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Book section
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ORIGINAL ARTICLE

Phnx Med J. March 2024, Volume 6 No 1
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The Effect of War Conditions and Social Stress on Semen Parameters in Syrian Refugees

Suriyeli Mültecilerde Savaş Koşulları ve Sosyal Stresin Semen Parametreleri Üzerindeki Etkisi

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Health Sciences University, Adana City Training and Research Hospital, Assisted Reproduction Unit, Andrology Laboratory Yüregir/Adana/Türkiye

ABSTRACT

Objective: *This study aims to evaluate the effects of stress due to war in Syrians exposed to after migration to Turkey on sperm parameters.*

Material and Method: *Syrian migrant patients who underwent spermiogram due to infertility were included in the study. The patients were divided into two groups according to the period when the migration started and the stress was felt intensely, and the period when the immigrants had settled down and had less stress. Sexual abstinence duration, age, ejaculate volume, sperm concentration, total motility, progressive motility, morphology according to Kruger Strict criteria, were evaluated.*

Results: *There were 300 patients in group 1 and 902 patients in group 2. There was a statistically significant relationship between the marital status of the refugee patients and the years. There was no statistically significant difference between the groups in terms of ejaculate volume, concentration, morphology and sexual abstinence parameters. The total motility values of the patients in group 2 were statistically significantly higher than the patients in group 1. The progressive motility values of the patients in group 2 were statistically significantly higher than the patients in group 1.*

Conclusion: *The heavy psychosocial stress created by the war and post-war migration affects both sperm motility and progressive motility.*

ÖZET

Amaç: *Bu çalışma, Türkiye'ye göç sonrası maruz kalan Suriyelilerde savaş kaynaklı stresin sperm parametreleri üzerine etkilerini değerlendirmeyi amaçlamaktadır.*

Gereç ve Yöntem: *İnfertilite nedeniyle spermiogram yapılan Suriyeli göçmen hastalar çalışmaya dahil edildi. Hastalar göçün başladığı ve stresin yoğun olarak hissedildiği dönem ile göçmenlerin yerleşip daha az stres yaşadıkları dönemlere göre iki gruba ayrıldı. Kruger Strict kriterlerine göre cinsel perhiz süresi, yaş, ejakülat hacmi, sperm konsantrasyonu, toplam hareketlilik, ilerleyici hareketlilik ve morfoloji değerlendirildi.*

Sonuçlar: *Grup 1'de 300, Grup 2'de ise 902 hasta vardı. Mülteci hastaların medeni durumu ile yıllar arasında istatistiksel olarak anlamlı bir ilişki vardı. Ejakülat hacmi, konsantrasyonu, morfolojisi ve cinsel perhiz parametreleri açısından gruplar arasında istatistiksel olarak anlamlı fark yoktu. Grup 2'deki hastaların total motilite değerleri grup 1'deki hastalara göre istatistiksel olarak anlamlı derecede yüksekti. Grup 2'deki hastaların ilerleyici motilite değerleri grup 1'deki hastalara göre istatistiksel olarak anlamlı derecede yüksekti.*

Sonuç: *Savaşın ve savaş sonrası göçün yarattığı ağır psikososyal stres, hem sperm hareketliliğini hem de ilerleyen hareketliliğini etkiliyor.*

Keywords:
Stress
Infertility
Sperm concentration
Sperm motility
Sperm morphology

Anahtar Kelimeler:
Stres
İnfertilite
Sperm konsantrasyonu
Sperm hareketliliği
Sperm morfolojisi

INTRODUCTION

Infertility is defined as the absence of pregnancy despite regular (two days a week) and unprotected sexual intercourse for one year (1,2). Infertility is a problem that affects 10-15 percent of couples of reproductive age, and the male factor alone accounts for about 50 percent of this problem (3,4).

Testicular and hypothalamo-pituitary diseases (such as cryptorchidism, orchitis, genital tract infections, varicocele, male genital tract obstructions and hypogonadism), genetic conditions (Kallmann or Klinefelter syndromes, globozoospermia and Y chromosome microdeletions), cancer, systemic diseases, medical treatments or endocrine disorders are the main causes of male infertility. In addition, lifestyle-related factors such as smoking, alcohol, drug use, high-energy nutrition, obesity and psychological stress negatively affect male reproductive potential (1,5,6).

The negative effects of stress (work stress, life stress, etc.) on sperm quality, sperm concentration, motility and morphology have been discussed in previous studies. However, studies investigating the effect of war on sperm parameters are limited (7, 8). How the war affects sperm parameters is not fully understood. However, exposures and traumas experienced during and after the war seem to cause this (7,8).

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Migration from Syria to Turkey began in March 2011 with the escalation of the civil war and violence in Syria. The civil war in Syria has displaced 6.5 million Syrians, hundreds of thousands have been injured or killed, and created a vacuum in basic infrastructures that will resonate across the region for years to come (9-11). With these events, there was an intense refugee influx to Turkey (12-15). Turkey hosts nearly four million Syrian refugees. It has been observed that refugees frequently experience various physical and mental health problems such as depression, anxiety and post-traumatic stress disorder (14,15).

This study aims to evaluate the effects of stress due to war in Syrians exposed to after migration to Turkey on sperm parameters.

MATERIAL AND METHOD

The study was started after the approval of the local ethics committee (Ethics committee decision no: 1994, date: 23.06.2022). In this retrospective study, the semen analyzes of 1202 Syrian refugees who applied to the IVF Center Andrology Laboratory of a tertiary hospital due to infertility between 2014 and 2021 were evaluated retrospectively. Ejaculate volume, sperm concentration, total and progressive motility, and morphology parameters were studied based on the 2010 semen analysis criteria of the World Health Organization (16). The patients were divided into two groups according to the years of admission to our clinic. The patients who applied in the first four years (2014-2017) when the effects of the stress caused by war and refugee were intense, were evaluated as the first group, and the patients who applied in the second four years (2018-2021) when the refugees settled in our country and the stress decreased (2018-2021) were considered as the second group.

Collection and Analysis of Samples

After 2-7 days of sexual abstinence, the patients delivered their semen samples in a sperm container by masturbation by ejaculating into a sterile, cylindrical container with a transparent, red cap in a dimly lit room. Received samples were kept in an incubator (Thermo Scientific®, USA) at 37°C for 30 minutes for liquefaction. Liquefied samples were taken into a sterile laminar cabinet (K-System®, USA) and the analysis process was started. After examining the macroscopic properties, the volumes of the samples were measured with the help of graduated conical tube. Then, 0.5 µl volume of semen from each sample was placed in the Counting Chamber Makler with a pipette. Counting was started under a phase contrast microscope (Olympus CX®, Japan) and at least 200 sperm were evaluated at 200X (20X objective, 10X ocular) magnification and sperm concentration, total and progressive motility were recorded. Semen samples with a concentration above 5 Million/ml were stained with a staining kit (Spermac Stain, FertiPro®, USA) and evaluated according to the Kruger morphology criteria. The semen samples of the first and second groups of Syrian refugee patients were compared according to the data obtained based on the 2010 Semen Analysis criteria of the World Health Organization.

Statistical Evaluation

Statistical analyzes were performed using a package program called SPSS (IBM SPSS Statistics 24). Frequency tables and descriptive statistics were used to interpret the findings. The "Mann-Whitney U" test (Z-table value) statistics were used to compare the measurement values of two independent groups in the data that did not have a normal distribution. "Pearson-$\chi 2$" crosstabs were used to examine the relationships between two qualitative variables.

RESULTS

A total of 1202 patients were included in the study. There were 300 patients in group 1 (applicants between 2014-2017) and 902 patients in group 2 (applicants between 2018-2021). The first patient application to the Andrology laboratory was in 2014. After the first application, patients' applications increased over the years. There was a statistically significant relationship between the marital status of the refugee patients and the years ($\chi 2=82.889$; $p=0.000$). While the rate of being married was significantly higher among refugee patients in all years, the rate of being married was the highest in 2015 (96.2%), and the rate of being single was the highest in 2021 (30.5%) (Table 1).

Table-2 shows the mean and median values of age, ejaculate volume, concentration, total and progressive motility percentage, percentage of normal morphology, and duration of sexual abstinence. A statistically significant difference was found between the groups in terms of age

Table 1: Examination of the relationship between the years of admission and marital status of refugee patients.

Variable	Married		Single		Total		Statistical analysis* Possibility
	n	%	n	%	n	%	
Years							
2014	65	92,8	5	7,1	70	5,8	
2015	51	96,2	2	3,8	53	4,4	
2016	77	92,7	6	7,3	83	6,9	
2017	88	93,6	6	6,4	94	7,8	$\chi 2=82,889$ $p=0,000$
2018	179	91,3	17	8,7	196	16,3	
2019	287	92,8	22	7,2	309	25,7	
2020	169	85,7	28	14,3	197	16,5	
2021	139	69,5	61	30,5	200	16,6	

**Pearson-$\chi 2$ crosstabs were used to analyze the relationships between two qualitative variables.*

Table 2: Comparison of sperm parameters according to year groups.

Variable	2014-2017 (n=302)		2018-2021 (n=900)		Statistical analysis* Possibility
	$\bar{X}$±S.D.	Median [Min-Max]	$\bar{X}$±S.D.	Median [Min-Max]	
Age (years)	29,46±6,24	29,0 [17,0-51,0]	28,39±6,40	28,0 [16,0-56,0]	Z=-3,013 p=0,003
Ejaculate Volume (ml)	3,49±1,68	3,3 [0,4-12,5]	3,69±1,72	3,5 [0,0-15,0]	Z=-1,773 p=0,076
Concentration (Million/ml)	23,81±32,78	10,8 [0,0-202,0]	27,23±34,99	13,0 [0,0-232,0]	Z=-1,940 p=0,052
Total motile sperm percentage (%)	35,34±26,21	38,5 [0,0-100,0]	41,04±23,35	43,0 [0,0-100,0]	Z=-3,267 p=0,001
Progressive motile sperm percentage (%)	29,36±24,74	28,0 [0,0-100,0]	33,75±29,20	33,0 [0,0-576,0]	Z=-2,639 p=0,008
Morphology (%)	1,34±1,93	1,0 [0,0-11,0]	1,43±1,87	1,0 [0,0-12,0]	Z=-1,278 p=0,201
Duration of sexual abstinence (Day)	4,09±1,15	4,0 [2,0-14,0]	4,12±1,44	4,0 [2,0-25,0]	Z=-0,146 p=0,884

**The "Mann-Whitney U" test (Z-table value) statistics were used to compare the measurement values of two independent groups in the data not having normal distribution.*

(Z=-3.013; p=0.003). The age of the patients in group 1 was statistically significantly higher than the age of the patients in group 2.

There was no statistically significant difference between the groups in terms of ejaculate volume (ml), concentration (Million/ml), morphology (percentage of normal sperm) and fasting time (days) parameters (p>0.05). A statistically significant difference was found between the groups in terms of percent total motility values (Z=-3.267; p=0.001). The total motility values of the patients in group 2 were statistically significantly higher than the patients in group 1.

A statistically significant difference was found between the groups in terms of progressive motility values (Z=-2.639; p=0.008). The progressive motility values of the patients in group 2 were statistically significantly higher than the patients in group 1.

DISCUSSION

It is not clear how war (psychological trauma and stress) affects sperm parameters. Psychological stress has been shown to be negatively related to various parameters associated with semen quality, including sperm concentration, motility, and morphology. Increased stress levels may be related to the direct effect of the war experience from acute stress, but also to marginalization, cultural problems, deterioration in physical infrastructure and socioeconomic conditions (14,17). The effects of stress may be through the hormonal component of spermatogenesis. There is evidence that such a phenomenon may be associated with hormonal changes observed in men during stressful events. Testicular biopsies from highly stressed prisoners showed complete spermatogenetic arrest in all cases (18).

The first patient included in our study admitted in 2014. The number of patients increased over the years. There was a statistically significant difference between the first years of war and the hardships of being a refugee (Group 1) and group 2, in which the refugees were partially settled, in terms of both the number of patients and their marital status. While the number of married patients was high in the first group, the number of single patient admissions was higher in the second group. The fact that families migrated by taking all kinds of risks together with the concern of protecting their spouse and children explains the excess of the married patient population in the first years, while the long duration of the war and the fact that the children who came with the families grew up and reached the age of marriage support the increase in the number of single patients in the second four years.

In our study, total motility and progressive motility values were statistically significantly lower in the first group compared to the second group. In a study examining the relationship between work stress and life stress and semen quality, it was reported that work stress was not associated with semen parameters, but life stress was negatively correlated with sperm concentration, motility and morphology (19). War is both life stress and psychosocial stress. This study is similar to our study in terms of motility, but differs in terms of concentration and morphology. In another study, it was shown that psychosocial stress reduces sperm concentration and motility, but does not affect morphology (20). However, in this study, it was limited to 3 months whether the patients experienced a major event related to stress or not. The fact that they found the change in sperm concentration with the acute effect significant may be due to the shortness of this period. In our study, we found that sperm motility was lower in group 1 patients, who migrated due to war, had more intense life and psychosocial stress from their place of residence and homeland, compared to group 2 patients, but their concentration and morphology were not significantly affected. The mechanism affecting semen

quality may be mediated by neuroendocrine factors affecting spermatogenesis (21). Stress causes an increase in seminal plasma reactive oxygen species, resulting in oxidative stress that affects semen quality and fertility (5,22). Most of the hypothetical pathways by which stress can affect semen quality operate under endocrine factors (23,24). Men living under stress have been shown to have lower testosterone and luteinizing hormone levels. However, while stress causes a secondary elevation in serum LH and FSH levels, it primarily lowers the serum total testosterone level, which alters the seminal quality (25). It has been reported that this situation impairs spermatogenesis, especially reduces sperm motility, and also negatively affects morphology and sperm count (23,24). Another study showed that an increase in post-stress levels of cortisol and adrenocorticotropic hormones may cause impaired conversion of androstenedione to testosterone in Leydig cells. This disruption results in lower mean semen volume, sperm concentration, and sperm cell motility, along with increased androstenedione and decreased testosterone levels (24). Stress can affect the level of many other factors in the plasma. For example, it has been shown that stress causes a significant increase in nitric oxide in plasma and a decrease in arginase activity, thus worsening semen quality (26). War is a psychosocial stress in which both the struggle for survival and severe traumas are experienced, perhaps it is an event where stress is experienced most intensely. While migrants struggle to survive in the country they migrate to, they are under stress with the anxiety that what they leave behind creates in them. In addition, the stress caused by the psychosocial effects of a new country and a new lifestyle will naturally affect all balances. Our study covers a long period. The significantly lower sperm motility in group 1 patients with intense stress may be due to stress-induced neuroendocrine mechanisms. The insignificance of concentration change in the long-term study may be due to the activation of adaptive mechanisms.

In a retrospective study that included 10000 semen samples to show the effects of the war on sperm parameters during and after the Lebanese civil war, it was determined that sperm concentration decreased during the war period compared to the 5-year period after the war. Again in this study, it was shown that the percentage of abnormal sperm morphology increased in the post-war period and that the war did not have a significant effect on semen volume and sperm motility (7). In this study, the authors stated that these changes in sperm parameters are due to the neuroendocrine mechanism created by war-related psychological stress. In our study, a significant difference was found between the groups in the percentages of total and progressive motile sperm. However, there was no significant difference between the two groups in terms of semen volume, concentration and sperm morphology. In a study conducted after the 10-day war in Slovenia in 1991, it was shown that there was a significant decrease in progressive motility and rapid progressive motility, and that sperm morphology and concentration did not change (27). Of course, the neuroendocrine changes caused by stress in the acute period and the changes that occur after long-term stress are different, and this situation also affects spermatogenesis differently. The effects of psychological stress on sperm parameters are still controversial and the mechanism by which it can affect semen quality is unclear. In addition to studies showing that stress negatively affects parameters related to semen quality, there are also studies showing that there is no relationship between the psychosocial status of men and semen parameters (28). This discrepancy may be explained by differences in population characteristics, study design, and assessment of psychosocial factors.

Fukuda et al. (29) evaluated only the short-term effects of the earthquake and found that sperm motility decreased in men who lost their homes as a result of the 1995 Kobe earthquake, but there was no significant difference between sperm concentrations before and after the earthquake. In our study, while there was a significant decrease in both total and progressive motility in the first group, who had to leave their homeland during the (acute) period of intense war and conflict, there was no significant difference in sperm concentrations between the two groups. The effects of disasters on semen parameters are a complex issue because many variables come into play that can affect semen parameters. Both the study of Fukuda et al (29) and our study showed that catastrophic events (civil war, loss of a family member during the war, and having to leave their homes) cause a disorder in sperm motility.

In a study conducted in the USA before and after a devastating natural disaster such as Hurricane Katrina, there was a significant difference between the two groups in terms of sperm motility and morphology, but no statistically significant difference was found in terms of concentration (30). In our study, we found that motility was significantly lower in the near period, which had devastating effects, compared to the distant period, and sperm morphology did not show a significant change. The study investigating the sperm effects of Hurricane Katrina was a study that included only normospermic patients and may explain this difference. The change in concentration between the two groups was not significant in our study either. War and post-war migration, living in a different place, pain and social stress cause significant differences between sperm parameters in both total and progressive motility. It also reveals that war conditions and social stress negatively affect sperm motility in Syrian patients. The high variability in semen parameters makes it difficult to obtain sufficient data in semen quality studies. More detailed studies are needed to clarify the information on this subject.

