incidentally on computed tomography of the thorax: the azygos lobe

Toraks Bilgisayarlı Tomografisinde Tesadüfen Saptanan Nadir Bir Anatomik Varyasyon: Azigos Lobu

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ABSTRACT

Aim: An azygos lobe is an unusual anatomical variation of the upper lobe of the right lung. During the formation of the lungs, the right azygos lobe develops as the precursor of the azygos vein, moving along the pleural surface rather than arcing forward above the origin of the lung to reach the upper vena cava, enters the apex of the right lung in the upper thoracic region. The purpose of this study was to assess the prevalence of the right azygos lobe in a sample of the Turkish population.
Methods: This study was conducted from February to October 2020. Each computed tomography (CT) scan was checked for the presence of a right azygos lobe and a fissure on the axial plane. The azygos lobe was described as the convex line in the paramediastinal portion of the upper right lung on the CT scan. Our exclusion criteria were previous thoracic surgery in the right lung or parenchymal distortion on CT examinations.
Results: One thousand nine hundred and sixty-five thorax CT scans were evaluated retrospectively. Nine hundred and forty-five of them were from men and the remainder were from women. A total of 15 right azygos lobe variations were detected (six females, nine males). The mean age of the patients was 52.87 ± 14.604 years (30–77). The azygos lobe frequency was 0.76%.
Conclusion: The azygos lobe is typically an uncommon variation of the right lung and is not really a distinct lobe. It is a very important variation that should be known about as it can mimic pathological conditions such as cysts, abscesses, or lung lesions. Since the azygos lobe is usually an incidental finding, radiologists and surgeons should be aware of this this potential anomaly during the imaging and interventional procedures.
Keywords: Azygos vein, prevalence, computed tomography

ÖZ

Amaç: Azigos lob, sağ üst lobun nadir bir anatomik varyasyonudur .Akciğerlerin oluşumu sırasında, sağ azigos lob, prekürsör sağ akciğerin apeksinden azigos venin üst torasik kısmına girerek akciğerin orjin noktasının üzerinde ileriye doğru kıvrılarak vena kavaya ulaşmak yerine plevral yüzey boyunca hareket ederken gelişir.Bu çalışmanın amacı, bir Türk popülasyonu örnekleminde sağ azigos lobunun prevalansını değerlendirmektir.
Yöntem: Bu çalışma Şubat 2020'den Ekim 2020 tarihleri arasında gerçekleştirildi. Bilgisayarlı tomografi (BT) görüntüleri, aksiyel düzlemde sağ azigos lob ve fissür varlığı açısından kontrol edildi. Azigos lob, BT taramasında sağ akciğerin üst paramediastinal kısmındaki konveks çizgi olarak tanımlandı. Dışlama kriterlerimiz, sağ akciğerden daha önce göğüs cerrahisi geçirmiş olan ya da BT incelemelerinde parankimal distorsiyonu bulunan vakalardı.
Bulgular: 1965 toraks BT taraması retrospektif olarak değerlendirildi. Vakaların 945'i erkek, kalanı kadındı. Toplam 15 sağ azigos lob varyasyonu tespit edildi (6 kadın, 9 erkek). Hastaların yaş ortalaması 52.87 ± 14.604 (30-77) idi. Azigos lob frekansı% 0.76 olarak bulundu.
Sonuç: Azigos lob, tipik olarak sağ akciğerin nadir görülen bir varyasyonu olup gerçek bir lob değildir. Kistler, apseler veya akciğer lezyonları gibi patolojik durumları taklit edebileceği için bilinmesi gereken oldukça önemli bir varyasyondur. Azigos lob genellikle insidental bir bulgu olduğundan, görüntüleme ve girişimsel işlemler sırasında radyologlar ve cerrahlar bu anomalinin varlığı açısından dikkatli olmalıdır.

Anahtar Kelimeler: Azigos veni, prevalans, bilgisayarlı tomografi

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INTRODUCTION

An azygos lobe, first defined by Heinrich Wrisberg in 1778, is an unusual anatomical variation of the upper lobe in the right lung [1]. Via the azygos fissure, containing the azygos vein and a four-folded pleura, the azygos lobe is split from other lung areas. During the formation of the lungs, the right azygos lobe is developed as the precursor enters the apex of the right lung into the upper thoracic portion of the azygos vein, termed the right posterior cardinal vein, moving along the pleural surfaces, rather than arcing forward above the origin of the lung to access the superior vena cava [2, 3]. A good understanding of the entity is essential, as it can be mistaken for some disease processes such as cysts, abscesses or pulmonary lesions. It is also essential in planning for medical interventions. An understanding of the imaging characteristics of the azygos lobe is critical to avoid misdiagnosis and unwarranted interventions [4]. Its prevalence ranges from 0.4% on chest x-rays to 1.2% on computed tomography (CT) and is generally incidentally identified by imaging [4, 5]. The purpose of this study is to assess the prevalence of the right azygos lobe in a sample of the Turkish population.