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ORIGINAL ARTICLE

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Evaluation Of Hearing Functions of Children with Type 1 Diabetes: A 2-Year Retrospective Study

Tip 1 Diyabetli Çocukların İşitme Fonksiyonlarının Değerlendirilmesi: 2 Yıllık Retrospektif Çalışma

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ABSTRACT

Objective: *Our study aims to investigate the differences in pure tone audiometry and tympanograms in terms of gender, disease duration, and right-left ear in children with Type 1 diabetes mellitus (Type 1 DM).*

Material and Method: *For our study, a total of 109 patients between the ages of 0-18 who were diagnosed with Type 1 DM between 2016-2017 were identified. Among these, 26 children, 52 ears, aged between 2-18, who applied to the Ear Nose Throat outpatient clinic for different reasons and had anamnesis and examination information, were included. Then, hearing thresholds and middle ear functions were evaluated by looking at pure-tone audiometers and tympanograms. Whether the patients met the study criteria was retrospectively determined by using the patient registration forms filled out by the pediatric endocrinology.*

Results: *In the measurements made for the right and left ears, the conduction threshold values at a frequency of 4000 and 8000 Hz were found to be statistically significantly higher values in the Type 1 DM patient group for 5 years and longer than the control group with Type 1 DM for less than 5 years ($p<0.05$). Both right and left ear bone canal threshold values of the patient group with diabetes for 5 years or longer were found to be statistically significantly higher at a frequency of 4000 Hz ($p<0.05$). A statistically significant and negative moderate correlation was found between the threshold values of speech discrimination (SD) in the right and left ears and the duration of the disease.*

Conclusion: *As a result of our study, individuals with Type 1 DM were found to have a higher risk of developing hearing loss. For this reason, it is recommended to examine hearing in detail and to establish a clinical examination protocol in this patient group.*

ÖZET

Amaç: *Çalışmamızın amacı; Tip 1 diabetus mellitus (Tip 1 DM) çocuklarda, saf ses odyometrileri ve timpanogramlarının; cinsiyet, hastalık süresi ve sağ-sol kulak yönünden farklılıklarının araştırılmasıdır.*

Gereç ve Yöntem: *Çalışmamız için 2016-2017 yıllarında Tip 1 DM tanısı alan yaşları 0-18 arasında değişen toplam 109 hasta belirlenmiştir. Bunların içerisinden, kulak burun boğaz (KBB) polikliniğine farklı sebeplerden başvuran, anamnez ve muayene bilgileri olan yaşları 2-18 arasında değişen, 26 çocuk 52 kulak dâhil edilmiştir. Ardından saf ses odyometrileri ve timpanogramlarına bakılarak işitme eşikleri ve orta kulak fonksiyonları değerlendirilmiştir. Hastaların çalışma kriterlerine uygun olup olmadığı ise pediatrik endokrinoloji tarafından doldurulan hasta kayıt formlarından geriye dönülerek belirlenmiştir.*

Bulgular: *Sağ ve sol kulak için yapılan ölçümlerde 4000 ve 8000 Hz frekansta hava yolu eşik değerlerinin 5 yıl ve daha uzun süre Tip 1 DM'li hasta grubunda, 5 yıldan daha az süre Tip 1 DM'li kontrol grubuna oranla istatistiksel olarak anlamlı bir şekilde daha yüksek değerlerde olduğu bulundu ($p<0,05$). 5 yıl ve daha uzun süre diyabetli olan hasta grubunun hem sağ, hem de sol kulak kemik yolu eşik değerlerinin 4000 Hz frekansta istatistiksel olarak anlamlı bir şekilde daha yüksek değerlerde bulundu ($p<0,05$). Sağ ve sol kulak konuşmayı ayırt etme eşik (SD) değerleri ile hastalık süresi değerleri arasında ise istatistiksel olarak anlamlı ve negatif yönlü orta düzeyde ilişki saptandı.*

Sonuç: *Çalışmamızın sonucunda; Tip 1 DM tanısı olan bireylerde işitme kaybı gelişme riskinin daha fazla olduğu bulunmuştur. Bu sebeple, bu hasta grubunda işitmenin ayrıntılı olarak incelenmesi ve klinik muayene protokolü oluşturulması tavsiye edilmektedir.*

Keywords:
Type 1 diabetes
Hearing loss
Pure-tone audiometry

Anahtar Kelimeler:
Tip 1 diyabet
İşitme kaybı
Saf ses odyometri

INTRODUCTION

Diabetes Mellitus is a genetically defined chronic metabolic disorder characterized by hyperglycemia resulting from autoimmune destruction of beta-cells of the pancreas (1,2). It is caused by reduced tissue responses to insulin in the absence or lack of secretion of the insulin hormone released from beta cells in pancreatic islets (3-6). It also causes vascular and neuropathic complications due to its metabolic effects (5).

Type 1 DM was also called 'insulin-dependent diabetes', 'juvenile diabetes', or 'childhood-onset diabetes' in the past. In type 1 diabetes mellitus, pancreatic beta cells are

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mostly seen in cases of absolute insulin deficiency due to autoimmune damage. Genetic predisposition and many environmental factors that have not been clearly revealed are thought to play a role in the development of type 1 DM (7).

In patients with DM, the mechanism of hearing impairment (as in retinal and kidney dysfunction) is largely unclear because it cannot be evaluated by intravital examination. However, there are many different hypotheses about how DM affects hearing. DM is characterized by the presence of insulin and beta-cell autoantibodies resulting from the autoimmune destruction of insulin-producing beta cells (8). Cochlear hypoxia due to diabetic microangiopathy, neuropathy due to vascular/metabolic events in the cochlear nerves, and changes in cochlear glucose levels are thought to cause auditory pathologies (9-12).

Glucose metabolism significantly affects the physiology of the inner ear, which is metabolically very active. The energy of the inner ear is not stored, so small changes in glucose in the blood affect the function of the inner ear and cause balance disorders. As a result of this mechanism, vertigo, tinnitus, hypoacusis, and ear fullness can occur (13).

Therefore, our study aims to investigate the differences in pure-tone audiometry and tympanograms in terms of gender, disease duration, and right-left ear in children with type 1 DM.

MATERIALS AND METHODS

This study was carried out in Başkent University, Department of Otorhinolaryngology, Audiology Clinic. Written informed consent was obtained from each patient for the tests to be performed in the audiology clinic. It has been declared by the Ethics Committee of Başkent University that there is no harm in conducting the study with the decision dated 24.01.2018 and numbered 3227.

In this study, among 109 patients aged 0-18 who were followed up and treated with a diagnosis of type 1 DM in Başkent University Hospital Pediatric Endocrinology Outpatient Clinic in 2016-2017, 26 patients and 52 ears with anamnesis and examination information, aged between 2-18, who applied to the Ear Nose Throat outpatient clinic for different reasons were included.

Whether the patients met the study criteria was determined by using the patient registration forms filled out by the Endocrinology. The glucose and Hemoglobin A1c (HbA1c) results of the patients were taken into account. With these results, the duration of the disease and the treatment adopted were used as study parameters.

Madsen Orbiter 922-2 Clinical Audiometry (Denmark) device was used for pure voice and speech audiometry. TDH-39 standard earphones were used for conduction hearing thresholds and speech tests, and a radioear B-71 vibrator was used for bone conduction hearing thresholds. Frequencies of 125-8000 Hz for conduction thresholds and 500-4000 Hz frequencies for bone conduction thresholds were evaluated. Conduction and bone conduction thresholds of 500-1000-2000 Hz as mean pure-tone (SSO) were calculated separately for both ears (14). The speaking threshold (SRT) test was performed with a three-syllable word list and speech discrimination (SD) test with a live voice using the monosyllabic phonetic balanced word list (FD-300). Only tympanograms of patients under 2 years of age were evaluated.

Statistical Analysis

The research data were evaluated using the SPSS 22.0 statistical package program. Descriptive statistics are presented as mean (±) standard deviation, median (minimum-maximum), frequency distribution, and percentage. The compliance of continuous variables to normal distribution was evaluated using visual (histogram and probability charts) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). For categorical variables, whether there was a difference between groups in terms of frequency was compared using Chi-square tests. Patients with type 1 DM were divided into two groups (<5 years and ≥ 5 years) as the type 1 DM patient group for 5 years or longer, and the control group with type 1 DM for less than 5 years.

RESULTS

52 ears of 26 patients (10 females, 16 males) diagnosed with type 1 DM were included in the study. The mean age of the patients was 12.5±3.8. The mean disease duration of the patients was 5.4±4.9 (1-16) years, and the mean HbA1c (%) value was 6.6±2.1 (4.7-12.4). When the groups were examined according to the duration of the disease, there were 15 patients (57.7%) with an illness of fewer than 5 years and 11 patients (43.3%) with 5 years or more.

Type A tympanogram was obtained in 21 (80.8%) of the patients and type B tympanogram in 5 (19.2%). SNHL (sensorineural hearing loss) was not detected in 20 (80%) of the patients. Hearing loss in the right and left ears was graded according to SSO (pure tone average); 19 (76.0%) patients and 20 (80%) patients had normal hearing in the right ears and left ears. 5 (20.0%) patients and 4 (16%) patients had mild hearing loss in the right ears and left ears, respectively. 1 (4%) patient had severe hearing loss in the right ear and 1 (4%) patient had severe hearing loss in the left ear.

Table 1 presents the comparison of pure-tone audiometry conduction and bone conduction threshold values of the research groups. In the measurements made for the right and left ears, conduction threshold values at a frequency of 4000 and 8000 Hz were found to be statistically significantly higher in the patient group with DM for 5 years or longer ($p<0.05$). Conduction threshold values at other frequencies were found to be similar in both groups. Besides, in the evaluation made for the bone conduction, it was found that the bone conduction threshold values of both the right ear and left ear at a frequency of 4000 Hz were statistically significantly higher in the patient group with DM for 5 years or longer ($p<0.05$). For both ears between groups, pure-tone threshold mean values and disturbing loudness levels were similar.

Speaking threshold and speech discrimination score values of the participants were compared. In the right and left ear evaluations, speech acquisition threshold values were found to be similar in the group with DM for less than 5 years and the patient with type 1 DM for 5 years or longer. In the right and left ear evaluations, the speech discrimination score % values had statistically significantly lower values in the patient group with type 1 DM for 5 years or longer ($p<0.05$).

Table 1: Comparison of pure-tone audiometry and conduction and bone ducts hearing threshold values of the study groups participating in the study.

		Children with Type 1 DM diagnosis <5 years (n=15)				Children with Type 1 DM diagnosis ≥5 years (n=11)				
	Frequencies	Mean±sd	median	minimum	maximum	Mean±sd	median	minimum	maximum	p
Right ear conduction	500 Hz (dB)	13.5±12.0	10.0	0	35.0	19.1±25.0	10.0	5.0	95.0	0.955
	1000 Hz(dB)	12.1±10.1	10.0	0	30.0	20.5±29.0	10.0	5.0	105.0	0.473
	4000 Hz(dB)	13.6±11.8	10.0	5.0	35.0	27.7±24.0	25.0	10.0	95.0	0.026
	8000 Hz(dB)	12.1±7.8	10.0	0	25.0	30.9±24.2	20.0	18.0	85.0	0.006
Left ear conduction	500 Hz(dB)	12.1±9.9	10.0	5.0	30.0	21.36±23.8	15.0	5.0	90.0	0.083
	1000 Hz(dB)	10.4±8.8	7.5	0	25.0	20.0±27.4	10.0	5.0	100.0	0.185
	4000 Hz(dB)	12.5±9.8	10.0	5.0	30.0	28.2±29.0	15.0	10.0	110.0	0.008
	8000 Hz(dB)	13.6±8.6	10.0	5.0	30.0	35.5±29.6	25.0	5.0	110.0	0.010
Right ear bone	1000 Hz(dB)	4.3±2.7	5.0	0	10.0	10.9±19.9	5.0	0	65.0	0.336
	4000 Hz(dB)	6.1±3.5	5.0	0	10.0	17.3±21.6	10.0	5.0	80.0	0.028
Left ear bone	1000 Hz(dB)	3.6±3.6	5.0	0	10.0	10.0±20.5	5.0	0	70.0	0.518
	4000 Hz(dB)	5.0±3.4	5.0	0	10.0	17.7±21.4	10.0	5.0	80.0	0.003
Pure-tone threshold mean value (dB)	Right ear	13.1±10.5	10.0	3.0	32.0	20.0±26.1	10.0	5.0	96.0	0.415
	Left ear	12.1±10.1	10.0	2.0	30.0	21.6±28.3	13.0	5.0	105.0	0.085
Uncomfortable sound level(dB)	Right ear	107.9±4.3	110.0	100.0	110.0	106.0±5.2	110.0	100.0	110.0	0.334
	Left ear	107.9±4.3	110.0	100.0	110.0	106.0±5.2	110.0	100.0	110.0	0.334

Hz: Hertz, dB: Decibel.

The relationship between disease duration, age, and HbA1c values of the participants in the study with pure-tone audiometric threshold values, speech acquisition threshold, and speech discrimination scores were examined (Table 2). No relationship was found between HbA1c and pure-tone audiometric threshold values, speech reception threshold, and speech discrimination scores. A statistically significant and positive moderate relationship was found between the threshold values of conduction measurements at 4000 and 8000 Hz in both the right and left ears of type 1 DM patients participating in the study and the duration of the disease. It was found that patients with high disease duration values had a high right and left ear conduction threshold values in both frequencies. A statistically significant and positive moderate correlation was found between the right and left ear bone conduction thresholds at a frequency of 4000 Hz and the duration of the disease. Both right and left ear bone conduction threshold values at 4000 Hz frequency were also high in patients with high disease duration values. There was no statistically significant relationship between the measurements at other frequencies and the duration of the disease. There was no statistically significant relationship between right and left ear SRT values and disease duration values. A statistically significant and negative moderate correlation was found between the right and left ear SD values and the duration of the disease ($p>0.05$). SD values of those with high disease duration values were found to be low in the study. A statistically significant and positive moderate correlation was found between the age values of the participants and the left ear bone canal threshold values at a frequency of 4000 Hz; no significant relationship was found between pure-tone audiometric threshold values, speech reception threshold, and speech discrimination scores at all other frequencies.

DISCUSSION

The relationship between hearing loss and type 1 DM started to be discussed by Jordao about 150 years ago after the report of hearing loss and sudden diabetic coma (15-17). Some studies have shown that patients with high blood glucose levels in type 1 DM follow-up have severe hearing loss, and bilateral sensorineural hearing loss may

Table 2: The relationship between the disease duration, age, and HbA1c values of the study participants with pure-tone audiometric threshold values, speech acquisition threshold, and speech discrimination scores.

Spearman's correlation coefficients n=26	Right ear conduction			Left ear conduction			Right ear bone		Left ear bone		Right Ear		Left Ear	
	1000 Hz(dB)	4000 Hz(dB)	8000 Hz(dB)	1000 Hz(dB)	4000 Hz(dB)	8000 Hz(dB)	1000 Hz(dB)	4000 Hz(dB)	1000 Hz(dB)	4000 Hz(dB)	SRT (dB)	SD (%)	SRT (dB)	SD (%)
Duration of Illness, years r(p)	0.128 (0.541)	0.430 (0.032)	0.487 (0.014)	0.218 (0.296)	0.493 (0.012)	0.479 (0.015)	0.192 (0.359)	0.567 (0.003)	0.182 (0.384)	0.681 (0.001)	-0.085 (0.692)	-0.429 (0.037)	-0.136 (0.525)	-0.411 (0.046)
Age r(p)	0.049 (0.815)	0.238 (0.253)	0.209 (0.317)	0.235 (0.259)	0.269 (0.194)	0.242 (0.244)	0.104 (0.619)	0.386 (0.057)	0.182 (0.384)	0.487 (0.014)	-0.179 (0.404)	-0.059 (0.783)	-0.203 (0.342)	-0.060 (0.780)
HgA1c r(p)	-0.118 (0.575)	0.129 (0.540)	0.078 (0.713)	0.018 (0.933)	0.066 (0.753)	0.215 (0.302)	-0.097 (0.646)	0.269 (0.194)	0.152 (0.467)	0.274 (0.185)	-0.135 (0.529)	-0.121 (0.573)	-0.018 (0.933)	0.044 (0.840)

Hz: Hertz, dB: Decibel, HgA1c: Hemoglobin A1c, SRT: Speech Reception Threshold, SD: Speech Discrimination

occur in middle frequencies (18-21).

Damage to the inner ear usually begins after the age of 40 and is noticed later when speaking frequencies (500 Hz, 1000 Hz, and 2000 Hz) remain intact. Nonetheless, it can occur at an earlier age in people exposed to noise and those with vascular and metabolic diseases. DM is responsible for overall metabolic sensorineural hearing loss (22). In this case, it is possible to notice the symptoms of hearing loss earlier in individuals with DM.

Although hearing loss has been observed for a long time in patients with type 1 DM, the cause-effect relationship has not been proven. The pathophysiology of otological findings in type 1 DM is not fully known, but it is thought that the auditory impairment is caused by damage to the inner ear cells or retrocochlear auditory pathways. There are also studies showing that there may be a relationship between glycemic control and hearing loss (24). In animal modeling by Timothy Smith et al., type 1 DM was created by applying streptozocin to mice. Subsequently, it was observed that mice developed cochlear microangiopathy. Thus, cochlear dysfunction occurred and hearing thresholds were affected (25). Based on all these studies, it is seen that DM can affect almost all components of hearing and cause different degrees of hearing loss (23).

In the study conducted on children with type 1 DM between the ages of 5-18 hearing threshold values of type 1 DM patients were higher than the control group. Besides, the auditory thresholds of the patients were positively correlated with the HbA1C concentration at frequencies of 250, 500, 1000, and 4000 Hz (17). In our study, a statistically significant and positive moderate correlation was found between the right and left conduction threshold values at 4000 Hz and 8000 Hz, and the right ear bone canal threshold value at 4000 Hz frequency and the duration of the disease.

In our study, HbA1c values and pure-tone audiometry thresholds were compared to support the negative effect of complications of diabetes on hearing. The relationship of disease duration, age, and HbA1c values with pure-tone audiometric threshold values, speech acquisition threshold, and speech discrimination scores were evaluated.

The significant correlation between HbA1c concentration and auditory thresholds in patient groups in the studies of Elamin et al. (19) and Pessin et al. (20) shows that, if glycemic control is insufficient, it may be an important factor in the development of hearing impairment in patients with type 1 DM. In our study, no relationship was found between HbA1c and pure-tone audiometric threshold values, speech acquisition threshold, and speech discrimination scores. This may be due to the small number of patients and their short duration of illness.

In a study conducted by Trevino-Gonzalez et al. on 84 children and adolescents with type 1 DM, it was observed that there was a prevalence of 14.3% (12 of 84 patients) sensorineural hearing loss. The most affected frequency is 8000 kHz (26). In our study, a statistically significant and positive moderate relationship was found between the threshold values of conduction measurements at 4000 and 8000 Hz in both the right and left ears and the duration of the disease.

In a study conducted by Silva et al. on the speech perception performance of patients with type 1 DM, it was found that pure tone threshold values were significantly higher at high frequencies and medium frequencies compared to partially healthy individuals. It was determined that there is a significant difference for all participants (n = 80 ears) between the thresholds of receiving speech in silence and speaking in the noise of the group with type 1 DM and the control group (27). In our study, the right and left ear speech discrimination score % values were statistically significantly lower in the patient group with DM for 5 years or longer.