MATERIAL AND METHODS

This study was approved by the Hitit University Ethics Committee and complies with the Helsinki Declaration (2020–353). Informed consent was waived due to the retrospective nature of the study as well as the fact that the assessment utilized anonymous research findings. The study included 1965 thorax CT scans, of which 945 subjects were men and the remainder were women. Many had respiratory symptoms, such as chest pain, shortness of breath and cough, while others were asymptomatic for respiratory problems but were routinely screened for several diseases, such as malignancies and lung nodules. This study was conducted over nine months, from February 2020 to October 2020.

CT studies were performed using a multidetector 16 row helical CT scanner (Alexion, Toshiba Medical Systems, Nasu, Japan). CT imaging was performed during a breath-hold at deep inspiration. Spirometric gating was not applied. The scans were obtained from the base of the neck down to the diaphragm. A supine or prone position was chosen. Parameters were 100–120 Kv, the field of view was 350 mm, beam collimation was 1 × 16 mm, gantry rotation time was 0.5 seconds and scan time was 11–13 seconds. Thin-section CT data was reconstructed at a slice thickness of 1 mm with 0.8-mm intervals. Image matrix size was 512 × 512. We used automatic tube-current modulation at a maximum of 225 mAs for exposure dose reduction. Intravenous contrast medium was not administered unless vascular pathologies, such as pulmonary embolism, were suspected. CT images were analyzed using a Vitrea workstation (Canon Medical Systems Corporation; Otawara, Japan) by a single radiologist, EG, who has seven years of CT experience.

Each CT scan was checked for the presence or absence of the right azygos lobe and fissure on the axial plane. The azygos lobe was described as the convex line in the paramediastinal portion of the upper-right lung, on the CT scan. Our exclusion criteria were participants who had previous thoracic surgery in the right lung or parenchymal distortion on CT examinations.

Statistical analysis was performed via the SPSS v.22 package program (IBM SPSS Statistics, Chicago, IL, USA). Participant age, gender, diagnosis and radiological features were recorded. Descriptive statistics were expressed as a mean, ± standard deviation (SD). Continuous variables were analyzed using the Mann-Whitney U test. A P-value of less than 0.05 was considered statistically significant.

RESULTS

From February 2020 to October 2020, 1,965 chest CT scans were evaluated retrospectively. Nine hundred and forty-five of them were from men and the remainder were from women. A total of 15 right azygos lobe variations were detected (6 females, 9 males). The mean age of the patients was 52.87 ± 14.604 (30-77). There was no statistically significant difference between the ages of men and women (p = 0.906). None of the patients had known congenital heart anomalies. The azygos lobe frequency was found to be 0.76% in the sample of 1,965 thorax CT. The prevalence of the right azygos lobe in males and females was 0.95% and 0.58%, respectively (Table 1). The most common

symptoms and other accompanying imaging findings were cough (8/15) and pneumonia (6/15). Symptoms and other accompanying CT findings of all cases are given in Table 2. Examples of the right azygos lobe from two different patients are shown in Figures 1 and 2.

Table 1: Prevalence of the right azygos lobe in patients.

	Male	Female	Total
Number of patients (n)	945	1020	1965
Patients with an azygos lobe (n)	9	6	15
Prevalence (%)	0.95	0.58	0.76

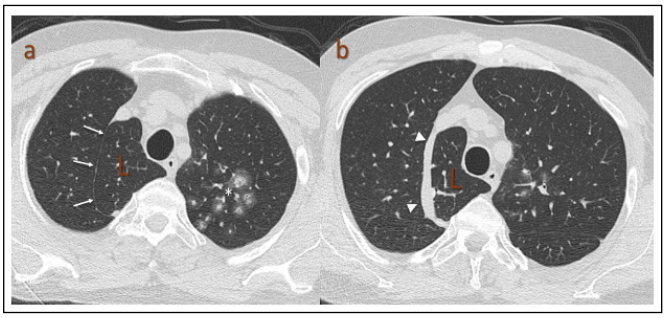

Figure 1: Parenchymal window images of consecutive axial non-contrast thoracic CT images of a 47-year-old female (a) shows pleural fold of azygos fissure at the arrow. Besides, nodular ground-glass opacities in the neighborhood of ' * ' in the left lung apicoposterior were found to be compatible with COVID pneumonia. (b) At the inferior sections, the view of the azygos vein is observed at the arrowhead. The azygos lobe (L) is located medial to the azygos vein and fissure.

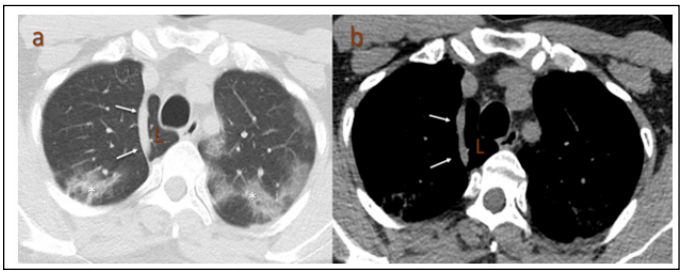

Figure 2: In a 48-year-old male patient who presented with shortness of breath, the azygos vein at the arrow and azygos lobe (L) are observed in the parenchyma (a) and mediastinum (b) window images of the non-contrast thoracic CT. Also, in the parenchymal window images, patchy ground-glass densities of both lungs are observed (*).

Table 2: Clinical symptoms and other CT findings in 15 adults accompanying the azygos lobe.