Based on the studies in the literature, although high-frequency audiometry was not performed in our study, the audiometric results of individuals with type 1 DM between 0.125 and 8 kHz were evaluated, and significant results were obtained. Possibly because patients with type 1 DM had a short disease duration and the patients were young, DM complications such as nephropathy or retinopathy were not observed in the present study. Therefore, the relationship between diabetic end-organ complications and hearing loss could not be evaluated.

CONCLUSION

In the light of current knowledge, there is evidence that DM can cause hearing loss, but we cannot fully state the cause-effect relationship. Considering the fact that hearing dysfunctions of patients with type 1 DM may be asymptomatic, care should be taken to question patients diagnosed with type 1 DM in terms of hearing. In addition to cardiological and neurological evaluations, it should not be ignored that progressive hearing loss can be prevented in line with the results obtained after a routine ear, nose and throat examination.

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ORIGINAL ARTICLE

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Effects of Duloxetine on Oxidant-Antioxidant System in Rat Brain Tissues

Duloksetinin Rat Beyin Dokularındaki Oksidan-Antioksidan Sistem Üzerine Etkisi

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ABSTRACT

Objective: *After the relationship between depression and oxidative stress (OS) was demonstrated, the effect of antidepressant drugs on OS has become important. In this study, we aimed to determine the effects of the antidepressant duloxetine on the activities of the superoxide dismutase (SOD), catalase (CAT), adenosine deaminase (ADA), xanthine oxidase (XO) and glutathione peroxidase (GSH-Px) enzymes as well as the lipid peroxidation (LP) product malondialdehyde (MDA) and nitric oxide (NO) levels in rat brains.*

Material and Method: *Twenty male Sprague-Dawley rats were used for the study. The first group was the control group (n=10) and the second group was the duloxetine group (n=10). Duloxetine was administered intragastrically once a day at a dose of 10 mg/kg for two weeks in the second group. Water was administered intragastrically once a day for two weeks in the first group. Rats were sacrificed at the end of the fourteenth day. The brain tissues were collected and then analyzes were performed.*

Results: *As a result of this study, we found that duloxetine increased the SOD (P=0.026) activity and decreased the ADA (P=0.041), XO (P=0.034) and CAT (P=0.006) activities significantly compared to the control group. We also found an increase in the GSH-Px enzyme activity and decrease in the NO and MDA levels at non-significant rates in the duloxetine group brain tissues.*

Conclusion: *The significant increase in the activity of the antioxidant enzyme SOD, the significant decrease in the activities of the XO and ADA enzymes, which can cause the formation of reactive oxygen products in the organism, and the insignificant decrease in the LP indicator MDA suggest that duloxetine can positively change the antioxidant status in rat brain tissues.*

ÖZET

Amaç: *Oksidatif stres (OS) ve depresyon arasındaki ilişki gösterildikten sonra antidepresanların OS üzerine etkisi önemli hale gelmiştir. Bu çalışmanın amacı duloksetinin rat beyin dokularındaki katalaz (CAT), süperoksit dismutaz (SOD), adenozin deaminaz (ADA), ksantin oksidaz (XO) ve glutatyon peroksidaz (GSH-Px) enzim aktiviteleri ile nitrik oksit (NO) ve malondialdehid (MDA) düzeylerine etkilerini araştırmaktır.*

Gereç ve Yöntem: *Çalışmaya birinci grup kontrol grubu (n=10) ve ikinci grup duloksetin grubu (n=10) olmak üzere toplam yirmi tane Sprague-Dawley cinsi erkek rat alındı. Duloksetin grubuna günde bir defa 10 mg/kg dozunda intragastrik yoldan iki hafta süreyle duloksetin verildi. Kontrol grubuna da günde bir defa iki hafta süreyle intragastrik olarak su verildi. On beşinci günde tüm ratlar sakrifiye edilerek beyin dokuları çıkarıldı ve incelemeler yapıldı.*

Bulgular: *Çalışmamızda duloksetinin rat beyin dokularında kontrol grubuna kıyasla SOD (P=0,026) enzim aktivitesini anlamlı düzeyde artırdığını ve XO (P=0,034), ADA (P=0,041) ve CAT (P=0,006) enzim aktivitelerini anlamlı düzeyde azalttığını saptadık. Ayrıca GSH-Px enzim aktivitesini anlamlı olmayan düzeyde artma ve MDA ile NO düzeylerinde ise anlamlı olmayan düzeyde azalma saptadık.*

Sonuç: *Çalışmamızda antioksidan bir enzim olan SOD enzim aktivitesinin anlamlı düzeyde artması, organizmada reaktif oksijen ürünleri oluşumuna neden olabilen XO ve ADA enzim aktivitelerinin anlamlı düzeyde azalması ve LP göstergesi olan MDA'nın artmayıp, anlamlı düzeyde olmasa da azalması duloksetinin antioksidan durumu olumlu yönde değiştirebileceğini düşündürmektedir.*

Keywords:
Duloxetine
Adenosine deaminase
Superoxide dismutase
Xanthine oxidase
Malondialdehyde
Catalase

Anahtar Kelimeler:
Duloksetin
Adenozin deaminaz
Süperoksit dismutaz
Ksantin oksidaz
Malondialdehit
Katalaz

INTRODUCTION

Reactive oxygen species (ROS) are continuously produced by all body tissues, especially during oxidative phosphorylation (1). Under physiological conditions, these ROS are eliminated by cellular antioxidant mechanisms. However, under pathological conditions, there is a shift towards an oxidative state due to an increase in oxidant markers, a decrease in antioxidant mechanisms, or both (2). Oxidative stress (OS), defined as the disruption of this balance in the oxidant-antioxidant system in favor of the

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oxidant system (3). A persistent increase in OS can lead to cell and tissue damage (2). In particular, the brain is more sensitive to ROS due to its high lipid content and high energy demand (4).

Evidence, especially in the last two decades, reveals that an imbalance between the oxidant system and antioxidant defenses is involved in the pathogenesis of depression (5). For example, studies in patients with depression have indicated that increased ROS production and decreased antioxidative defense systems are responsible for the altered brain structure of these patients. (6,7). The mechanisms of action of antidepressants are still not fully understood despite their use for many years. The hypothesis that these drugs regulate noradrenergic and serotonergic neurotransmitter systems has been dominant until today (8). However, recent studies have shown that, these drugs also have antioxidant effects, based on which a new concept of antidepressant mechanism of action has been proposed (9).

Duloxetine is a drug, that acts by inhibiting the reuptake of both serotonin and noradrenaline in the central nervous system (CNS) (10). The positive role of duloxetine in the activation of antioxidant defense and its anti-inflammatory properties have been demonstrated in some studies (11-14). In an animal study, duloksetin significantly increased the expression of antioxidant enzyme Cu-Zn-SOD in gerbil hippocampal pyramidal neurons (14), in a cell culture study conducted with PC 12 neuronal cells, duloxetine increased the GSH-Px enzyme levels (11), in a mice study, duloxetine reduced the stressor-induced increased brain MDA levels, and increased the SOD and CAT enzyme levels (15), in an another rat study, it was found that duloxetine reversed the hippocampal methamphetamine-induced increased MDA levels and decreased SOD and GSH-Px enzyme activities (16). No study has been found in the literature examining the effects of duloxetine on ADA and XO enzymes. The data obtained from these studies suggest that duloxetine may activate mitochondrial antioxidant systems and plays a role in neuroprotection against some neurotoxic agents. However, there are very few studies directly demonstrating this neuroprotective effect. We thought that, in addition to its effect on neurotransmitters such as serotonin and noradrenaline, duloxetine may have positive effects on the oxidant-antioxidant system balance by strengthening antioxidant defense systems. In this study, we aimed to investigate the effects of duloxetine on SOD, XO, GSH-Px, ADA and CAT activities, which are enzymes related to the oxidant-antioxidant system, MDA levels, which is an indicator of LP, and NO levels, an inorganic radical, in rat brain tissues.

MATERIAL AND METHOD

This study was carried out in Süleyman Demirel University Faculty of Medicine Experimental Animal Research Laboratory and Department of Medical Biology Laboratory. The study was approved by the Local Ethics Committee for Animal Experiments of Süleyman Demirel University Faculty of Medicine (decision no. 04 dated 27.05.2010) and supported by Süleyman Demirel University Scientific Research Projects Management Unit under project no. 2183-TU-10.

Experimental Animals

The total number of animals included the study (sample size) was calculated by using the resource equation method (17). In this study, a total of 20 male Sprague-Dawley rats aged 8-12 weeks and weighing 200-250 g were used. They were obtained from Süleyman Demirel University Faculty of Medicine Experimental Animal Research Laboratory Production Unit. During the experiment, the rats were kept under standard light, humidity, and temperature (25º C) conditions. Feed and water were not restricted throughout the experiment. They were divided into 2 groups as control group and duloxetine experimental group. In the duloxetine experimental group, duloxetine was dissolved in water and administered intragastrically once daily at a dose of 10 mg/kg for two weeks. The control group was given intragastric water at a single dose of 10 ml/kg for two weeks (18). The weight of the animals was monitored every three days and the drug dose was adjusted accordingly.

Anesthesia and Tissue Samples

Feeding of all rats was discontinued overnight except water, and anesthesia was induced by i.p. administration of a ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) mixture approximately 24 hours after the last treatment, i.e., on the 15th day of the experiment.

All rats were sacrificed after anesthesia and brain tissues were removed. The brain tissue samples were stored at -20°C.

Preservation, Homogenization and Preparation of Samples for Experiment

Brain tissue samples were homogenized in 50 mM Tris-HCl buffer (pH 7.4) and transferred to glass tubes while maintaining their cold temperatures. After that, the samples were centrifuged at 16 000 rpm for 3 minutes. The homogenates were placed in Eppendorf tubes without increasing the temperature, and the NO, MDA and protein levels were determined. The homogenates were centrifuged again at 5000 rpm at +4°C for 30 minutes, and the supernatant was obtained. ADA, XO, and CAT detection assays were performed. The supernatants were diluted and vortexed with chloroform/ethanol to 1/1 (v/v) and then centrifuged at 3200 rpm at +4°C for 40 minutes. Protein levels and GSH-Px, SOD activities were determined on the supernatant.

Detection of Catalase Activity

The rate of degradation of hydrogen peroxide (H2O2) by CAT was measured spectrophotometrically by Aebi's method using the light absorption of H2O2 at a wavelength of 240 nm (19). The results obtained were calculated as k/gr protein.

Detection of Glutathione Peroxidase Activity

GSH-Px catalyzes the oxidation of reduced glutathione to oxidized glutathione. Oxidized glutathione is converted to reduced glutathione with the help of glutathione reductase and NADPH. The GSH-Px activity was calculated by measuring the change in absorbance at 340 nm due to the decrease in NADPH. The activity was recorded as units per gram of protein (U/gr) (20).

Detection of Superoxide Dismutase Activity

Detection of SOD activity is based on the inhibition by SOD of the reduction of nitroblue tetrazolium by superoxide anions in the medium. The resulting superoxide radicals

Table 1: SOD, CAT, XO, ADA and GSH-Px activities and MDA and NO levels in rat brain tissues.

Groups	SOD (U/mg protein)	CAT (k/g protein)	GSH-Px (U/g protein)	MDA (nmol/g protein)	NO (µmol/g protein)	XO (U/g protein)	ADA (U/g protein)
I-Control (n=10)	0.736 ± 0.120	0.083 ± 0.013	68.52 ± 17.40	15.78 ± 0.617	0.244 ± 0.089	0.454 ± 0.093	0.320 ± 0.040
II-Duloxetine (n=10)	0.858 ± 0.153	0.069 ± 0.005	86.24 ± 22.19	15.05 ± 1.123	0.168 ± 0.083	0.342 ± 0.106	0.276 ± 0.038
P values	0.026	0.006	0.089	0.112	0.140	0.034	0.041

The results are shown in arithmetical values mean ± standard deviation. SOD: Superoxide dismutase, CAT: Catalase, ADA: Adenosine deaminase, XO: Xanthine oxidase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde, NO: Nitric oxide

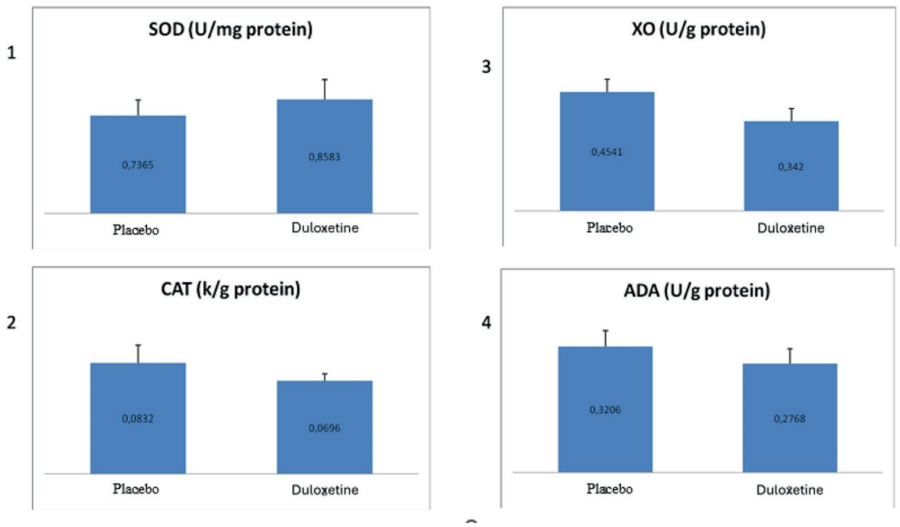

Figure 1: The duloxetine group was compared with the control (placebo) group. 1. The SOD activity was higher in duloxetine group (p= 0.026). 2. The CAT activity was lower in duloxetine group (p= 0.006). 3. The XO activity was lower in duloxetine group (p= 0.034). 4. The ADA activity was lower in duloxetine group (p= 0.041).

reduce nitroblue tetrazolium in the medium and form a colored complex. This complex formation was measured in a spectrophotometer at a wavelength of 560 nm. When SOD is present in the medium, there is no reduction, and a light color is observed in relation to the activity of the enzyme. The results obtained were calculated as U/mg protein (21).

Detection of Adenosine Deaminase Activity

The absorbance of ammonia by the reaction of ADA with adenosine was measured with a spectrophotometer at a wavelength of 628 nm. The results obtained were calculated as U/gr protein (22).

Detection of Xanthine Oxidase Activity

The absorbance of uric acid formed from xanthine was determined spectrophotometrically at a wavelength of 293 nm. The results were calculated as U/gr protein (23).

Detection of Nitric Oxide Levels: The NO levels in the tissues were measured by the Griess method (24). The results obtained were calculated as µmol/g protein.

Detection of Malondialdehyde Levels

Increased free radical formation at the end of LP was measured using the method of Draper and Hadley (25). MDA reacts with thiobarbituric acid to form a colored complex with maximum absorbance at 532 nm. The MDA concentration was calculated as nmol/g protein.

Protein Detection in Samples: The protein content was measured using bovine serum with albumin as the standard. This method is a combination of the biuret reaction and the Folin-Ciocalteau reaction under alkaline conditions. Formation of a dark blue color is characteristic. The darkness of the color is directly proportional to the protein concentration in the medium (26).

Statistical analysis

A Windows-compatible computer program SPSS 9.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Since the distributions of the groups were not normal, the Mann-Whitney U test, one of the non-parametric tests, was used to compare the groups. Values are given as arithmetic mean ± standard deviation and for statistical significance; $p<0.05$ values were considered significant.

RESULTS

Table 1 shows the SOD, CAT, GSH-Px, XO and ADA activities as well as the NO and MDA levels in the rat brain tissues of the two groups in our study. Duloxetine group revealed a statistically significant decrease in the CAT (p=0.006), XO (p=0.034) and ADA (p=0.041) activities, whereas a statistically significant increase was shown in the SOD activity (p=0.026), compared to the control group. There was a statistically non-significant decrease in the MDA and NO levels and a statistically non-significant increase in the GSH-Px activity in the duloxetine group compared to the control group. The graphical representation of the SOD, CAT, XO and ADA activities measured in the brain tissues are given in Figure 1, respectively.

DISCUSSION

With regard to the etiology of depression, evidence from the last two decades reveals an imbalance between the oxidant system and antioxidant defenses (5). Therefore, another potential target of antidepressant drug regulation should be intracellular antioxidant enzymes because antioxidant enzymes act to lower the OS levels of cells by scavenging free radicals, thereby working to prevent cell damage and neuronal death (27). Antioxidant enzymes are important for the brain as the brain is more vulnerable to OS (5). This study is the first in the literature to examine certain parameters of both oxidant and antioxidant systems of duloxetine in the same environment. The most important finding in our study was that we found a significant increase in the SOD activity and a significant decrease in the CAT, XO, and ADA activities in the duloxetine group. We also found a non-significant increase in the GSH-Px activity and a non-significant decrease in the MDA and NO levels in the duloxetine group.

The literature involves several studies showing that duloxetine has a protective effect against OS (11,12,16,28,29). In an in vitro experimental study, it was found that TRPM2 and TRPV1 channel activities involved in Ca2+ entry-induced oxidative neuronal death in rat hippocampus and DRGs decreased with duloxetine treatment, and it was suggested that this was the mechanism for apoptosis and the neuroprotective effect (12). In an in vitro experimental study in human neuroblastoma SH-SY5Y cells, it was shown that duloxetine had the potential to reduce ROS damage through the Akt/Nrf2/HO-1 protective signaling pathway and exhibited neuroprotective effects (28). In an ischemia-reperfusion animal experiment, pretreatment with duloxetine protected gerbil hippocampal pyramidal neurons from ischemia associated delayed neuronal damage. Pretreatment with duloxetine did not increase LP markers and significantly increased the SOD, an antioxidant enzyme, after ischemia-reperfusion, in neurons. As a result, it has been suggested that duloxetine has a neuroprotective effect against transient global cerebral ischemia, which may be due to the reduction of OS (14). In our study, in support of these findings in the literature, the duloxetine group demonstrated a significant increase in the activity of SOD, an antioxidant enzyme, and a decrease, although non-significant, in the levels of MDA, a LP product that is an OS indicator. These findings suggest that duloxetine may change the antioxidant status positively, hence may contribute positively to the imbalance between the oxidant system and antioxidant defenses, which is suggested to be involved in the etiology of depression. In a cell culture study in PC 12 neuronal cells, duloxetine was shown to be beneficial against apoptotic cell death and OS, which appear to be due to increased intracellular Ca+2 levels through activation of voltage-gated Ca+2 and TRPM2 channels, and the antioxidant GSH-Px and GSH levels were determined significantly higher in the duloxetine group (11). Although there was no statistically significant increase in the GSH-Px activity in our study, in the duloxetine group, the fact that it tended to increase supports this finding. That is because, SOD enzymes are involved in the catalytic dismutation of the toxic superoxide radical, and H2O2 is produced in this process. H2O2, the resulting reactive oxygen product, is eliminated by peroxidases such as GSH-Px (30).