No	Age	Gender	Symptoms	Other CT findings
1	39	M	Cough and weakness	Emphysema
2	30	F	Cough, fever, and joint pain	Pneumonia
3	47	F	Cough and shortness of breath	Pneumonia
4	71	M	Cough and diarrhea	Atelectasis
5	77	F	Weakness and fever	Pneumonia
6	62	M	Sore throat, cough, and weakness	Pneumonia
7	54	M	Cough	Atelectasis
8	48	M	Shortness of breath	Pneumonia
9	37	M	Fever and joint pain	None
10	60	M	Headache, sore throat, and cough	Pneumonia
11	73	M	Shortness of breath and cough	Signs of heart failure
12	51	F	Cough	Atelectasis
13	64	F	Weakness and joint pain	None
14	35	M	Sore throat and weakness	None
15	45	F	Cough, weakness, and shortness of breath	Aortic aneurysm

DISCUSSION

The azygos lobe is an unusual anatomical variation of the upper lobe in the right lung, which ranges in frequency from 0.4% on chest x-rays, to 1.2% on CT scans; it is generally identified incidentally via imaging [6-8]. The azygos lobe is located in the medial part of the upper lobe of the right lung and is separated from the other parts of the upper lobe of the right lung through the azygos fissure. The fissure contains the azygos vein and a double layer of the pleural leaf. Since it does not have its bronchus and does not represent a unique bronchopulmonary segment, the azygos lobe is not a specific lobe [4, 9].

The azygos vein is typically formed by the union of the right subcostal vein and the right lumbar ascending vein. It continues to the thorax from the diaphragmatic aortic cavity, ascends through the thoracic vertebra, at T4, takes an anterior curve and joins the superior vena cava. In normal conditions, the posterior cardinal vein moves through the apex of the right lung to its usual position in the mediastinum. The azygos lobe is formed due to the penetration of the right posterior cardinal vein to the apex of the right lung

rather than normal migration during embryonic development. The azygos lobe is the outcome of a medial movement defect of the azygos vein to the right tracheobronchial junction, above the lung apex [10, 11].

In our study, the frequency of azygos lobes in thorax CT images was found to be 0.76%. The incidence in men was 0.95%, while the frequency in women was 0.58%. There are few studies in the literature on the azygos lobe and fissure, and these are mostly case reports. In the study performed by Özdemir et al., the frequency of the azygos lobe was found to be 1.54% [12]. In this study, the prevalence was found to be 1.39% in women and 1.64% in men. In the study conducted by Al-Mnayyis et al. with 1709 CT scans, the prevalence of the azygos lobe was found to be 1.01%, 0.62%, and 0.88% in men, women, and all cases, respectively [13]. In the study conducted by Perincek et al., the frequency of azygos lobes was found to be 1.09%, and the incidence was found to be higher in men than in women (approximately 56% and 44%, respectively) [8]. Our findings are consistent with these studies.

Despite the uncommon frequency of the azygos lobe, there may be a relationship with pathologies such as accessory fissures, extrapulmonary sequestration, masses, pneumothoraces, bullae, and vascular anomalies [7, 9, 14, 15]. Additionally, a consolidated azygos lobe can be misinterpreted as a mass [12, 16]. In our study, we did not identify any of the azygos-lobe-accompanying pathological conditions mentioned above; however, pneumonia was the most common accompanying imaging finding. Although the azygos lobe is a coincidental finding, all physicians should be aware of azygos lobe variation and anatomy to avoid misinterpretations; thoracic surgeons should also be aware of it as it may affect surgical approaches [6].

Limitations of Study:

Our study has two major limitations. First, since there was no contrast in our CT examinations, accompanying vascular anomalies could not be clearly evaluated. Second, our study includes only a limited number of patients and is single-centered. Future multi-center studies with higher patient numbers will provide more accurate information.

Conclusion: The azygos lobe is an uncommon variation of the right lung and is not a distinct lobe. It is necessary to have clear knowledge about the azygos lobe, as it can resemble pathological disorders such as cysts, abscesses or lung lesions. Since the azygos lobe is usually an incidental finding, radiologists and surgeons should be aware of this this potential anomaly during the imaging and interventional procedures. Our study is important in terms of demonstrating the successful detectability of this variation on CT without contrast.

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Diagnosis of Covid-19 in Children and Nursing Approach: A Systematic Review

Çocuklarda Covid-19'un Tanılanmasında Hemşirelik Yaklaşımı: Sistematik Bir İnceleme