An animal study with mice, evaluating the effect of duloxetine on chronic immobilization stress (CIS)-induced cognitive impairment and neurodegeneration, also assessed its effect on OS, as a result of which it was found that duloxetine pretreatment at doses of 10 and 20 mg/kg provided a dose-dependent decrease in elevated brain MDA levels and an increase in reduced brain GSH, SOD and catalase enzyme activities. Based on these findings, the authors concluded that the reduction of OS may be one of the mechanisms of the protective effect of duloxetine against neuropsychiatric symptoms caused by the CIS (15). Another study with rats showed that duloxetine treatment may have a protective effect against OS by reversing the increase in the level of MDA, which is a marker of methamphetamine-induced increased LP in animal brains, and the decrease in GSH-Px, SOD, and Glutathione reductase enzyme activities, which are the enzymes of the antioxidant defense system in hippocampal tissues (16). In a study analyzing the possible protective role of single-dose duloxetine against pentylenetetrazol (PTZ)-induced convulsive seizures in mice, brain OS parameters were also evaluated. There was a statistically significant decrease in both SOD and CAT activities and a statistically significant increase in LP in the cerebral cortex of PTZ-administered mice. However, in the group receiving a single dose of 20 mg/kg duloxetine, this effect of PTZ was not observed and SOD and CAT activities were preserved, and it was suggested that this modulation of SOD and CAT enzymes may have a role in antioxidant protection (31). We also found results that support these data in the literature. In our study, we found a significant increase in the brain's total SOD activity, an antioxidant enzyme. Although the significant decrease in CAT activity suggests that duloxetine may have a negative effect on the antioxidant system, an increase in the level of GSH-Px, another antioxidant enzyme that removes H2O2 in the environment like CAT, although not at a significant level, and not an increase but conversely a decrease in the level of MDA, a LP product, which is one of the important markers of OS in tissues, although not at a significant level, suggest that duloxetine may have positive effects on the antioxidant system.

XO forms reactive oxygen products in living organisms. It converts hypoxanthine into xanthine and xanthine into uric acid. In these reactions, molecular oxygen is converted to superoxide. In the brain tissue, which is rich in oxygen and requires a lot of energy, this enzyme is activated for the destruction of the ATP used and produces free radicals that damage the tissue as a result of their reactions (32). ADA is also an aminohydrolase in purine metabolism (33). In a study conducted in patients with major depression, ADA and XO levels were found to be high before treatment. Significant decrease in XO levels and increase in ADA levels were observed after eight weeks of antidepressant treatment (34). In another study conducted by the same researchers in panic patients, ADA and XO levels were

found to be significantly higher in patients, and after eight weeks of antidepressant treatment, ADA activity increased and XO activity significantly decreased (35). As a result, it has been stated that increased purine metabolism in depression and panic patients can be controlled with antidepressant treatment. Also in our study, the significant decreases in the XO and ADA activities of the duloxetine group support that purine catabolism is decreased in the organism, thereby reducing radical formation. These results can be considered as a supportive parameter that duloxetine may help increase the resistance of the brain against oxidative damage.

NO is an inorganic free radical that the form of a colorless gas and has an odd number of electrons. In vivo studies have shown that NO regulates the levels of serotonin, dopamine, GABA and glutamate in the CNS. However, excessive NO synthesis has been found to damage neurons (36). Studies evaluating the effect of duloxetine on NO are available in the literature (37). For example, in a study using the comet assay on mouse liver and brain cells, duloxetine caused significant DNA damage and increased DNA, lipid, protein and NO oxidation in both organs mainly after 9 hours. Even at a dose of 2 mg/kg, duloxetine has been reported to have the capacity to damage DNA, and it has been suggested that this effect may be due to its oxidative potential (37). In our study, we found a non-significant decrease in rat brain NO levels after duloxetine treatment for 14 days. This non-significant decrease caused by duloxetine on the NO levels suggested that it would not have a negative effect on the oxidant-antioxidant system at least through NO, which is an inorganic free radical. Perhaps duloxetine may have an oxidative potential by increasing NO oxidation in the acute period in short-term applications (37), however, in long-term treatments such as the one in our study, this oxidative effect it has through NO may be eliminated.

The most important limitation of our study is that the results are preliminary for clinical use because it was performed on rats. The superiority of our study over similar studies in the literature is that it is the only study that evaluated the direct effect of duloxetine on the oxidant-antioxidant system in such a wide range. The studies on this subject are predominantly laboratory studies similar to ours, thus there is a need for further clinical studies.

CONCLUSION

The importance of the antioxidant system is indisputable, especially in the brain tissue, which is weak against oxidant radicals formed due to high oxygen utilization. Increase in SOD activity by duloxetine, the antidepressant drug we use, may strengthen the protective system thereby rendering the brain more resistant and stronger against stress. Decreases in XO and ADA activity, which are involved in purine catabolism, are also supportive parameters related to the increase of this resistance. In addition, a decrease in the level of MDA, a LP product, and NO, an inorganic free radical, although not at a significant level, suggests that duloxetine may have a protective effect on the brain against OS.

Conflict of Interest: No conflict of interest was declared by the authors.

Ethics: The study was approved by the Local Ethics Committee for Animal Experiments of Süleyman Demirel University Faculty of Medicine (Decision no: 04 Date: 27.05.2010)

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ORIGINAL ARTICLE

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Relationship Between Pulmonary Thromboembolism and Neutrophil Albumin Ratio

Pulmoner Tromboemboli ve Nötrofil Albümin Oranı Arasındaki İlişki

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ABSTRACT

Objective: *Pulmonary Thromboembolism (PTE) is a serious clinical condition and is common all over the world, requiring early diagnosis and treatment. Laboratory data and radiological imaging provide support in its diagnosis. The purpose of the present study is to investigate the clinical significance of neutrophil to albumin ratio (NAR) in patients who are diagnosed with PTE.*

Material and Method: *A total of 150 cases who of 100 patients with a diagnosis of PTE and 50 healthy volunteers without a history of smoking or any comorbidities were included in the study. Complete Blood Count (CBC) and biochemical data were evaluated retrospectively. NAR was calculated as the ratio of neutrophil to albumin.*

Results: *A total of 150 cases (84 male (56%) and 66 (44%) female) were included in the study. Neutrophil Albumin Ratio (NAR), were found to be statistically and significantly elevated in the patient group (p<0.001).The Serum NAR value showed a positive correlation with the Serum d-dimer value (r:0.488, p<0.001).*

Conclusion: *It is possible to argue that NAR, which is a novel, cheap, and easily calculable biomarker, can be a useful parameter for the diagnosis of patients with PTE.*

ÖZET

Amaç: *Pulmoner tromboemboli (PTE), tüm dünyada sık görülen ve erken tanı ve tedavi gerektiren ciddi bir klinik tablodur. Tanı koymada, hem laboratuvar verileri hem de radyolojik görüntüleme destek sağlamaktadır. Çalışmanın amacı, PTE tanılı hastalarda nötrofil albumin oranının (NAR) klinik önemini araştırmaktır.*

Gereç ve yöntem: *Çalışmaya, PTE tanılı 100 hasta ve sigara içme öyküsü ve komorbidite öyküsü olmayan 50 sağlıklı gönüllü olmak üzere toplam 150 olgu dahil edildi. Tam kan sayımı ve biyokimyasal veriler retrospektif olarak elde edildi. NAR, nötrofilin albumine oranı olarak hesaplandı.*

Bulgular: *Çalışmaya 84'ü erkek (%56) ve 66'sı (%44) kadın olmak üzere toplam 150 vaka dahil edildi. NAR, hasta grubunda istatistiksel olarak anlamlı düzeyde yüksek bulundu (p<0,001). Serum NAR değeri, serum d-dimer değeri ile pozitif korelasyon gösterdi (r:0,488, p<0,001).*

Sonuç: *Yeni, ucuz ve kolay hesaplanabilen bir biyobelirteç olan NAR, PTE hastalarının tanısında yararlı bir parametre olabilir.*

Keywords:
Pulmonary embolism
Inflammation
Albumin
Neutrophil

Anahtar Kelimeler:
Pulmoner emboli
İnflamasyon
Albumin
Nötrofil

INTRODUCTION

Pulmonary Thromboembolism (PTE) is a serious clinical condition and is common all over the world, requiring early diagnosis and treatment. PTE is characterized by pulmonary circulatory disorder that results from partial or complete occlusion of the pulmonary artery by thrombi that originate from the deep veins of the lower extremities (1). Laboratory data and radiological imaging provide support in its diagnosis (2,3).

Intense inflammation develops in PTE after the rapid release of inflammatory cells in the pulmonary artery wall and peaks in two days (4). It was reported in previous studies that some novel biomarkers in this inflammatory response play important roles in the diagnosis of PTE and predicting its prognosis (5-7). Neutrophils initiate the early inflammatory response after the acute infection. In this respect, an elevated neutrophil count is an important marker for systemic infection (8). Albumin is a negative acute phase reactant, decreases in acute infection, and is an important marker of mortality (9). Many studies are emphasizing the importance of neutrophils and albumin in the pathogenesis of PTE (10,11).

Neutrophil Albumin Ratio (NAR) is a novel inflammatory biomarker and was reported in many previous studies to be an important prognostic biomarker (12-14). When the literature was reviewed, no study was detected examining the relationship between PTE and NAR. The purpose of the present study is to investigate the NAR value in patients who are diagnosed with PTE and to pioneer future studies to be conducted on this subject.

MATERIAL AND METHOD

A total of 120 patients who were followed up with a diagnosis of PTE in our hospital between June 2019 and May 2023 were reviewed in this retrospective study. The

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study was approved by the Institutional Ethics Committee of Harran University, Faculty of Medicine (Approval No. HRU/23.12.19, Date: 10.07.2023). The patients who were under 18 years of age, those with acute or chronic inflammatory diseases, active cancer, a history of acute attacks of Chronic Obstructive Pulmonary Disease (COPD), and patients with a history of nephrotic and/ or hepatic disease that would cause hypoalbuminemia were excluded from the study. A total of 20 patients were excluded from the study according to these criteria. As a result, a total of 100 patients and 50 healthy volunteers without a history of smoking or any comorbidities were included. The demographic data and laboratory parameters of the patients were taken from the digital archive and recorded. The diagnosis of PTE was made with pulmonary angiographic computed tomography.

Blood samples were taken from the patients on the first day of their hospitalization. Complete Blood Count (CBC) and biochemical data were evaluated retrospectively. NLR was calculated as the ratio between the number of neutrophils and lymphocytes, LMR as the ratio between the number of lymphocytes and monocytes, CAR as the ratio of CRP to albumin, and NAR as the ratio of neutrophils to albumin.

Statistical Analysis

The SPSS for Windows version 22.0 (SPSS Inc., IL, USA) was used for statistical analyses. The Kolmogorov-Smirnov Test was used to evaluate whether the continuous data were distributed normally. The continuous data were expressed as Mean ± SD or Median (25-75 IQR) and were compared by using the Student's t or Mann-Whitney U Tests depending on their distributions. Receiver Operating Characteristics (ROC) Curve Analysis was used to determine the optimal cutoff value of NAR to predict PTE. The correlation between NAR and d-dimer parameters was determined by using the Spearman Test and a p-value of <0.05 was considered statistically significant.

RESULTS

A total of 150 cases (84 male (56%) and 66 (44%) female) were included in the study. The demographic and laboratory data of the patients are given in Table 1. When compared to the Control Group, C-Reactive Protein (CRP), White Blood Cell (WBC), leukocyte and d-dimer values were significantly higher in the patient group, and albumin and lymphocyte values were statistically lower. Among the patients who were diagnosed with PTE, 28% had Congestive Heart Failure (CHF), 18% had Hypertension (HT), 18% had Chronic Obstructive Pulmonary Disease (COPD), and 15% had Diabetes Mellitus (DM).

As novel biomarkers, Neutrophil Lymphocyte Ratio (NLR), Lymphocyte Monocyte Ratio (LMR), C-RP Albumin Ratio (CAR), and Neutrophil Albumin Ratio (NAR), were found to be statistically and significantly elevated in the patient group ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, respectively) (Table 1).

The correlation between the variables was given by using the Spearman Test. The Serum NAR value showed a positive correlation with the Serum d-dimer value (r:0.488, $p<0.001$) (Table 2).

The ROC Curve Analysis was performed to determine the cut-off value of NAR in predicting PTE, which was found to be ≥ 1.09 with 81% sensitivity and 72% specificity (AUC: 0.859, $P < 0.001$) (Figure 1).

Table 1: Comparison of demographic and laboratory data between groups.

	PATIENT GROUP (n=100)	CONTROL GROUP (n=50)	p
Age, year	63.0 (47.2-73.7)	56.0 (47.7-59.0)	0.16
Gender, (m/f)	43/57	41/9	<0.001
Urea, mg/dL	39.0 (29.0-54.5)	26.5 (23.0-34.0)	<0.001
Creatinine, mg/dL	0.8 (0.6-1.0)	0.8 (0.7-1.0)	0.82
Albumin, g/ dL	3.4 (3.1-3.8)	4.4 (4.1-4.7)	<0.001
CRP, mg/dL	19.3 (10.5-47.2)	0.3 (0.1-0.4)	<0.001
WBC, x103/mL	10.7 (7.6-14.0)	6.6 (6.0-7.6)	<0.001
Neutrophil, x103/mL	8.0 (5.0-11.3)	3.7 (3.1-4.9)	<0.001
Lymphocyte, x103/mL	1.7 (1.0-2.4)	2.2 (1.8-2.5)	0.001
Monocyte, x103/mL	0.7 (0.5-1.0)	0.4 (0.3-0.5)	<0.001
Platelet, x103/mL	249.5 (190.2-316.5)	253.8 (221.5-331.0)	0.350
MCV, fL	85.9 ± 9.4	87.3 ± 5.4	0.236
RDW, %	14.4 ± 3.5	12.6 ± 1.5	0.001
d-dimer	3.6 (1.4-6.6)	0.2 (0.1-0.3)	<0.001
CAR	5.8 (3.6-13.2)	0.06 (0.02-0.93)	<0.001
NLR	4.6 (2.2-8.4)	1.7 (1.3-2.2)	<0.001
LMR	2.9 (1.6-4.3)	5.2 (3.9-8.0)	<0.001
NAR	2.2 (1.6-3.5)	0.9 (0.7-1.1)	<0.001

CRP, C-reactive protein; WBC, white blood cell; MCV, mean corpuscular volume; RDW, red cell distribution width; CAR, C reactive protein to albumin ratio; NLR: Neutrophil to lymphocyte ratio; LMR: Lymphocyte to monocyte ratio; NAR: Neutrophil to albumin ratio.

Table 2: Spearsman correlation of variables.

		NAR
D-dimer	Correlation Coefficient	.488*
	p	<0.001
	n	150

** p<0,001.*

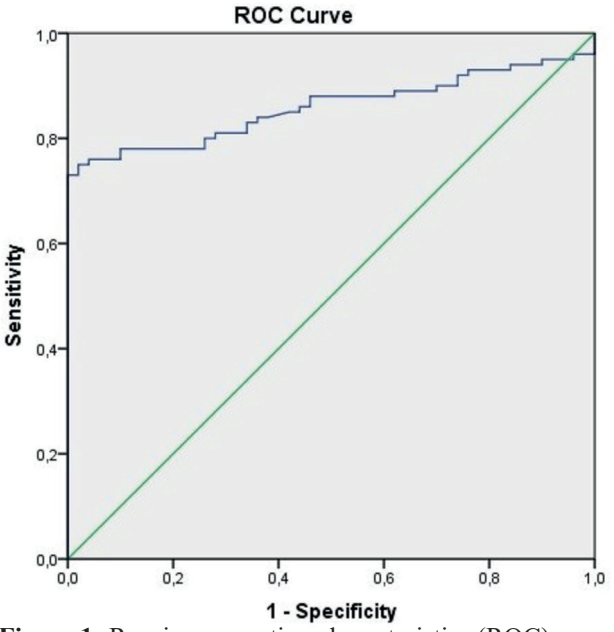

Figure 1: Receiver operating characteristics (ROC) curve of NAR for predicting the PTE.

DISCUSSION

In the present study, the clinical importance of NAR, which is a novel inflammatory marker, was investigated in patients with PTE. The important results of the study were that NAR had high sensitivity in the diagnosis of PTE and showed a positive correlation with d-dimer. Also, this study is the first to investigate the relationship between NAR and PTE.

The role of systemic inflammation in PTE was investigated in many previous studies. It was reported that an inflammatory reaction characterized by increased cytokines and inflammatory cell influx occurs in the pulmonary artery wall because of endothelial damage caused by thrombus (15). Therefore, it is possible to argue that biochemical and hematological parameters can play important roles in the diagnosis, follow-up, and prognosis evaluation of PTE. In their study, Gülen et al. compared the NLR, MPV, and WBC values in patients with PTE at the time of diagnosis and after treatment and found that these values decreased significantly at the end of treatment, and they argued that NLR, MPV, and WBC could be important biomarkers that can be used in the diagnosis and follow-up of PTE (16). Çaltekin et al. argued that LMR can be used as a prognostic biomarker in stroke cases (17). Several studies examining the relationship between CAR and patients with PTE emphasized that CAR may be both a risk-increasing factor for the disease and an independent predictive factor of mortality (7,18,19). In the present study, as a result of the examination of the blood samples taken at the time of diagnosis of patients with PTE, the NLR, LMR, and CAR values were found to be significantly higher than in the Control Group. Novel inflammatory markers can be used in the diagnosis, follow-up, and prognosis evaluation of PTE because this result supports previous studies.

NAR is a novel inflammatory biomarker and was considered an important prognostic factor in different pathologies such as cancer, sepsis, and diabetic retinopathy (20-22). It is an inflammatory cell initiating the stimulus for the development of neutrophils and thrombus and is necessary for their spread (23,24). In addition to the anti-inflammatory characteristics of albumin, which is a negative acute phase reactant, it also plays important roles in antioxidant, anticoagulant, and anti-platelet aggregation (25,26). Many studies emphasized that elevated serum neutrophil and low serum albumin values are strong and independent markers for mortality in patients who are diagnosed with PTE (27-29). However, no study has been conducted yet on NAR in patients diagnosed with PTE. In the present study, the relationship between PTE and NAR was investigated for the first time, and statistically significant high NAR values were detected in PTE patients. According to the results of the present study, we think that NAR may be an important marker in the diagnosis of PTE. Early diagnosis and treatment are vital in PTE because it is associated with high mortality. The d-dimer, a fibrin degradation product formed by the endogenous fibrinolytic system, is among the most frequently used laboratory parameters in its diagnosis and follow-up (30). The relationship between PTE and d-dimer was reported in many previous studies (31,32). In the present study, d-dimer levels were found to be significantly elevated in patients with PTE. For the first time, the present study showed a positive correlation between NAR as a novel inflammatory parameter and d-dimer levels. Based on this result, evaluating both parameters together may be more effective and helpful in the diagnosis of PTE.

The present study had some limitations, which include the fact that the study had a single-centered design, the lack of long-term follow-up data of the patients, and the inability to make clinical classifications because of insufficient data.

In conclusion, it is possible to argue that NAR, which is a novel, cheap, and easily calculable biomarker, can be a useful parameter for the diagnosis of patients with PTE. We also think that the present study, which investigated the relationship between PTE and NAR for the first time, will guide future studies to be conducted with larger populations.

Conflict of Interest: No conflict of interest was declared by the authors.

Ethics: This study was conducted with the approval of the Harran University Faculty of Medicine Clinical Research Ethics Committee (HRU/23.12.19, date: 22.07.2023).

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ORIGINAL ARTICLE

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T2*-ADC Comparison in Liver Iron Quantification in Thalassemia Patients

Talasemi Hastalarında Karaciğer Demir Ölçümünde T2*-ADC Karşılaştırması

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ABSTRACT

Objective: *Repetitive transfusion is a treatment option in patients with thalassemia, but this causes iron accumulation in various organs, especially the liver. In this study, it is aimed to present our own experience in the correlation of serum ferritin concentrations with liver T2* MR measurements and ADC values in thalassemia patients.*

Materials and methods: *Seventy-four consecutive patients who underwent T2* MR examination of the liver due to beta thalassemia were included in the study. Liver T2* and ADC measurements and ferritin measurements of the patients included in the study were compared. MRI examination was performed with 3T scanner. Multiecho gradient echo was used for T2* MRI examinations. T2* and ADC measurements were made from 4 different regions of the liver, one each from the medial and lateral segments of the left lobe and one each from the anterior and posterior segments of the right lobe. Spaerman correlation analysis was performed to evaluate the correlation between serum ferritin levels and R2* and ADC measurements.*

Results: *Thirty-two patients (43.24%) were female and and forty-two (56.76%) were male. There was moderate correlation between serum ferritin and liver T2* measurements (r= -0.52 p<0.01). The highest T2 value was measured in the left lobe medial segment of the liver as 7.85 ms and the lowest was measured in the right lobe posterior segment of the liver as 6.5 ms. There was weak correlation between serum ferritin and ADC (r= -0.41, p<0.01). The highest ADC value was measured in the left lobe medial segment of the liver as 908.90 mm2/s and the lowest was measured in the right lobe anterior segment of the liver as 766.78. There was a moderate-high correlation between liver T2* measurements and ADC measurements. This correlation was higher than the correlation between serum ferritin and liver T2* measurements.*

Conclusion: *Correlations between the serum ferritin measurements and both ADC and T2* measurements are lower than those found with 1.5T in the literature. The correlation of ADC with serum ferritin is lower than the correlation between serum ferritin and T2*MR, so we do not think ADC is as useful as T2* measurements in assessing liver iron accumulationin in thalassemia patients.*

ÖZET

Amaç: *Talasemi, hastalarında tekrarlayıcı transfüzyon bir tedavi opsiyonudur, fakat bu durum başta karaciğer olmak üzere değişik organlarda demir birikimine neden olmaktadır. Bu çalışmada talasemi hastalarında, serum ferritin konsantrasyonları ile karaciğer T2* MR ölçümleri ve ADC değerlerinin korelasyonu konusundaki kendi deneyimimizin sunulması amaçlanmıştır.*

Gereç ve yöntem: *Beta talasemi nedeni ile karaciğere yönelik T2* MR tetkiki yapılan ardışık 74 olgu çalışmaya dahil edilmiştir. Olguların karaciğer T2* ve ADC ölçümleri ile ferritin ölçümleri karşılaştırıldı. MR incelemesi 3T tarayıcı ile yapıldı. T2* MR incelemelerinde multieko gradyan eko kullanıldı. Karaciğer sol lob medial ve lateral segmentten birer ve sağ lob anterior ve posterior segmentlerden birer adet olmak üzere toplam 4 farklı bölgeden T2* ve ADC ölçümleri yapıldı. Serum ferritin düzeyleri ile R2* ve ADC ölçümleri arasındaki korelasyonu değerlendirmek için Spearman korelasyon analizi yapıldı.*

Bulgular: *Hastaların 32'si (%43,24) kadın, 42'si (%56,76) erkekti. Serum ferritin ile karaciğer T2* ölçümleri arasında orta düzeyde negatif bir korelasyon vardı (r= -0,52 p<0,01). En yüksek T2* değeri 7,85 ms ile karaciğerin sol lob medial segmentinde, en düşük T2* değeri ise 6,5 ms ile karaciğerin sağ lob arka segmentinde ölçüldü. Serum ferritini ile ADC arasında negatif zayıf bir korelasyon vardı (r= -0.41, p<0.01). En yüksek ADC değeri 908,90 mm2/s ile karaciğerin sol lob medial segmentinde, en düşük ADC değeri ise 766,78 ile karaciğerin sağ lob ön segmentinde ölçüldü. Karaciğer T2* ölçümleri ile ADC ölçümleri arasında orta-yüksek korelasyon mevcuttu. Bu korelasyon serum ferritin ile karaciğer T2* ölçümleri arasındaki korelasyondan daha yüksekti.*

Sonuç: *Serum ferritin ölçümleri ile hem ADC hem de T2* ölçümleri arasındaki korelasyonlar literatürde 1,5T ile bulunanlardan daha düşüktür. ADC'nin serum ferritin ile korelasyonu, serum ferritin ve T2*MR arasındaki korelasyondan daha düşük olduğundan, talasemi hastalarında karaciğer demir birikiminin değerlendirilmesinde ADC'nin T2* ölçümleri kadar yararlı olduğunu düşünmüyoruz.*

Keywords:
T2 MRI*
ADC
Iron-loaded liver

Anahtar Kelimeler:
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ADC
Demir yüklü karaciğer

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INTRODUCTION

Thalassemia is a heterogeneous group of diseases characterized by anemia caused by damaged synthesis of one or more of the hemoglobin chains (1). According to the severity of anemia, recurrent transfusion is a treatment option in these patients (2). Repetitive transfusions show iron accumulation in several organs in the body (3). Accumulating iron damages tissues and organs (4). Liver are one of the target organs for iron accumulation (5,6). Chelator drugs are used in the treatment of iron accumulation (7). This treatment significantly improved survival in patients with thalassemia (8).

The most reliable method to calculate body iron deposition is histochemical or biochemical assessment of iron in a liver biopsy specimen but that is an invasive procedure (2). Serum ferritin levels are widely used to monitor chelation therapy and to assess iron accumulation (9). However, it should be noted that serum ferritin level may be affected by many inflammatory conditions (10).

In early 1990s it has been reported a very close correlation between T2 relaxation rate and liver iron accumulation on magnetic resonance imaging (MRI) (11). In the early 2000s, T2* MRI techniques were used to evaluate liver iron accumulation (12). Nowadays, the amount of liver iron accumulation can be calculated by measuring liver T2* signals using multiecho gradient echo sequences with MRI. In recent years there have been reports that diffusion-weighted imaging (DWI) is a useful method for evaluating liver changes and iron accumulation (13).

Standards have been established in 1.5T MR scanners used in liver T2* measurements for many years. In 3T scanners, the situation is not as clear as in 1.5T scanners. Due to the increased magnetic field strength, the susceptibility effect of iron has increased significantly. In this case, it makes it difficult to measure T2* reliably in patients with iron accumulation in the liver (14). The aim of this study was to evaluate the correlation between serum ferritin concentrations and liver T2* measurements and apparent diffusion coefficient (ADC) values in thalassemia patients in 3T MR scanners.

MATERIALS AND METHODS

Study Population and characteristics

This retrospective study was approved by Muğla Sıtkı Koçman University Human Research Ethic Committee (Number 85/2019). The study was conducted from January 2018 to November 2018. Seventy-four patients who examined T2* MRI due to beta-thalassemia were included in the study. Beta-thalassemia was diagnosed by complete blood count, hemoglobin electrophoresis test, and clinical evaluation by an experienced hematologist. Only patients with complete MRI examination (including T2* MRI and DWI sequences) were included. All patients were follow-up cases of our center. Four patients with prominent motion artifacts and 2 patients with inconclusive liver T2* (the serum ferritin levels were significantly elevated) were excluded from the study. There were 74 patients in the study population. A total of 38 patients had splenectomy. A total of 67 patients, 27 women and 40 men, were receiving chelation therapy. Patients characteristics are summarized (Table 1).

Liver T2* and ADC measurements and ferritin measurements of the patients included in the study were compared. Serum ferritin levels were measured on the same day with MRI examination. Serum ferritin level was measured by electrochemiluminescence method (Roche Diagnostics). Since ferritin is an acute phase reactant, detailed clinical examination and c-reactive protein (CRP) measurements were performed to rule out possible infectious/ inflammatory processes. Additionally, transaminase results such as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were reviewed to evaluate processes that could affect liver measurements such as hepatitis. There was no infectious/inflammatory process in the study population.

MRI acquisition and measurements

MRI examination was performed with 3T scanner (Siemens Skyra, Erlangen, Germany). Multiecho gradient echo was used for T2* MRI examinations. A breath-hold sequence was used to reduce respiratory artifacts. Liver

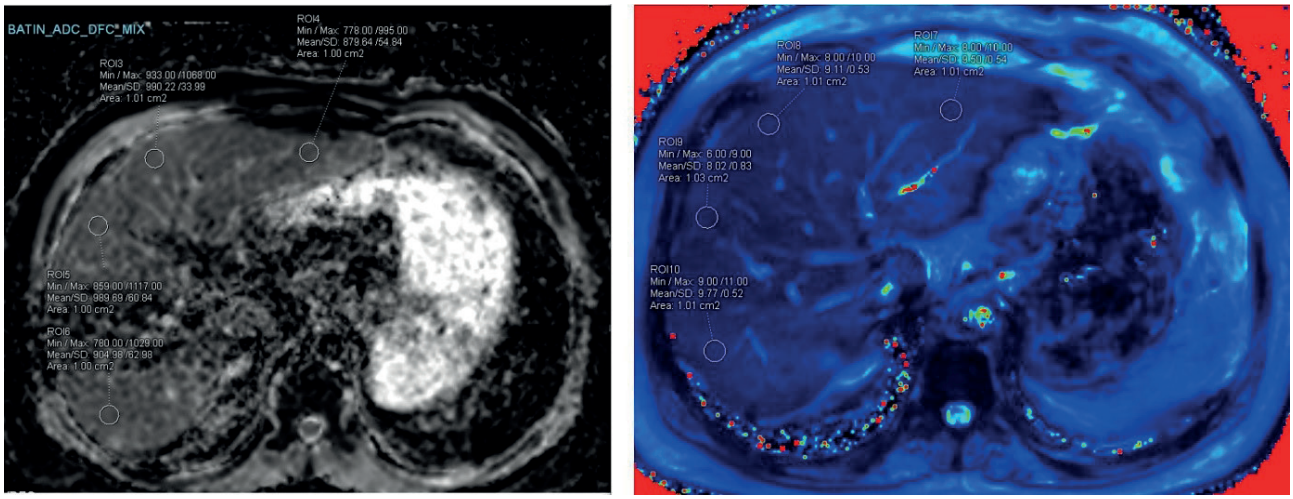

Figure 1: Both ADC and T2 measurements were made from four different regions of the liver. The illustration shows the areas where the measurements are made.

Table 1: Patients characteristics.

Characteristic		
Age (year) median (min-max)		25.30 (9-53)
Gender n (%)	Female	32 (43.24)
	Male	42 (56.76)
Splenic size (mm) median (min-max)		144.75 (101-200)
Ferritin level (ng/ml) median (min-max)		753.45 (127-2540)

examination parameters: Field of view 25x18 cm, TR 177 ms, slice thickness 5 mm, spacing between slices 5.5 mm, matrix 256x192, number of excitation 1, flip angle 60˚. There were 7 different echo times in protocol (TE 2.54-17.22 ms, increasing by increments of 2.45 ms). T2* and ADC measurements were made from 4 different regions of liver left lobe medial and lateral segment and right lobe anterior and posterior segments (figure 1). Each measurement was made using approximately 1 cm2 ROI. All four T2* and ADC measurements were averaged. Data analysis was performed using Syngovia workstation (Siemens, Germany). R2* calculated from measured T2* values (R2*=1/T2*). The equation ((R2* × 0.0254) +0.202) was used in the LIC calculation from R2* (15)

Statistical analysis

All statistical analysis was performed by SPSS version 22. Descriptive analysis of quantitative variables was performed. Spaerman correlation analysis was performed to evaluate the correlation between serum ferritin levels and R2* and ADC measurements. Correlation coefficients were accepted as negligible correlation between 0.00-0.30, weak correlation between 0.30-0.50, moderate correlation between 0.50-0.70, high correlation between 0.70-0.90 and very high correlation above 0.90 (16). Student's t-test was used to compare the mean of the groups. Results were assessed within 95% confidence intervals and $p < 0.05$ was considered as significant.

Table 2: Comparison of measurements by gender.

	Female n=32	Male n=42	p
Ferritin level (ng/ml)	879.41	660.35	0.57
Liver T2* (ms)	7.50	6.75	1.00
Liver iron concentration	6.20	5.76	1.00
Liver ADC (mm2 /s)	784.13	845.1	0.87

RESULTS

Thirty-two patients (43.24%) were female and mean age was 27.18 years old, and forty-two (56.76%) were male and mean age was 23.48 years old. The male to female ratio was 1.3/1. There was no statistically significant difference in serum ferritin, liver T2*, LIC concentration, liver ADC values between women and men (Table 2).

There was moderate correlation between serum ferritin and liver T2* measurements ($r= -0.52$ $p<0.01$). The highest T2 value was measured in the left lobe medial segment of the liver as 7.85 ms and the lowest was measured in the right lobe posterior segment of the liver as 6.5 ms. There was weak correlation between serum ferritin and ADC ($r= -0.41$, $p<0.01$). The highest ADC value was measured in the left lobe medial segment of the liver as 908.90 mm2/s and the lowest was measured in the right lobe anterior segment of the liver as 766.78. There was a moderate-high correlation between liver T2* measurements and ADC measurements. This correlation was higher than the correlation between serum ferritin and liver T2* measurements. All correlations is given in table 3.

DISCUSSION

The correct measurement of liver iron accumulation is important in the treatment of diseases that cause iron accumulation in the body, such as thalassemia. Different methods have strengths and weaknesses in monitoring iron accumulation in the body. Serum ferritin measurements are low-cost and easily accessible, but their accuracy in short-term follow-up is low. MRI is an expensive method and not easily accessible, however, its accuracy is high in short-

Table 3: Relationship between serum ferritin, liver T2 * and liver ADC values. The correlation of each variable with the other can be cross-compared.

		Serum ferritin	Median ADC	Liver T2*	LIC
Serum ferritin	CC	1.000	-0.410	-0.520	0.517
	Sig.		-0.009	0.001	0.001
	N	80	80	80	80
Median ADC	CC	-0.410	1.000	0.598	-0.599
	Sig.	0.009		0.000	0.000
	N	80	80	80	80
Liver T2*	CC	-0.520	0.598	1.000	-1.000
	Sig.	0.001	0.000		0.000
	N	80	80	80	80
LIC	CC	0.517	-0.599	-1.000	1.000
	Sig.	0.001	0.000	0.000	
	N	80	80	80	80

CC: Correlation coefficient, LIC:Liver iron concentration, ADC: Apperent diffusion coefficient

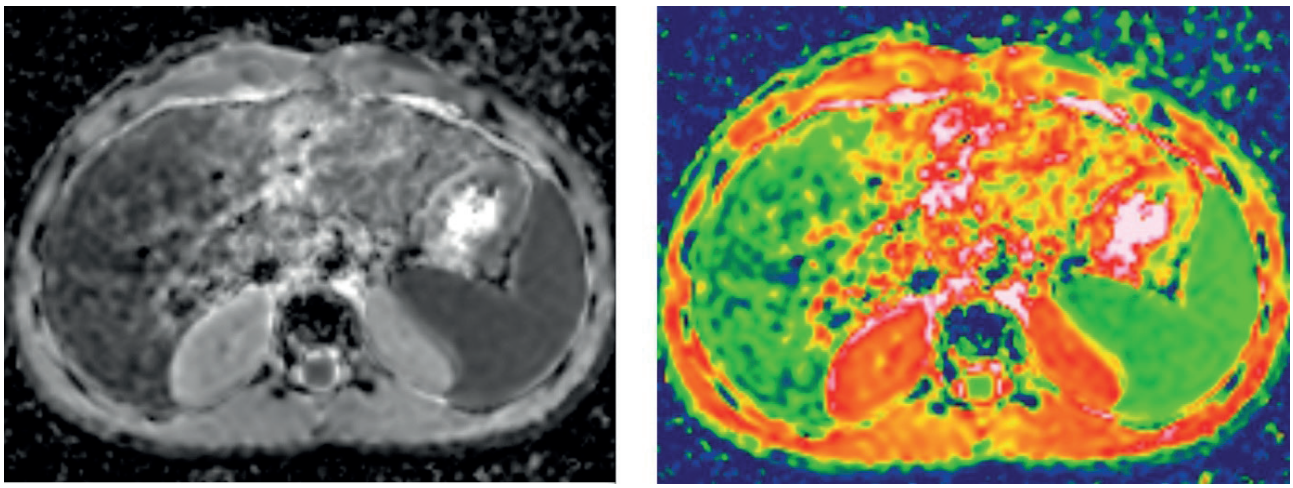

Figure 2: The picture shows heterogeneity in the left lobe in liver ADC measurements. This is evident in the color maps on the left.

term follow-ups and it can measure iron accumulation in each organ individually (17). Therefore, MRI has become the standard imaging method for evaluating iron deposits in the liver and heart of thalassemia patients. A good correlation between serum ferritin levels and liver T2* measurements has been reported in the literature (18). In this study, a moderate correlation was found between serum ferritin level and liver T2* level. In this study, serum ferritin levels were found as 956 ng/mL and 2540 ng/mL in two patients who had a very similar T2* values as 2.07 and 2.16 respectively. T2* values were close to each other in these two cases ferritin values were very different from each other.