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ABSTRACT

Aim: This study was carried out to summarize the systematic literature review on current information about Serious acute respiratory syndrome 2 (SARS-CoV-2 or Covid-19 in children and to determine nursing approach.
Methods: Regarding the research, 156 publications were examined between 21 January and 15 November 2020. The review was conducted with the key words of "SARS-CoV-2", "coronavirus", "Covid-19", "child", "nursing", which are openly accessible on databases such as PubMed, Science Direct and the WHO.
Results: Covid-19 infection may be asymptomatic or characterized by fever and fatigue in children; they can be potential carriers of the disease. Several upper respiratory symptoms have been seen, such as nasal congestion and a runny nose. In some patients, abdominal pain, nausea, vomiting and diarrhea occurs. Fever and cough were evident in pediatric patients. As the condition progresses, dyspnea, cyanosis and other signs may arise after typically one week of the disease, along with systemic toxic signs, including restlessness or malaise, decreased appetite, poor feeding and reduced activity. Children's condition might progress quickly and turn to respiratory failure, which cannot be improved by conventional oxygen within 1-3 days. Metabolic acidosis, septic shock, irreversible bleeding and coagulation dysfunction can take place in such severe cases. However, Covid-19 may cause an inflammatory reaction in some children. Those starting with gastrointestinal symptoms may progress to severe conditions and newborns whose mothers are infected with Covid-19 could have severe complications.
Conclusion: This systematic review has shown that children generally develop mild Covid-19 disease and these infections are often acquired through community sources. Diagnosis of the disease is difficult in children and there is limited data on children with Covid-19. The disease mainly causes fever, respiratory symptoms and other flu-like manifestations in children. The signs and symptoms of the disease should be carefully monitored. Nurses should know the course and symptoms of the disease well in children and take precautions.

Keywords: 2019-nCoV, care, Covid-19, child, nursing

ÖZ

Amaç: Bu çalışma, çocuklarda ciddi akut solunum sendromu 2 (SARS-CoV-2 veya Covid-19) hakkındaki güncel literatürü sistematik olarak özetlemek ve hemşirelik yaklaşımını belirlemek amacıyla yapılmıştır.
Yöntem: Araştırmaya konu olan kısım ile ilgili olarak, 21 Ocak ve 15 Kasım 2020 tarihleri arasında 156 araştırmaya ulaşılmıştır. İnceleme PubMed, Science Direct ve WHO veri tabanlarında "SARS-CoV-2", "koronavirüs", "Covid-19", "çocuk", "hemşirelik" anahtar ke-limeleri ile gerçekleştirilmiştir.
Bulgular: Covid-19 enfeksiyonu, çocuklarda asemptomatik olabilir ve hastalığın potansiyel taşıyıcıları olabilirler. Burun tıkanıklığı ve burun akıntısı gibi çeşitli üst solunum yolu semptomları görülür. Bazı hastalarda karın ağrısı, bulantı, kusma ve ishal görülür. Pediatrik hastalarda daha çok ateş ve öksürük görülmektedir. Durum ilerledikçe, huzursuzluk veya halsizlik, iştahsızlık, zayıf beslenme ve daha az aktivite gibi sistemik toksik belirtilerle birlikte tipik olarak hastalığın bir haftasından sonra dispne, siyanoz ve diğer belirtiler ortaya çıkabilir. Çocukların durumu hızlı bir şekilde ilerleyebilir ve 1-3 gün içinde geleneksel oksijenle düzeltilemeyen solunum yetmezliğine dönüşebilir. Metabolik asidoz, septik şok, geri döndürülemez kanama ve pıhtılaşma disfonksiyonu bu tür ağır vakalarda meydana gele-bilir. Bununla birlikte, Covid-19 bazı çocuklarda inflamatuar bir reaksiyona neden olabilir. Gastrointestinal semptomların görüldüğü çocuklarda ve anneleri Covid-19 ile enfekte olan yenidoğanlarda hastalığın ilerlemesiyle birlikte ciddi komplikasyonlar oluşabilir.
Sonuç: Bu sistematik derleme sonuçları çocuklarda Covid-19 hastalığının genellikle hafif geliştiğini ve bu enfeksiyonun genellikle toplum kaynaklı edinildiğini göstermiştir. Çocuklarda hastalığın tanısı zordur ve Covid-19'lu çocuklar hakkında sınırlı sayıda veri vardır. Bu hastalık, çocuklarda temel olarak ateş, solunum semptomları ve diğer grip benzeri belirtilere neden olur. Bu yüzden, hastalığın semptom ve belirtileri dikkatle izlenmelidir. Hemşireler çocuklarda hastalığın seyrini, semptomlarını iyi bilmeli ve önlem almalıdır.

Anahtar Kelimeler: 2019-nCoV, bakım, Covid-19, çocuk, hemşirelik

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INTRODUCTION

Serious acute respiratory syndrome 2 (SARS-CoV-2 or Covid-19), which was reported in Wuhan, China in December 2019, has spread rapidly. The sequence of the causative virus was shared by Chinese scientists at the beginning of January 2020. As soon as January 30, approximately 10 000 cases were confirmed and 15 000 cases were identified as suspects [1].

The Chinese government made quick and radical decisions, by canceling many events, flights, trains and bus schedules [2]. However, the measures taken were insufficient and the outbreak spread throughout the world, from Australia to Europe. On March 11th 2020, the epidemic was declared a pandemic by the World Health Organization [3].

According to the "Weekly epidemiological update" published by the World Health Organization (WHO) on the 8th and the 15th of December 2020, the number of Covid-19 cases had exceeded 70 million cumulative cases worldwide [4,5].

Common complaints such as fever, dry cough, dyspnea and fatigue, were reported in adults and considerable death statistics were reported in the elderly population [6]. In Covid-19 case statistics, it was determined that infants and children did not stand out prominently. Children's infections represent 10% of the Covid-19 cases. Since the beginning of the pandemic, over 1 million children, ages 0-17 years, have been infected with the Covid-19 virus in the United-States. The immature immune system have been linked to increased risk of infectious diseases, particularly respiratory viral infections in infants [7]. Current research suggests that the lowered susceptibility in children is likely due to the scarcity of the SARS-CoV-2 angiotensin-converting enzyme 2 (ACE2) receptor in the respiratory tract in children, meaning that the virus has less receptors to bind to and take hold within a child's respiratory tract. It is also hypothesized that higher rates of prior infection with other human coronavirus in children, may provide protection against severe SARS-CoV-2 infection in this population. Child cases are mostly affected by family members or other adult individuals. Young adults and children have a high likelihood of developing Covid-19 as a result of household transmission, once a family member tests positive to Covid-19. However, there is limited evidence of secondary infection from children to others and as a result, the role children play in the transmission pathways of Covid-19 remains unclear [8]. The clinical findings of the children are often atypical and milder [9]. Data on seriously affected pediatric patients is unknown [10]. A large proportion of the young children with the Covid-19 infection are asymptomatic, underpinning the need for ongoing surveillance to monitor Covid-19 disease epidemiology in this population, strengthening prompt laboratory identification for case isolation and clinical management.

As there are limited data on children with Covid-19, there is an urgent need to define the clinical characteristics and severity of the disease, especially in those countries that lack pediatric patient data. At present, China has made initial progress in containing the spread of Covid-19, however many countries are still suffering from the disease.

Nurses play a key role in pandemics as they are at the forefront of the fight against the disease. With their professional training, they can manage crises and make decisions. Pediatric nurses interact with children and parents of all ages and the provide effective health care to children and their families. They are accustomed to approaching parents and patients with evidence-based nursing information and alleviating the lack of information. Pediatric nurses can be advocates by increasing awareness of the need for clinical management and diagnosis, for children and parents affected by Covid-19. Children diagnosed with Covid-19 in this process may be anxious and nurses are able to be honest with children and reassure them [11]. Accurate and appropriate information about the process should be provided. The child should be protected against destructive news appearing on social media, television or heard on the radio [12]. Pediatric nurses can promote children's wellbeing by administering care, educating patients and families, as well as leading campaigns to increase awareness for Covid-19. This study was carried out to summarize the systematic literature review on current information about coronavirus disease 2019 (Covid-19) in children and ascertain nursing approaches.

MATERIAL AND METHOD

Design

This research is a literature review for the identification of the Covid-19 outbreak in children.

Research method and outcome

Regarding the research, 236 publications were accessed between 21 January and 15 November 2020. Covid-19 prevention plans and guides published by featured centers were also included in these studies. As they were unrelated or concerning adults (n = 51), out of the health field (n = 38) and duplicates (n = 43), 92 studies were excluded from the study. Of the remainder, 64 were accepted to this review and analyzed in detail. The review was conducted with the keywords of "SARS-CoV-2", "coronavirus", "Covid-19", "child", "nursing", which are openly accessible on databases such as PubMed, Science Direct and the WHO (Figure 1).

Methodological evaluation

Due to the importance of the subject, no methodological evaluation was made and all publications that could be accessed were included. Descriptive studies, case reports, letters, WHO reports and guides of institutions were all examined.

Data

In this research, the definition of Covid-19 in children and the nursing approach were synthesized according to the subject. Two reviewers conducted the search (AS, ST, and ES). ES compiled all articles identified through a literature search. Two reviewers (AS, and ST) independently screened the title. All of the articles published about children were examined for use.

RESULTS

Etiology

Covid-19 is a new human coronavirus alongside 229E, NL63, OC43, HKU1, MERS-CoV and SARS-CoV. Covid-19 is a pleomorphic single-strand RNA virus with a diameter of 60-140 nm, circular or elliptical. There is an 86.9% to 89% genome similarity to bat-SL-CoVZC45 coronavirus occurring in bats. The physicochemical feature of Covid-19 has not been clarified yet [13].

Epidemiology

People of all ages are sensitive to Covid-19. The elderly and those with chronic diseases are more likely to turn into serious cases and it was found that children have a better prognosis. According to the data reported by China's Center for Disease Control on 11 February 2020, 44,672 cases were confirmed. Of these, 416 (0.9%) were found to be 0-10 year-olds and 549 (1.2%) were between 10-19 years old [14]. In a meta-analysis where 65 studies from 11 different countries were analyzed, a total of 1,214 children with RT-PCR confirmed Covid-19 infection (further mentioned as Covid-19 cases) were included. It was stated that 474 Covid-19 cases from China, 720 from the United States of America and Canada, eight from the United Kingdom, five from Iran, two from Malaysia, and one each from Vietnam, Lebanon, Iraq, France and Germany. Of 1,214 Covid-19 cases, age-distribution data were available for 1,135 (93%) cases. The age of 1,135 Covid-19 cases ranged from zero days to less than five years and 596 (53%) were less than one year (infant). Among the 596 Covid-19 infant cases, five (1%) were newborns [7]. However, the proportion of severe and critical cases was 10.6% under 1 year of age, compared to 7.3, 4.2, 4.1, and 3.0% among the 1-5, 6-10, 11-15, and > 15-year subsets, suggesting that infants may be at higher risk of severe respiratory failure than initially thought. It is not known why the disease is less common in children. A possible reason may be that children have less outdoor activity and travel. Another reason may be that children have a more active immune system and a healthier respiratory system. Perhaps children are also less exposed to cigarette smoke and air pollution [15].