The correlation between serum ferritin and liver T2* measurements were -0.58 ($p < 0.001$ in Karakaş et al.' study. Majd et al. found a good correlation between ferritin levels and liver T2* levels ($r = 0.698$, $p < 0.001$) (19). In our study, these rates were found as -0.52 ($p < 0.05$). Although the value we found for the liver is slightly lower, it is just about similar to that of the studies of the Karakaş et al. and lower than found Majd et al. 1.5T scanners were used in the studies of both Karakaş and Majd. In our study, 3T scanner was used. The difference may be related to the magnet power. In their study to compare 1.5T and 3T MRI scanner, Storey et al. suggested that liver T2* values in patients with iron overload could be significantly shortened and this would make precise measurement difficult (20). They suggested that 1.5T imaging should be preferred to 3T because of the increase in magnetic field strength. In order to prevent this, they reported that if there is more tissue iron concentration than 37 mg Fe/g dry weight, sequences using shorter TE durations may be needed for precise measurement of T2* in strong magnetic fields. Patel et al. found the correlation between serum ferritin and liver T2* moderately significant ($r = 0.41$), which is lower than the rate in our study (21).

In this study, the correlation between ADC measurements and serum ferritin levels were found weak. The correlation of ADC with serum ferritin is significant lower than the correlation between serum ferritin and T2* MRI. Akpınar et al. suggested that DWI may be a sensitive method to assess the severity of liver iron accumulation. It may be thought that it could be due to the paramagnetic effect of iron rather than diffusion restriction. However, multiple measurements are required to perform T2* mapping of a tissue. In this way, the exponential decay curve of the tissue can be extracted with multiple samples. ADC measurements do not correlate as highly as T2* measurements (22). ADC measurements appear not to be useful for follow-up in cases of liver iron accumulation in 3T MR scanners.

There are some limitations in this study. Firstly it was retrospective and relatively small size cohort. T2* measurements were higher in the medial segment of the left lobe of the liver than in the other parts of the liver. We think that this is due to the heterogeneity of cardiac and aortic pulsations (motion artifacts) in this region rather than heterogeneous distribution of iron (figure 2).

CONCLUSION

In conclusion, correlations between the serum ferritin measurements and both ADC and T2* measurements are lower than those found with 1.5T in the literature. The correlation of ADC with serum ferritin is lower than the correlation between serum ferritin and T2*MR, so we do not think ADC is as useful as T2* measurements in assessing liver iron burden.

Conflict of Interest: No conflict of interest was declared by the authors.
Ethics: The study was approved by the Muğla Sıtkı Koçman University Ethics Committee (Decision no: 2019/95).
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ORIGINAL ARTICLE

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Diagnosis of Tuberculosis by Conventional and Molecular Methods in Our Laboratory: A 4-Year Assessment

Laboratuvarımızda Konvansiyonel ve Moleküler Yöntemlerle Tüberküloz Tanısı: 4 Yıllık Bir Değerlendirme

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ABSTRACT

Objective: *The aim of this study was to compare direct microscopy, culture and Polymerase Chain Reaction (PCR) methods and to present the antibiotic resistance profile of the last 4 years comparatively by conventional and molecular methods.*

Material and Method: *Bacterial culture, EZN and PCR methods were applied to all samples. Direct rapid resistance test was performed for EZN positive samples.*

Results: *968 patients were included in the study. Culture was positive in 81 (8%), PCR in 78 (8%) and EZN in 39 (46%) patients. PCR performed on the same day in both respiratory and other samples showed very good agreement with culture, while EZN staining showed moderate agreement. It was observed that the rapid resistance test detected rifampicin resistance which was not detected in culture, and in the case of INH, culture antibiogram and rapid resistance test were fully compatible. Application of the rapid resistance test to every patient with positive EZN staining resulted in very early detection of resistance.*

Conclusion: It was concluded that PCR tests are useful in the rapid diagnosis of tuberculosis and resistance in suspicious clinical samples.

ÖZET

Amaç: *Bu çalışmanın amacı, tüberküloz tanısında direkt mikroskopi, kültür ve Polimeraz Zincir Reaksiyonu (PCR) yöntemlerini karşılaştırmak ve son 4 yılın antibiyotik direnç profilini konvansiyonel ve moleküler yöntemlerle karşılaştırmalı olarak sunmaktır.*

Gereç ve Yöntemler: *Tüm örneklere Ehrlich-Ziehl-Neelsen (EZN) boyama, Mycobacterium kültürü ve PCR testleri yapıldı. EZN boyama ile aside dirençli basil (ARB) saptanan örneklere direkt hızlı direnç testi yapıldı.*

Bulgular: *Çalışmaya 968 örnek dahil edildi. Bunların 81'inde (%8) kültür, 78'inde (%8) PCR ve 39'unda (%46) EZN pozitif bulundu. Hem solunum hem de diğer örneklerde aynı gün yapılan PCR kültür ile çok iyi uyum gösterirken, EZN boyaması orta düzeyde uyum gösterdi. Hızlı direnç testinin kültürde saptanmayan rifampisin direncini saptadığı, INH durumunda ise kültür antibiyogramı ile hızlı direnç testinin tam uyumlu olduğu görülmüştür. Hızlı direnç testinin EZN boyaması pozitif olan her hastaya uygulanması, direncin çok erken tespit edilmesini sağlamıştır.*

Sonuç: *Şüpheli klinik örneklerde tüberküloz ve direncin hızlı tanısında PCR testlerinin yararlı olduğu sonucuna varılmıştır.*

Keywords:
Mycobacterium tuberculosis
Polymerase chain reaction
Rapid diagnosis
Rapid resistance test

Anahtar Kelimeler:
Mycobacterium tuberculosis
Polimeraz zincir reaksiyon
Hızlı teşhis
Hızlı direnç testi

INTRODUCTION

Tuberculosis has been an important health problem and cause of death for centuries. Its control is difficult since it is transmitted by droplet infection. In order to reduce transmission, patients should be identified as soon as possible (1,2). For definitive diagnosis of tuberculosis, Mycobacterium tuberculosis should be isolated from clinical specimens. The fastest and cheapest method for this is direct microscopic examination of clinical specimens stained with the EZN method. However, it has low sensitivity (35-80%) (3). When the number of mycobacteria in the clinical sample is less than 104 bacillus/ml, it cannot be detected, and also the differentiation between tuberculosis and non-tuberculous mycobacteria cannot be made.

The gold standard in diagnosis is culture. The detection limit of *M. tuberculosis* in cultures is 100 bacillus/ml. It is necessary to wait for a long period of 4-8 weeks for reproduction. Although this period is reduced to 10-12 days with automatic controlled liquid media, even this

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period can be long for many patients. For this reason, real-time Polymerase Chain Reaction (PCR) test that can be performed directly on the samples, which is a fast, specific and sensitive method have been developed for diagnosis of M.tubeculosis (4-7). PCR is a diagnostic method, where unlike culture method the number and viability of the microorganism in the sample to be examined is unimportant, and a small number of genetic materials can be reproduced (8-15). However, factors such as clinical sample type, method, contamination, evaluation as well as personal factors affect the performance of the tests.

Although we have a gold standard diagnostic method like the reason for investigating another method is the need for rapid diagnosis in clinical cases caused by this slow-growing bacterium. With the PCR method, diagnosis can be made with a sensitivity of 15-30 bacilli/ml, and antibiotic susceptibility tests can also be studied from the sample at the same time.

The aim of our study is to compare the direct microscopy, culture and PCR methods studied in the samples that were sent to our laboratory for the diagnosis of tuberculosis and to reveal the antibiotic resistance profile of the last 4 years. The study was approved by the Non-Interventional Health Research Ethics Committee of Düzce University Faculty of Medicine with the date 07.11.2023 and number E-050.99-360085.

MATERIALS AND METHODS

In our study, microscopic examination, culture and PCR test results of sputum, bronchoalveolar lavage (BAL), deep tracheal aspirate (DTA), biopsy, urine, sterile body fluid samples examined between January 2017 and December 2020 in the Tuberculosis Laboratory of Düzce University were examined. Only one of the multiple samples from the same patient, which was examined by all three methods, was included in the study. Patients were divided into two age groups as above and below 65 years of age. The samples were compared by dividing them into two groups as respiratory tract samples and other. EZN staining (Merck, Turkey), culture [Löwenstein-Jensen(LJ) (RTA Laboratories, Turkey) and BACTEC MGIT 960 (Becton, Dickinson and Company Sparks, USA)] and FluoroType® MTB (Hain Lifescience, Germany) methods were applied to the samples. Growth times in culture were recorded. The culture was accepted as the gold standard and compared with PCR, EZN and clinical findings. Steptomycin, INH, rifampicin and ethambutol susceptibilities were determined by the BACTEC MGIT 960 (Becton, Dickinson and Company Sparks, USA) method of those in whom *M. tuberculosis* growth was detected in the culture. In addition, rapid resistance test [GenoType® MTBDRplus (Hain Lifescience, Germany)] was performed directly from the samples that were positive only in the EZN dye. The presence of wild type probes and mutation probes in the katG and inhA gene regions for INH resistance and in the rpoB gene regions for rifampicin resistance were investigated by rapid resistance test.

Statistical analysis:

SPSS 17 (SPSS Inc, Chicago, IL, USA) was used for statical evaluation. Categorical data were summarized as frequency and percentage. The compatibility of the diagnostic methods used was determined by McNemar and Kappa methods. Pearson Chi-square and Fisher Exact tests were performed for the relationships between categorical variables. $p<0.05$ was considered statistically significant.

RESULTS

A total of 968 patients, 645 (67%) male and 323 (33%) female, were included in the study. The mean age of the patients was 55.5 ± 19.8 (min:2-max:95).

Of patients with growth in culture, which is accepted as the gold standard method, 56 (69%) were male and 25 (31%) were female, with a mean age of 55.6 ± 19.5 (min: 9-max: 94). Of the patients, 54 (67%) were under the age of 65 and 27 (33%) were over the age of 65. There was no difference between these two groups in terms of *M. tuberculosis* culture positivity (p=0.817).

When the samples that were sent to our laboratory was examined, the distribution was as follows: 18% (179) BAL, 70% (686) sputum, 4.9% (48) sterile body fluid, 1% (11) urine, 1% (18) other samples (gastric fasting fluid, wound, tissue). The evaluation of *M. tuberculosis* growth according to age groups, sample types and sex is shown in Table 1.

Bacterial culture, EZN and PCR methods were applied to

Table 1: Association of age group, gender and sample type with M. tuberculosis positivity

		Culture positive sample n(%)	Culture negative sample n(%)	p value
Age	>65 Old Year	27 (%8)	307(%92)	0.817
	<65 Old Year	54 (%9)	580(%91)	
Gender	Women	25(%8)	298(%92)	0.618
	Men	56(%9)	589(%91)	
Sample type	Sputum	42(%6)	644(%94)	0.000
	Bronchoalveolar lavage	34(%19)	145(%81)	
	Biopsy	4(%15)	22(%85)	
	Urine	0(%0)	11(%100)	
	Sterile Body Fluid	0(%0)	48(%100)	
	Other*	1(%6)	17(%94)	

**: Gastric lavage, wound*

Table 2: Diagnostic values of EZN and PCR results according to the culture results of the samples

	EZN	PZR
Respiratory system samples	Total: 865	
Sensitivity (%)	46	82
Specificity (%)	99	98
PPV*(%)	97	84
NPV(%)**	95	98
Harmony with culture	Middle	Too big
Non-respiratory system samples	Total: 103	
Sensitivity (%)	40	80
Specificity (%)	100	100
PPV*(%)	100	100
NPV(%)**	97	99
Harmony with culture	Middle	Very good

**PPV: positive predictive value ** NPV: negative predictive value*

Table 3: Antibiotic resistance status in M. tuberculosis specimens with growth

Antibiotic	Sensitivity n%	Resistant n %	p value
Streptomycin	68 (%84)	13(%16)	0.000
INH	70 (%86)	11(%14)	
Rifampin	81(%100)	0(%0)	
Ethambutol	72 (%89)	9(%11)	

Table 4: Evaluation of INH rapid susceptibility tests

		INH rapid susceptibilty test (GenoType® MTBDRplus)	
		Sensitive(n)	Resistant(n)
INH rapid susceptibilty test (Bactec 960 TB Culture)	Sensitive (n)	39	0
	Resistant (n)	0	8

Table 5: Evaluation of Rifampin rapid sensitivity tests

		Rifampin susceptibilty test (GenoType® MTBDRplus)	
		Sensitive(n)	Resistant(n)
Rifampin rapid susceptibilty test (Bactec 960 TB Culture)	Sensitive (n)	45	2
	Resistant (n)	-	-

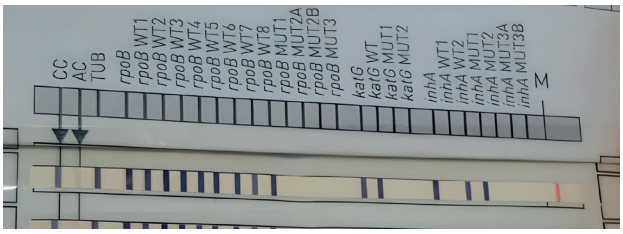

Figure 1: INH resistance detected in the inhA locus (loss of InhA wild type 1)

all samples. Culture was positive in 8% (81), PCR in 8% (78), EZN in 46% (39) of patients.

The mean growth period of *M. tuberculosis* in culture was 12.56±8.24 days (min:3-max:36). With the PCR method, the result was obtained on the day of sample arrived.

Out of 81 patient samples with *M. tuberculosis* growth 37 (46%) were detected by EZN staining and 66 (81%) by PCR method. Sensitivity, specificity, positive predictive value and negative predictive values of EZN staining were determined as 42%, 85%, 95% and 16%, respectively. For the PCR test, these values were determined as 80%, 98%, 84% and 98%, respectively. The gold standard culture method was found to be moderately compatible with EZN staining and very well compatible with PCR method. The diagnostic values of EZN and PCR results according to the culture results of respiratory and non-respiratory samples are shown in Table 2.

PCR method was positive in 12 patients (1%) while culture and EZN methods were negative. 5 of these 12 patients were clinically and radiologically compatible with tuberculosis and cured with treatment. Four of them had malignancy and died. The PCR positivity in the remaining 3 patients was not clinically compatible. EZN method was positive in one patient while culture and PCR tests were negative. This patient had no clinical and radiological findings and was considered as contamination.

Antibiogram of 81 specimens with *M. tuberculosis* growth showed that all specimens were susceptible to rifampicin, while 13 (17%) were resistant to streptomycin, 11 (14%) to isoniazid (INH), and 9 (11%) to ethambutol. Rifampin was found to be statistically more sensitive than other antibiotics (p=0.000). Antibiotic sensitivities are shown in Table 3.

In our study, rapid resistance test was performed on 47 (58%) of 81 samples with culture growth. While there was 100% correlation between rapid resistance test and antibiotic susceptibility tests for INH; Incompatibility was detected in two samples for rifampin (Tables 4 and 5).

In the INH resistance study with the rapid resistance test, it was observed that “low level INH resistance detected in the inhA locus” was also detected in the antibiotic susceptibility test (Figure 1)

While the mutations detected in the rpoB locus are detected both by rapid resistance test and antibiotic resistance tests; It was observed that wild type probe deletions could not be detected. This showed that rifampin antibiotic resistance could be detected earlier with rapid resistance tests (Figure 2,3).

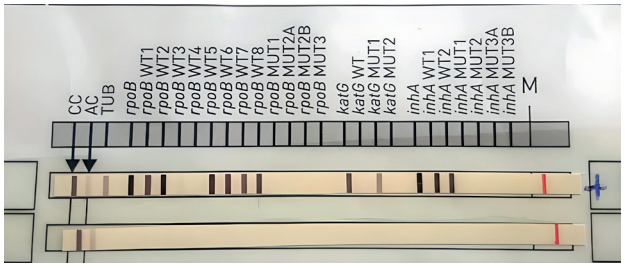

Figure 2: Rifampin resistance by mutations in the rpoB locus (loss of rpoB wild type2 and 3)

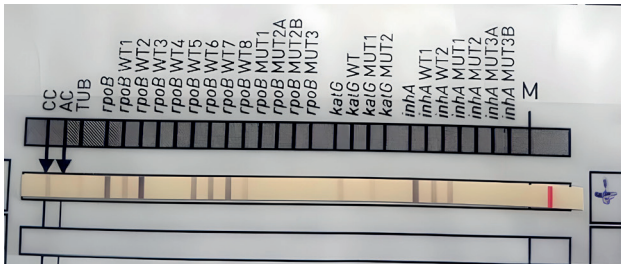

Figure 3: Rifampin resistance by mutations in the rpoB locus (loss of rpoB wild type2 and 3) and INH resistance detected in the katG locus (loss of katG wild type 1) and detection of kat G MUT 1)

DISCUSSION

Tuberculosis is a life-threatening disease for centuries. Diagnosis of this disease and determination of antibiotic susceptibility will provide treatment with the right drug regimens and reduce the infectiousness (16). There are many methods used for diagnosis. Although staining with EZN is the fastest method, its sensitivity is low (3). With this method, it is not possible to distinguish between species and live/dead bacteria. In order to detect positivity, it is necessary to have at least 103-104 bacteria / ml in the sample (16,17).