Transmission route and physiopathology

Covid-19 infection is transmitted between people as a result of contact, droplet delivery (by caregivers, family members, visitors) and nosocomial infections [16]. It has been reported that the Covid-19 pathogen can be found in the stool and it is also thought that children carry this pathogen longer in their stools [17]. It was found that the incubation stage of the disease is between

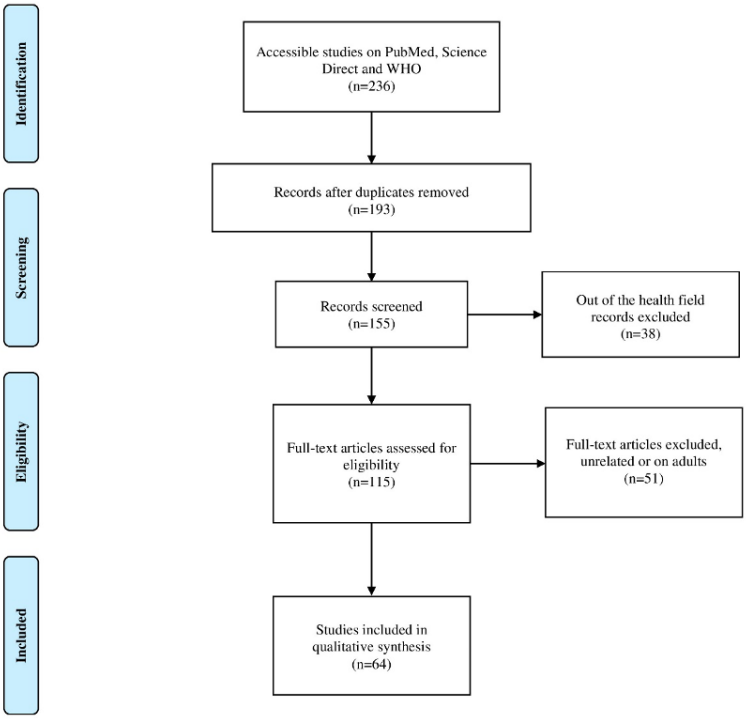

Figure 1. Flow diagram of the studies included in the review.

7-14 days [18], and mostly changes between 3-7 days [19]. The binding of a receptor expressed by host cells is the first step of viral infection, followed by cell membrane fusion. Lung epithelial cells are thought to be the primary target of the virus. Thus, it has been reported that human-to-human transmissions of SARS-CoV occur by the binding between the receptor-binding domain of virus spikes and the cellular receptor, which has been identified as an angiotensin-converting enzyme 2 (ACE2) receptor. Importantly, the sequence of the Covid-19 spiked receptor binding domain is similar to that of the SARS-CoV. This data reveals that entry into host cells is most likely through the ACE2 receptor [20].

Symptoms and Signs

According to data obtained from adults with Covid-19, fever, dry cough, dyspnea, muscle pain, fatigue and leukopenia problems occur. In acute cases, severe respiratory distress, septic shock, metabolic acidosis and coagulation disorders, were observed a week after the onset of the disease [21].

Covid-19 infection may be asymptomatic or characterized by fever, dry cough and fatigue in children. Several upper respiratory symptoms have been observed, such as cough, nasal congestion and runny nose. In some patients, abdominal pain, nausea, vomiting and diarrhea occurs. Most infected children have mild clinical signs and the prognosis is good. Many pediatric patients recover 1-2 weeks after the onset of the disease [9].

The characteristics of the symptoms of pediatric patients as reported by some select studies are provided in Table 1 [10,21–29]. It was determined that fever and cough were evident in pediatric patients (Table 1). In chest computerized tomography (CT) studies, pediatric patients showed signs of glass opacities and thickened lung tissue [30].

Laboratory and imaging

In the clinical findings of children infected with Covid-19 that have been described, diagnosis is made with swabs from sputum or secretions. However, Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay can detect Covid-19 cases in the blood, where the C-reactive protein (CRP) is high. Creatine kinase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine can be increased in the presence of Covid-19 [31].

Table 1. Symptoms of children infected with Covid-19.

Author(s)	Age (%)	Value	Gender (%)	Fever (%)	Nasal Congestion (%)	Cough (%)	Dyspnea (%)	Abdominal Pain (%)	Vomiting (%)	Diarrhea (%)	Fatigue (%)	Headache (%)
Sun et al. [10]	0-3 years	8	6 (75) males 2 (25) females	6 (75)	Not specified	6 (75)	Not specified	Not specified	4 (50)	3 (37.5)	1 (12.5)	1 (12.5)
Zheng et al. [21]	0-3 years (40)	10	14 (56) males 11 (44) females	13 (52)	2 (8)	11 (44)	2 (8)	2 (8)	2 (8)	3 (12)	Not specified	Not specified
	3-6 years (24)	6										
	≥6 years (36)	9										
Qui et al. [22]	≤5 years	10	23 (64) males 13 (36) females	13 (36)	1 (3)	7 (19)	1 (3)	Not specified	1 (3)	1 (3)	Not specified	3 (8)
	>5	28										
Le et al. [23]	3 months old	1 (case report)	Female	Yes	Yes	No	No	No	No	No	Yes	-
Ji et al. [24]	15 years old	1 (case report)	Male	Yes	Yes	No	No	No	No	No	No	No
	9 years old	1 (case report)	Male	No	No	No	No	No	No	Yes	No	No
Cui et al. [25]	5 years old	1 (case report)	Female	Not specified	Yes	Yes	Yes	Not specified	Not specified	Not specified	Not specified	Not Specified
Wang et al. [26]	1 month -7 years	31	Not specified	20 (65)	Rare	14 (45)	Not specified	Not specified	Rare	3 (10)	3 (10)	Rare
	<1 year	31 (18)	104 (60) males 67 (40) females	71 (42.5)	9 (5.3)	8 (48.5)	4 (2.3)	Not specified	11 (6.4)	15 (8.8)	13 (7.6)	Not specified
	1-5 years	40 (23)										
	6-10 years	58 (34)										
	11-15 years	42 (25)										
Xu et al. [28]	2 months old-15 years old	9	5 (55) males 4 (45) females	6 (66)	2 (22)	4 (44)	Not specified	Not specified	Not specified	2 (22)	Not specified	Not Specified
Liu et al. [29]	1-11 months' old	9	2 (22) males 7 (78) females	4 (44)	1 (11)	2 (22)	Not specified	Not specified	Not specified	Not specified	Not specified	Not Specified

It has been determined that some tests taken from swab or blood were negative, but that there was the presence of opacity and thickening in the chest CT. Repeat tests have reported cases that negative results are actually positive. Therefore, chest CT has a vital importance as it is very sensitive and effective in detecting signs of pneumonia [30].

Culture analysis can be used in diagnosis because of stool transmission. Nucleic acid testing from respiratory samples or serum are effective in detecting the virus and results can usually be obtained in 4-6 hours [17,31].

It is difficult to diagnose in children with mild or atypical findings. Different imaging and laboratory tests should be applied to children who are suspected [28].

Treatment

The evidence in terms of care for Covid-19 is ever-changing and there exists no substantive evidence about treatment, at this point in time. However, some interventions are recommended.

Most disease cases have a history of life in the epidemic region or contact with individuals who have travelled there [32]. This situation should not be ignored. General management comprises in homoeostasis maintenance, close follow-up of blood work and chest radiography, and initialization of respiratory support if required [33]. Contact and droplet isolation measures should be applied to suspected or infected patients, in single rooms [34]. There is no definitive evidence whether breastfeeding can cause Covid-19 infection, however, it has been stated that infected mothers should not breastfeed their infants until they recover [33]. Liquid and electrolyte balance should be maintained, routine blood tests should be performed and chest radiography should be reviewed regularly [35]. Respiratory support should be administered with non-invasive oxygen therapy or invasive ventilation [18]. Oxygen, like other drugs, should be applied only in a specific and appropriate concentration [36]. In the drug treatment of Covid-19, some antiviral drugs whose effectiveness is not fully known have nonetheless been recommended [37]. The effectiveness of these drugs in children continues to be investigated [38]. In newborns, high dose nitric oxide inhalation, surfactant and high frequency ventilation are recommended, in respiratory distress [39]. Glucocorticoids or immunoglobulins can be administered intravenously to newborns who show no improvement [40]. Interferon nebulization and gamma globulin infusion have been reported to be successful in treatment [41].

Nursing Approach

Virus mutations and germ diversity have created many pandemics in the world [42]. Contagion is high, as most of the epidemic diseases are unpredictable. In the process of obtaining information about an epidemic, healthcare professionals need to work in coordination [43]. Edmonson et al. stated the importance of nursing interventions in global outbreaks [12]. Nurses make an important contribution to the prevention, intervention and management of diseases and they offer expert perspectives in implementing emergency response plans in outbreaks. Their understanding and expertise regarding care delivery models, complex systems, resources, infection prevention and control principles, in addition to biopsychosocial human needs, make them valuable assets as leaders and members of the interprofessional healthcare community and in legislative advocacy preparedness.

On March 11th 2020, the World Health Organization (WHO) declared Covid-19 as a pandemic [44]. Since the start of Covid-19, a race against time has begun to treat and care for patients. During this period, doctors, nurses and all health workers have worked with great devotion. In the process, patients have presented with requirements such as oxygen and mechanical ventilation, intensive care and drug therapy. Nurses play a key role in the success of these procedures [36]. They successfully provide both basic care to these patients and the delivery of the psychosocial process. Therefore, it is a great challenge for nurses to fight Covid-19, but their ongoing work in the pandemic is making nursing history.

It was determined that Covid-19, an RNA virus, is highly contagious and that 2-3 more people from an infected patient can be infected [45]. Many studies have proven that Covid-19 can be transmitted from person to person in hospital and family settings [17,32]. Control measures have been reported to be of vital importance in the early stage of the outbreak [13]. Preventive treatments have an important role to control and avoid the spread of respiratory disease, especially in children and this prevention may not be easy to achieve, as preventive measures can be difficult to define. Patients and their families may not be aware that hospitals are either restricting or not allowing visitors to hospitals for all patients, and not only for those hospitalized with Covid-19. This fact may have significant implications for the type of care that patients who are at an elevated risk of mortality wish to receive, and this should be clearly communicated, ensuring patients and loved ones/family can be well informed regarding signs and symptoms of deterioration, as well as how to self-monitor the patients' condition on a daily basis. Nurses can provide information sessions for residents on Covid-19 to inform them about the virus, the disease it causes, and how to protect themselves from infection.