In a study by Abdulmajed et al(15), the sensitivity and specificity of the EZN staining method was 32% and 66% respectvely. And they found a low level of agreement between the culture and the EZN method. Sensitivity rates were similar to our study, but moderate agreement was found between culture and EZN in our study. Although by EZN staining method results can be obtained within twenty-four hours and it is a cheap test, it has low sensitivity rates that vary depending on the quality of the microscope used, the type of specimen, the thickness of the smear, the decolorization time during staining, the speed of the centrifugation process and the experience of the person evaluating the smear preparation, and the prevalence of tuberculosis in the population studied (16)

Tuberculosis culture method is the accepted gold standard method (18,19). For maximum efficiency, liquid and solid media should be used together. For culture growth an average of 2 weeks required (7-30 days) and the detection limit is approximately 100 bacteria/ml (16). Detection and the planning the treatment strategy for tuberculosis patients as soon as possible is of great importance (16). For this reason, nucleic acid methods have been developed in recent years (20-25). With these methods, the presence of *M. tuberculosis* and antibiotic susceptibility can be studied directly from the patient sample by PCR method (26-30). With the PCR method, detection can be made with a sensitivity of 15-30 bacillus/ml. In our study, 5 of 12 patients (41%) with culture negative and PCR positive were clinically and radiologically compatible with tuberculosis and healed with cure. Detection of positivity by PCR in all of these patients is valuable in terms of not to miss the patients who expelled a small number of bacilli or could not be detected due to being under treatment. PCR test is an important diagnostic tool in cases where there is no growth in culture (7,8,9,10). If there is clinical suspicion in a patient with a positive PCR test, beginning treatment and taking precautions without waiting for culture results will provide early infection control (27). It is also emphasized that it will be very useful in the differential diagnosis of tuberculosis and non-tuberculosis and also in the diagnosis of patients receiving inadequate tuberculosis treatment (12,13).

In this study, culture, PCR and microscopic examination methods performed on samples in our tuberculosis laboratory were compared. In our study, similar to the studies in the literature, the PCR methods of the samples received both from respiratory and non-respiratory systems performed on the same day had high agreement with the culture and has moderate agreement with the EZN staining method. (9,10, 21, 25, 28, 29)., The sample type and the amount of bacillus in the sample besides the PCR method used also play an important role in obtaining different sensitivity results. In theory, even a few bacilli in the sample is enough for PCR positivity. However, in practice, many studies have shown that the sensitivity of PCR for tuberculosis is not that high. Inability to reveal the *M. tuberculosis* DNA, loss of the bacilli during procedure and the presence of inhibitory substances in the sample may be shown as reason (20,21).

In the antibiogram of the samples with *M. tuberculosis* growth, 59 of 81 (72.8%) samples were found to be sensitive to all drugs and all samples were found to be sensitive to rifampin, while 13 (17%) were resistant to streptomycin, 11 (14%) to INH, 9 (11%) to rifampin. Rifampin was found to be statistically more sensitive than other antibiotics. In a study conducted in our laboratory in 2005, the rates of streptomycin, INH, rifampin, ethambutol resistance were reported as 11.3%, 8%, 4.8%, and 0%, respectively. Accordingly, streptomycin, INH, ethambutol resistance rates increased; It was observed that the rate of rifampin resistance decreased (11). In a study by Abdulmajed et al. (15), the survival rates for antituberculosis drugs were 12%, 4%, 13.2% and 4% for streptomycin, INH, rifampin, ethambutol, respectively; In the study of Saygan et al. (26), resistance rates were found to be 9.1%, 13.2%, 4% and 3.3% for streptomycin, INH, rifampin, ethambutol, respectively, to antituberculosis drugs. It has been observed that there may be regional differences in antibiotic resistance rates.

Studies show that both automated and manual systems are good in detecting INH and rifampin sensitivity, but are not so as for ethambutol and streptomycin (28,29).

In the rapid resistance test, incompatibility with the

antibiogram was detected in 2 patients (4%). While mutation probes detected in the rpoB locus with rapid resistance test are also detected with antibiotic resistance tests; wild type probe deletions could not be detected. This showed that rifampicin antibiotic resistance could be detected in the early period with rapid resistance tests. In the INH resistance study, it was observed that "low level INH resistance detected in the inhA locus" was also detected in the antibiotic susceptibility test. Early detection of mutations is important for these two drugs, that are very important in tuberculosis treatment. Acharya et al. (25) in their review; found the sensitivity of the rapid resistance test as 98% in rifampicin resistance and 84% in INH resistance. In a study by Barnand et al.(14), the sensitivity of Genotype MTBDR plus test in detecting rifampin and INH resistant strains was 99% and 94%, respectively, in 536 EZN positive sputum samples; the specificity is 99% and 100%; On the other hand, Ling et al.(27) determined that the specificity and sensitivity were 98% and 99%, respectively; Dorman et al.(30) determined the sensitivity of the test to determine rifampin resistance 86%, specificity 97%; They found INH resistance to be 62% and 98%, respectively. The findings support the recommendations that the GenoType MTBDR plus assay should not be used in sputum specimens where microscopy is negative or bacilli are rare. The use of rapid resistance tests is beneficial not only can be performed on the same day, but also for detecting the mutations that not have yet been reflected in antibiotic susceptibility tests. This guides the clinician during the treatment.

In conclusion; it should be considered that PCR tests are useful in the rapid diagnosis of tuberculosis in suspicious clinical samples in routine practice and that these tests definitely should not be used for screening purposes, but they are thought to be valuable in supporting the clinic together with conventional tests. In our study, it was observed that it is very important using staining and nucleic acid tests together, as well as culture methods.

Conflict of Interest: No conflict of interest was declared by the authors.
Ethics: The study was approved by the Non-Interventional Health Research Ethics Committee of Düzce University Faculty of Medicine with the date 07.11.2023 and number E-050.99-360085.
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Approval of final manuscript: All authors.

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REVIEW

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Genel Anestezi Sırasında Farkındalık

Awareness During General Anesthesia

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ABSTRACT

The aim of general anesthesia is to induce unconsciousness and amnesia during the surgery, and to provide adequate analgesia and muscle relaxation. Awareness during anesthesia refers to the state of the patient being conscious or able to remember the surgical procedure under general anesthesia. This may be an indication of insufficient anesthesia and may lead to potential psychological complications. Also, about 2% of claims against anesthesiologists have been related to the awareness complications during anesthesia. The incidence of awareness is rare, but the risk factors depend on the patient and technical factors. Factors such as difficulty in intubation, obesity, previous awareness during anesthesia, drug tolerance may increase the risk. In addition, technical errors, misuse of anesthesia machines and anesthetic administration errors may also cause awareness. Some surgical procedures and total intravenous anesthesia technique are also among the risk factors. Being aware of these risk factors is crucial for preventing, recognizing and managing awareness. An optimal strategy for avoiding awareness in anesthesia practice is a combination of individual patient preparation and careful anesthesia management. This complication can be reduced by identifying patients at risk, careful preparation and administration of drugs, and the use of appropriate strategies for anesthesia management. Routine use of devices that measure the amount of medication, neuromuscular block level and depth of sleep will help reduce awareness during surgery. In this article, the risk factors, prevention, recognition and management of awareness during anesthesia will be discussed.

ÖZET

Genel anestezinin uygulanma amacı ameliyat sırasında bilinç kaybı ve amnezi oluşturmak, yeterli analjezi ve kas gevşemesi sağlamaktır. Anestezi sırasında farkındalık, genel anestezi altında hastanın bilinçli olması veya cerrahi işlemi hatırlayabilmesi durumunu ifade eder. Bu durum, anestezinin yetersiz olduğunun bir göstergesi olabilir ve potansiyel psikolojik komplikasyonlara yol açabilir. Ayrıca, anestezistlere yönelik iddiaların yaklaşık %2'si anestezi sırasında farkındalık komplikasyonlarıyla ilgilidir. Farkındalığın insidansı nadirdir, ancak risk faktörleri hastaya ve teknik faktörlere bağlıdır. Entübasyon zorluğu, obezite, daha önce anestezi sırasında farkındalık yaşama öyküsü, ilaç toleransı gibi faktörler riski artırabilir. Ayrıca, teknik hatalar, anestezi makinelerinin yanlış kullanımı ve anestezik uygulama hataları da farkındalığa neden olabilir. Bazı cerrahi prosedürler ve total intravenöz anestezi tekniği de risk faktörleri arasındadır. Bu risk faktörlerinin farkında olmak, farkındalığı önlemek, tanımak ve yönetmek için önemlidir. Anestezi uygulamasında farkındalık halini önlemek için optimal bir strateji, hastaların bireysel hazırlığı ve dikkatli anestezi yönetiminin kombinasyonudur. Risk altındaki hastaların belirlenmesi, ilaçların dikkatli bir şekilde hazırlanması ve uygulanmasıyla birlikte, anestezi yönetimi için uygun stratejilerin kullanılmasıyla bu komplikasyon azaltılabilir. İlaçların kullanım miktarını, nöromuskuler blok düzeyini ve uyku derinliğini ölçen cihazların rutin kullanımı ameliyat sırasındaki farkındalığı azaltmaya yardımcı olacaktır. Bu yazıda anestezi sırasında farkındalık durumunun risk faktörleri, önlenmesi, tanınması ve yönetimi tartışılacaktır.

Keywords:
Intraoperative awareness
Anesthesia complications
General anesthesia
Monitoring

Anahtar Kelimeler:
İntraoperatif farkındalık
Anestezi komplikasyonları
Genel anestezi
Monitörizasyon

GİRİŞ

Ameliyat sırasında meydana gelen olayların bilinç kaybı ve amnezi durumu, genel anestezinin ana hedeflerini oluşturur. Bu hedeflere hemen hemen her zaman ulaşılsa da intraoperatif olayların hatırlanabilmesi nadiren meydana gelebilir. Hastanın genel anestezi altında uyanık olması veya cerrahi işlemi hatırlayabilmesine "anestezi sırasında farkındalık' olarak tanımlanan genel anestezinin korkutucu ve hafife alınan bir komplikasyonudur. Bu durum ameliyatın bitiminden hemen sonra veya daha sonra hasta tarafından bildirilebilir veya postoperatif yapılan görüşme sırasında ortaya çıkabilir (1-3).

Bu komplikasyon nadir görülmesine rağmen, klinik özellikler anestezi başarısızlığının göstergesidir, oysa potansiyel psikolojik komplikasyonlar akut stres bozukluğundan geçerek travma sonrası stres sendromlarına kadar subsendromal tablolara yol açarak yıkıcı olabilir (1,4-6). Ayrıca, bir mediko-legal analize göre, anestezistlere yönelik iddiaların yaklaşık %2'sinin farkındalık komplikasyonlarıyla ilgili olduğu belirtilmiştir (7,8). Beşinci Ulusal Denetim Projesi (NAP5) tarafından elde edilen sonuçlar, çalışma metodolojisi (örneğin, yapılandırılmış görüşmelerin olmaması) geniş çapta eleştirilse de genel olarak yaklaşık 1:19000 (%0,005)

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insidansını göstermektedir (1,9,10). Olguların sayısal olarak anlaşılmasını amaçlayan retrospektif değerlendirme çok karmaşık görünmektedir ve yalnızca tam bilgi akışının mevcut olduğu belirli ortamlarda güvenilirdir (7,8). Hastalar tarafından bildirilen olayların titizlikle yeniden bir araya getirilmesi, belirli intraoperatif anların epizotlarının rapor edilmesini sağlar. Bu nedenle komplikasyon, anestezinin üç fazından biri olan indüksiyon, idame ve uyanmadan birinde meydana gelebilir.

Bir olayın hatırlanması, epizodik ve semantik hafıza tiplerini içeren uzun süreli dekleratif hafızanın (açık hafızanın) aktivasyonunu gerektirir (5,6). Başka bir farkındalık alt grubu, açık belleği atlayarak gerçekleştirilir. Robert Veselis'e göre "var olan ancak sahip olduğumuzu bilmediğimiz" ve daha doğru bir şekilde bildirimsel olmayan örtük bellek olarak adlandırılan bir tür bellek olan "gizli" belleğin aktivasyonunu varsayar (5). Bu nedenle bu farkındalık alt tipi, açık hatırlama olmadan farkındalık olarak adlandırılır. Başka bir deyişle, anestezi sırasında kasıtsız bir bilinç epizodu, farklı uzun süreli bellek modlarında birleştirilen farklı bellek işleme yollarını izleyebilir. Bildirimsel bellek yoluyla konsolidasyon yolu, anestezi sırasında farkındalığı yapılandırır. Anestezinin sonunda veya sonrasında spontan veya indüklenmiş bir durum olarak kendini gösterir. Bu durum, anestezi altında rüya görmenin olası telkinlerinden veya fenomenlerinden açıkça ayırt edilmelidir (3,11,12). Alternatif olarak, intraoperatif bilincin tüm epizotları açık belleğin yolunu izlemez ve bilinçdışı veya örtük bellek açısından pekiştirilebilir. Belirgin epizotlardan sayısal olarak daha önemli olan bu epizotlar, ameliyatın sonunda kendiliğinden veya indüklenmiş biçimde bildirilmez ve klinik olarak davranış veya performans değişiklikleri olarak ortaya çıkabilir (1,12).

Bu bölümde anestezi sırasında farkındalık durumunun risk faktörleri, önlenmesi, tanınması ve yönetimi tartışılacaktır.

RİSK FAKTÖRLERİ

Hasta Kaynaklı Risk Faktörleri

Herhangi bir cerrahi prosedürde entübasyon zorluğu, muhtemelen uzun süreli entübasyon girişimleri sırasında yetersiz anestezi nedeniyle farkındalığa yol açabilir (14,16,17). Potansiyel olarak zor hava yolu olan bir hasta için "uyanık entübasyon" planlanıyorsa, hastanın genel anestezi indüksiyonundan önce tam amnezi beklentisini önlemek için planlanan sedasyon tekniğini hastaya açıklamak kritik derecede önemlidir (16).

Obezite ile daha yüksek anestezi sırasında farkındalık insidansı, entübasyon güçlüğü ile ilişkili olabilir (17).

Anestezi sırasında farkındalık öyküsü olan hastalar, gelecekteki anestezilerde daha yüksek risk altında olabilir. Bir gözlemsel çalışma, daha önce bir bu durumu yaşamış hastalarda beş kat daha yüksek insidans saptamıştır (18). NAP5 çalışması, anestezi sırasında farkındalık durumu yaşayan 20 hastadan 1'inin daha önce bu durumu yaşamış veya farkındalık hali yaşamış bir akrabaya sahip olduğunu bildirdi. Genetik varyasyonlar, belirli anestezik ajanların hipnotik veya amnezik etkilerine dirençle sonuçlanabilir, ancak bu tür varyasyonlar insanlarda tanımlanmamıştır (5,19).

Anestezik maddelere karşı kazanılmış tolerans oluşabilir. Sitokrom P450 2E1, alkol ve izoniazid tarafından indüklenir. İnhalasyon ajanları, benzodiazepinler ve opioidler sitokrom P450 enzim kategorisi tarafından metabolize edildiğinden, alkol alımı alışkanlığı olan hastalarda bu ajanların daha yüksek dozları gerekebilir. Bununla birlikte, alkol bir merkezi sinir sistemi depresanı olduğundan, akut alkol zehirlenmesi tipik olarak anestezi gereksinimlerinin azalmasıyla ilişkilidir (20,21). Benzodiazepin yoksunluk sendromunda gözlemlenen semptomların altında yatan, kronik maruziyetten sonra benzodiazepin kesilmesi üzerine uyarıcı glutamaterjik reseptörlerin ekspresyonunda artış vardır. Reseptörlerin bu aşırı ekspresyonu teorik olarak anestezik gereksinimini ve farkındalık riskini artırabilir. Opioid toleransı ve/veya opioid kaynaklı hiperaljezi, perioperatif dönemde opioid dozlama gereksinimlerini artırabilir. Cerrahi prosedür sırasında yetersiz analjezi, ağrı nedeniyle daha yüksek düzeyde kortikal stimülasyona neden olur ve farkındalık riskini artırabilir. Birçok reçeteli ve reçetesiz ilaç, opioidlerin metabolizmasında yer alan sitokrom P450 3A'yı (efavirenz, nevirapin, barbitüratlar, karbamazepin, glukokortikoidler, fenitoin, rifampisin) indükler. Kronik opioid kullanımına benzer şekilde, bu ilaçların kronik olarak uygulanması opioid dozlama gereksinimlerini artırabilir (21,22).

Literatürde bazı çalışmalar, anestezide farkındalık insidansının çocuklarda biraz daha yüksek olabileceğini (%0,2-1,2 arasında) öne sürse de NAP5 raporu çocuklarda ihmal edilebilir bir insidansa dikkat çekti (2,5,23). Çocuklarda farkındalık varlığının değerlendirilmesi, yaşa bağlı gelişimsel faktörler ve ameliyat sonrası görüşmelerin şüpheli doğruluğu nedeniyle özellikle zordur (2,23).

Teknik Nedenlere ve Uygulayıcıya Bağlı Risk Faktörleri

Anestezi makinesinin arızalanması veya yanlış kullanılması, farkındalığın nadiren görülen bir nedenidir. Anestezi makineleri ile ilgili problemler genellikle uygun alarmlar ve makine kontrolleri ile tespit edilir. Total intravenöz anestezi tekniği (TİVA) sırasında, damar içi infüzyon pompalarının arızalanması veya yanlış kullanılması, amaçlanan anestezik ajanın verilmemesine veya olası farkındalıkla düşük dozun verilmesine yol açabilir. Ayrıca, venöz kateter takılı ekstremite sıkıştırıldığında, cerrahi örtüler nedeniyle sürekli olarak görünmediğinde, venöz kateterin serum hattından ayrılması veya damar içi kateterin damardan çıkarak subkutan infiltrasyonu nedeniyle amaçlanan ilacın verilmemesi sonucu meydana gelebilir. Bu gibi durumlarda uyku derinliğini izleme (örn. elektroensefalogram ile) yetersiz anestezi derinliğinin tanınmasına yardımcı olabilir (2,3,24).

Anestezik uygulama veya yeterli doz ayarlama hatalarında insan faktörü, teknoloji başarısızlığından daha yaygındır. Damar içi anestezik ajanların uygulanması sırasında dikkatsizlik anestezik indüksiyon ajanından önce bir nöromüsküler blokaj uygulanmasına neden olabilir. Ayrıca klinisyenin anestezik konsantrasyonunu yanlış hesaplaması veya infüzyon pompasını yanlış programlaması da risk faktörüdür. İnhalasyon anestezik ajanı kullanıldığı durumlarda vaporizatörü açmayı unutmak da bu duruma yol açabilir. (24,25).

Ameliyat İlişkili Risk Faktörleri

Anestezi sırasında farkındalık riski bazı cerrahi

uygulamalarla ilişkili olabilir. Belirli prosedürler uygulanan hastalar (örneğin, travma ve acil cerrahi prosedürler, kardiyopulmoner baypas ile kalp cerrahisi, sezaryen doğum) özellikle yüksek risk altındadır (26).