Available data indicates that the incubation period of Covid-19 varies from 1 to 14 days. This is a

serious risk, however it is possible to prevent the spread of the outbreak by preventing contact with infected cases [19]. Medical staffs are at particular high risk of acquiring newly emerging infectious diseases while treating patients. Such risks arise during the first encounter with a patient, at the onset of an outbreak, when faced with a large number of patients. Nurse should wear personal protective equipment when caring for infected/ suspicious cases [46]. They should also take appropriate insulation measures [34]. Disposable equipment should be used as much as possible. If devices need to be reused, they should be sterilized or disinfected after each patient and should be disposed of appropriately [47].

Covid-19 infection is mostly asymptomatic in children [48]. However, the existence of another condition in children can exacerbate symptoms and pediatric patients whose condition has deteriorated should be treated in intensive care. Loutfy et al. reported that approximately 20% of their patients required intensive care during the SARS outbreak [49]. Pediatric patients may need respiratory support with either non-invasive oxygen therapy or invasive ventilation during a Covid-19 infection [18]. In the case of respiratory distress that occurs despite nasal catheter or mask oxygenation, heated humidified high-flow nasal cannula (HHHFNC), non-invasive ventilation, such as continuous positive airway pressure (CPAP), or non-invasive high-frequency ventilation, can be used. An emergency transfer situation plan has been prepared to ensure the safe transfer of newborns with suspect and definite Covid-19 [50]. Special vehicles should be used to transfer infected patients and strict protection for staff of transportation and disinfection for the vehicles, are of vital importance [51]. After the transfer is complete, ambulances, transfer equipment and personnel disinfection are recommended.

Infectious diseases can easily spread if proper medical care is not provided and it can cause harmful effects. Providing emergency management in an infectious disease requires professionalism in nursing. It is not known what kind of problems the outbreak will cause in children in the future, though it has been suggested that the long-term effects of Covid-19 on children will be worse than the initial phase of pandemic [52]. Although children have a low risk of disease, the daily life of families may be greatly affected: isolation measures applied to the family or child cause stress and they may also be concerned about the infection. They may be afraid of what could happen if they or another family member got sick with Covid-19 and nurses can ensure family members and children have access to psychosocial support.

Children with special health needs, chronic conditions and weakened immune system require special health precautions during coronavirus pandemics. Their routines and social environments may change; they may be kept away from their friends and loved ones. Nurses should be honest and help give confidence to these children. A comfortable environment should be created for them to feel safe. Honest answers, appropriate for the age of development, should be given to them in a calm fashion. Being open and sharing information can reduce anxiety, confusion and false perceptions [11]. The anxiety of a child who has lost a relative should be met with understanding. Stress management can be achieved through relaxation methods and breathing exercises and children should be protected from negative publications related the outbreak on social media, television, newspaper and radio. Adults should limit children's exposure to these media as they may misinterpret what they hear and be particularly afraid that they do not understand.

Limitations of Study : Some publications were in Chinese and treatment options have not been clarified yet and these were important limitations in our study. In addition, analyzing the research with limited examples can be considered another limitation.

Conclusion:

The Covid-19 pandemic has spread rapidly and it can be fatal in elderly patients and those with chronic diseases. There is limited data on children with Covid-19 and although those with Covid-19 have had milder clinical symptoms and better clinical outcomes, the incubation period of this highly contagious virus for children has been shown to be longer than that of adults. There is an urgent need to define the clinical characteristics and severity of the disease, especially in those

countries that lack pediatric patient data. The pandemic of Covid-19 has had a milder course in children, therefore the diagnosis of the disease has been difficult in this population. The signs and symptoms of the disease should be carefully monitored as treatment options have not yet been clarified.

Nurses are at the forefront with their professional training. They have an important role in the prevention and control of the disease. They should be well aware of the course and symptoms of the disease in regards to children and take precautions. They are a sensitive age group, they require special care and nurses should support and protect them. Separation from family members could increase the risk of psychiatric disorders in children with Covid-19 [53]. Offering honest responses appropriate for the child's developmental age can reduce their anxiety, confusion, and misperceptions. In the face of Covid-19, entire families can fall into great panic, and a hospitalization may lead them to suffer from separation anxiety. Pediatric nurses can provide family members with psychological support, Covid-19 related knowledge and methods of channeling children's negative emotions and answering their questions. Coordinated efforts are required to promote the physical and psychological well□being of vulnerable populations, including pediatric Covid-19 patients and their families, during the pandemic and the considerable uncertainties of the future. Excessive interventions can be useful during an emergency when the dynamics related to the way the virus spreads are still not fully known, but urgent considerations and studies are needed to understand which of these interventions are truly effective and useful for these patients and their families. It is hoped that these suggestions could help other countries to identify possible preventive and therapeutic strategies.

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