Travma veya acil cerrahide anestezi indüksiyonu ile cerrahi insizyon arasındaki zaman aralığı zorunlu olarak kısadır (örn. kanamayı kontrol etme ihtiyacından dolayı). Ayrıca, hemodinamik dengeyi sağlayabilmek için anestezi derinliği kasıtlı olarak azaltılabilir. Bazı kurumlarda, mesai saatleri dışında acil durumlarda kıdemsiz bir anestezi ekibi üyesi tarafından gerçekleştirilebilecek hızlı sıralı indüksiyon ve entübasyon da bu riski artırmaktadır (27,28). Anestezi indüksiyonu sırasında tiyopental kullanılması ve indüksiyon sırasında opioid (örn. fentanil) kullanılmaması diğer risk faktörlerindendir (26,29).

Total İntravenöz Anestezi İlişkili Risk Faktörleri

Total intravenöz anestezi (TİVA), inhalasyon anestezik ajanına dayalı tekniklerle karşılaştırıldığında daha yüksek farkındalık riski ile ilişkilidir. Bir TİVA tekniğinin kullanımıyla artan risk, muhtemelen yetersiz doz verilmesine yol açabilecek intravenöz anestezik ajanların kan konsantrasyonu monitörlerinin bulunmamasından kaynaklanmaktadır. Bu, end-tidal anestezik konsantrasyonunun (ETAK) sürekli izlenmesinin gerçek zamanlı doz ayarlamalarına izin verdiği inhalasyon anesteziklerinin tersidir. TİVA sırasında, farkındalık yetersiz anestezi derinliğini belirtmek için kısmen hastanın ağrılı uyaranlara verdiği tepkiler nedeniyle fark edilir. Bununla birlikte, hastada yeterli kas gevşemesi için nöromüsküler blokaj uygulanmışsa, hareket önlenebilir ve farkındalık anestezist tarafından fark edilmeyebilir (25). Ayrıca, TİVA uygulaması sırasında venöz kateter takılı ekstremite sıkıştırıldığında, ekstremite cerrahi örtüler nedeniyle sürekli olarak görünmediğinde, venöz kateterin serum hattından ayrılması veya damar içi kateterin damardan çıkarak cilt altına infiltrasyonu nedeniyle de meydana gelebilir (25,30).

Bir klinisyenin yanlış ilacı veya yanlış konsantrasyonu uygulaması hala mümkün olsa da akıllı damar içi infüzyon pompalarının kullanılması pompa programlama hatalarını teorik olarak önleyebilir. Teorik olarak, hedef kontrollü infüzyon (HKİ) cihazlarının kullanılması, yetersiz anestezi ile sonuçlanabilecek yanlış dozaj riskini azaltabilir. Anestezi uzmanlarının geniş bir araştırmasında farkındalık durumu oluşan hastaların hiçbirinde TİVA uygulamak için bir HKİ cihazı kullanılmamıştır. Ancak, hiçbir çalışma HKİ kullanımının TİVA sırasında anestezi altında farkındalık riskini azalttığını kesin olarak göstermemiştir. Ayrıca, HKİ teknolojisi ABD Gıda ve İlaç İdaresi tarafından ABD'de kullanım için onaylanmamıştır (30).

Elektroensefalografi monitörleri gibi beyin izleme, TİVA (veya diğer anestezik teknikler) kullanımı sırasında genel anestezinin bilinç kaybına yol açmasını sağlamak için klinik yeteneği geliştirebilir. Gelişen diğer teknolojiler, solunan havadaki konsantrasyonunu belirleyerek serumdaki propofol konsantrasyonlarının tahmin edilmesine izin verebilir, ancak bu tür yöntemler yaygın olarak kullanılmamaktadır (25,30).

Nöromüsküler Blokaja Bağlı Risk Faktörleri

Anestezi sırasında farkındalık hali için en önemli risk faktöründen biri bir nöromüsküler bloke edici ajanın kullanılmasıdır. Kas felci, hastanın farkındalığının fizyolojik belirtilerinden birini (yani amaçlı hareket) ortadan kaldırır. Tam felç, bir farkındalık deneyiminin psikolojik travmasını ağrıdan bile daha fazla kötüleştirdiğinden, uzun vadeli psikolojik sekel potansiyeli artabilir. Operasyonun sonunda anestezinin derlenme aşamasında anestezik ajanın etkisinin ortadan kalkmasına rağmen kas gevşeticinin etkisinin kısmen de olarak devam etmesi farkındalık durumuna yol açabilir. Nöromüsküler blokaj sugammadeks veya neostigmin ile ortadan kaldırılabilir. Nöromüsküler blokajın kaldırılması solunum fonksiyonunun iyileşmesine yardımcı olmanın yanı sıra farkındalık potansiyelini azaltır (2,3,5,24).

Anestezide Farkındalık ve Hukuki Önemi

Anestezi sırasında farkındalık hakkındaki risk hastaya detaylı anlatılmalıdır. Ancak bilgi hekim tarafından çok incelikli ve dikkatli bir şekilde verilmelidir. Bu risk, hukuki olarak yurtdışında çok önemli bir konudur. Örneğin bugüne kadar Almanya'da farkındalıkla ilgili sadece 3 mahkeme kararı olmuştur. Tüm davalarda iddialar reddedilmiştir. 2013 yılında, farkındalık durumunda bilgilendirilmiş onamla ilgili bir mahkeme kararı yoktur (31). Türkiye'de de bu konu ile ilgili hukuki şikâyet ve davalar başlamamış olsa da sosyal medyaya yansıyan şikayetler mevcuttur (32). Bu risk önlem alınmadığı zaman önemli bir sorun haline gelebilir. Özellikle farkındalık riski olduğu bilinen hastalarda yeterli denetim yapılması, anestezinin hazırlanmasında veya kontrolünde teknik hatalardan kaçınılması ve farkındalık oluşmuşsa yeterli terapötik müdahale başlıca alacağımız önlemlerimizden olmalıdır.

Anestezide Farkındalık Durumunun Önlenmesi

Anestezideki farkındalığının önlenmesi için birkaç pratik öneri sunulabilir. Bu önlemler preoperatif ve intraoperatif dönemde uygulanabilir (14). Preoperatif olarak risk altındaki hastaların belirlenmesi, daha önce farkındalık yaşayanlar gibi risk altındaki hastalara ilişkin dikkatli bilgi alınması ve bilgilendirme yapılmasını, değiştirilebilir risk faktörlerinin düzeltilmesini, anestezi cihaz ve aletlerinin kontrolünü içermektedir (2,3,14).

İntraoperatif yönetim ise nöromüsküler izlemenin kullanımı (niceliksel>niteliksel) bilmek, nöromüsküler blok tamamen iyileşene kadar yeterli anestezi durumunun sürdürülmesini sağlamak, nöromüsküler bloke edici ilaçların dikkatli doz ayarlamasını yapmaktır. Bispektral indeksi (BİS) monitorizasyonu bu doz ayarlama yöntemlerinden biridir ve BİS kullanımının ameliyat sırasında uyanıklığı engelleyebileceği bildirilmiştir (33). BİS bir EEG parametresidir ve çeşitli elektrotlar kullanılarak EEG sinyallerini algılamaktadır. BİS indeksi 0-100 arasında değişen bir sayıdır ve bu değerin 100 civarında olması hastanın uyanık olduğunu belirtirken, 0 olduğunda izoelektrik EEG'yi göstermektedir. Bu değer takip edilerek hastanın uyanıklık durumu kontrol edilebilir (34). Ayrıca tamamen hastanın bilinci açılmadan ekstübasyondan kaçınmak ve özellikle yüksek riskli hastalarda uyku derinliğini ölçen beyin izleme cihazlarının kullanımı önerilmektedir (3,5)

SONUÇ

Anestezi uygulamasında farkındalık halini önlemek için en iyi strateji her hastaya göre iyi bir hazırlık ve dikkatli bir

anestezi yönetiminin kombinasyonu gibi görünmektedir. Bununla birlikte, alınabilecek tüm önlemlere rağmen, anestezi sırasında farkındalık ataklarının sınırlı bir yüzdesinin önlenemeyeceği unutulmamalıdır. Bu paradoks, özellikle henüz aydınlatılmamış birçok yönü olan çok karmaşık bir nörofizyolojik süreci içermektedir. Optimal yaklaşım, anestezi yönetimi için uygun stratejilerle birlikte risk altındaki hastaların belirlenmesini, ilaçların özenli ve doğru hazırlanması ve dikkatli bir şekilde uygulanmasıdır. İlaçların kullanım miktarını, nöromuskuler blok düzeyini ve uyku derinliğini ölçen cihazların rutin kullanımı ameliyat sırasındaki farkındalığı azaltacaktır.

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LETTER TO EDITOR

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Definition of Sepsis and Novel Biomarkers for Sepsis

Sepsisin Tanımı ve Sepsis İçin Yeni Biyobelirteçler

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Dear Editor,

Sepsis is a clinical syndrome with high mortality that can progress with multiple organ failure as a result of the body's abnormal and inappropriate host response to infection. In order to determine some definitions that could be used in sepsis patients and make it easier for clinicians to recognize and categorize these patients, a conference was held for the first time in Northbrook in 1991 by the American College of Chest Physicians/Society of Critical Care Medicine. SIRS (systemic inflammatory response syndrome) criteria were defined for the first time (Sepsis-1 criteria) (1). Since there is no gold standard method for the diagnosis of sepsis, the definition of sepsis was insufficient and unclear for many clinicians until 2001, the 'International Sepsis Definitions Meeting' was held in Washington in December 2001 in order to correct the existing definitions and increase their accuracy and reliability (Sepsis-2 criteria) (2). These criteria were used until 2016. Sepsis definition was updated in 2016 by the European Intensive Care Medical Association and the Intensive Care Medical Association due to the correct understanding of the pathophysiology of sepsis, the terms described in 1991 and 2001 being used interchangeably or unnecessarily, the SIRS criteria not having high specificity in sepsis patients, and the understanding that they only address the excessive inflammatory response (Sepsis-3 criteria) (3). With these updates, the diagnosis of sepsis was changed to the diagnosis of the body's inappropriate inflammatory response to infection, it was accepted that the SIRS criteria do not always indicate the infection status and that it can occur in many hospitalized patients, and these criteria were abandoned. In addition, the diagnosis of severe sepsis was also abandoned. According to these criteria, sepsis is defined as a clinical syndrome with high mortality that can progress with multiple organ failure as a result of the body's abnormal and uncontrolled host response to infection.

There is no specific biomarker for sepsis. In the literature, there are many ideal biomarker studies but the definition of sepsis is so vague. Since there is no standard for distinguishing infection, it is difficult to distinguish sepsis from SIRS that is especially non-infectious.

Platelets are the basic cells of hemostasis. However, recent studies have shown that they also play a role in inflammation (4,5). Studies have shown that mortality increases in patients with decreased platelet function and thrombocytopenia due to sepsis, and that it plays a role in determining the prognosis in patients who stay in the ICU for more than five days and subsequently develop thrombocytopenia (6).

Lactate is formed as a result of the catabolism of the intermediate metabolite pyruvate, which occurs as a result of glycolysis, by the lactate dehydrogenase enzyme under anaerobic conditions (7). Due to tissue hypoperfusion in sepsis, hyperlactatemia occurs as a result of the decrease in oxygen delivery and the shift of the primary energy source for cells to anaerobic glycolysis, and it also occurs as a result of the reprogramming of glucose metabolism seen in immune system cells (8).

Procalcitonin is produced by C cells of the thyroid gland in healthy individuals in the absence of inflammation. Serum procalcitonin levels rise 2-4 hours after an inflammatory stimulus. After reaching its peak value at the 6th hour, it maintains its plateau value for up to 8-24 hours and its plasma half-life is 24 hours. Apart from systemic infection, causes such as shock, trauma, surgery, burn injury, pancreatitis, and chronic kidney disease can also induce procalcitonin production. Among all these reasons, the highest levels were detected in sepsis. Procalcitonin is also used as a marker of serious bacterial infections and organ failure due to sepsis (9,10). In 2016, its use was approved by the Food and Drug Administration (FDA) on the grounds that procalcitonin monitoring helps predict 28-day mortality in patients with sepsis and septic shock.

SCUBE-1 is a cell surface protein identified by recent studies within the SCUBE gene family. EGF-like repetitive structures have been shown to function as an adhesive module in mediating platelet-matrix and platelet-platelet interactions. Although these soluble EGF-like portions of SCUBE-1 do not induce platelet aggregation per se, they

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can potentiate ristocetin-induced platelet agglutination (11,12). In a study conducted in Turkey, including 187 patients, it was reported that SCUBE-1 is an independent prognostic factor in septic patients (13).

CD14 is a co-receptor located on the membrane of myeloid cells that helps the presentation of lipopolysaccharides to toll-like receptor-4 and lipopolysaccharide-binding protein. These lipopolysaccharides are found especially in the cell walls of bacteria phagocytosed by macrophages, monocytes, and neutrophils. After stimulation of the receptor, the membranous part of CD14 is destroyed and its level decreases, while sCD14 is secreted from the cell. It is then converted to presepsin, sCD14-ST (subtype), by cathepsin D and other proteases. The characteristics of presepsin are that it is measured in healthy individuals, increases in the early stage of infection, and is directly proportional to the activity of innate immunity (14). Presepsin has recently attracted the attention of various clinical research groups as a prognostic biomarker in sepsis. The biological activity of presepsin has not been elucidated in detail. However, it has been identified as a moderating factor. Plasma presepsin levels can be considered an indicator of activated innate immune effector cells in response to invasive pathogens (15).

Copeptin is a glycopeptide molecule consisting of 39 amino acids located at the C-terminus of pre-pro-vasopressin and was first identified in 1972. During the release of arginine vasopressin from the pituitary, they are released in equal molar amounts together with a peptide molecule called neurophysin. It is not yet clear whether copeptin has a physiological role or whether it is a nonfunctional protein found as a residue after arginine is separated from vasopressin (16). High copeptin levels serve as a prognostic marker for adverse outcomes in sepsis, shock, pneumonia, stroke, acute coronary syndrome, and diabetes (17).

As a result, the definition of sepsis is newly taking shape in the literature. However, there is no ideal sepsis biomarker. Researchers should be encouraged to work on new markers in this field.

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CASE REPORT

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Prolonged Air Leak After Pleurectomy/Decortication Surgery in Two Patients with COVID-19 Pneumonia

COVID-19 Pnömonili İki Hastada Plörektomi/Dekortikasyon Ameliyatı Sonrası Artan Uzamış Hava Kaçağı

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ABSTRACT
Surgical treatment is a part of multimodal treatment in patients with malignant pleural mesothelioma in appropriate clinical stage and medical condition. The pleurectomy/decortication is a surgical technique for mesothelioma and its challenging morbidity is prolonged air leak. In case of prolonged air leakage, both the duration of tube thoracostomy and the risk of its complications increase. An increase in this complication is expected due to reasons such as pleural adhesions developing as a result of exaggerated inflammation in COVID-19 pneumonia and increased susceptibility to tearing in the alveoli. Here, we present the treatment of prolonged air leak after pleurectomy-decortication surgery in 2 cases of malignant pleural mesothelioma who were scheduled for surgery after induction therapy and who developed COVID-19 pneumonia during the preparation process.

ÖZET
Malign plevral mezotelyomada uygun klinik evre ve medikal kondisyonlu hastalarda cerrahi tedavi, multimodal tedavinin bir parçasıdır. Plörektomi-dekortikasyon yöntemi, mezotelyoma için uygulanan cerrahilerden biridir ve bu yöntemdeki korkulan morbidite uzamış hava kaçağıdır. Uzamış hava kaçağında tüp torakostomi süresi de uzamakta ve buna bağlı komplikasyon riski de artmaktadır. COVID-19 pnömonisinde abartılı inflamasyon sonucu gelişen plevral yapışıklıklar ve alvelollerdeki yırtılmaya yatkınlık artışı gibi sebeplerle bu komplikasyonda artış beklenen bir durumdur. Burada malign plevral mezotelyoma tanısıyla indüksiyon tedavisi sonrası cerrahi planlanan ve hazırlık sürecinde COVID-19 pnömonisi gelişen 2 olguda, plörektomi-dekortikasyon ameliyatı sonrası uzamış hava kaçağı ve uzamış tüp torakostomi tedavisi sunulmuştur.

Keywords:
COVID-19
Malignant pleural mesothelioma
Prolonged air leak

Anahtar Kelimeler:
COVID-19
Maling plevral mezotelioma
Uzamış hava kaçağı

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a pleural malignancy with poor prognosis. Treatment of MPM is multimodal including surgery, chemotherapy and radiotherapy. Surgical therapy can be performed before or after induction chemotherapy in suitable patients. Primary aim of surgery is cytoreduction and it plays an important role in staging of MPM (1). There are two surgical option called pleurectomy/ decortication (P/D) and extrapleural pneumonectomy (EPP) for MPM (2). When the pericardium and/or diaphragm are included in P/D surgery, extended pleurectomy decortication is called EP/D. In surgery Mediastinal lymph node dissection or sampling is also important for staging of MPM (3). Prolonged air leakage due to alveolization after visceral pleurectomy in P/D procedure is a feared complication.

Covid-19 pneumonia causes a strong inflammatory response with proinflammatory cytokine release, oxidant stress and damaging of alveolar epithelium so risk of alveolization is increased in P/D procedure due to dense pleural adhesions in patients (4). Cytokine storm is an uncontrolled release of cytokines leading to hyperinflation in patients with COVID-19 pneumonia and it can be accompanied by further immune cell activation. The higher levels of inflammatory cytokines such as IL-6, IL-10, and TNF-α, in patients with COVID-19 pneumonia who developed pleural effusion is also indicate an intense cytokine storm (6,7). The developing cytokine storm induces extensive alveolar damage that makes the alveoli more vulnerable to rupture (8). Here, we presented two patients with prolonged air leak after P/D for MPM following induction chemotherapy and developed COVID-19 pneumonia during treatment.

CASE 1

A 66-year-old male patient was referred to us with complaints of fatigue and dyspnea. Pleural thickening and nodulation were seen in the right hemithorax on thorax computed tomography (CT). PET-CT showed a pathological increased uptake of 18f-FDG at pleural thickening areas (Figure1, SUV max:10,2). We planned VATS pleural biopsy with MPM pre-diagnosis. Histopathologic evaluation revealed mix type mesothelioma and he referred to department of medical oncology for induction chemotherapy (ChT). After the

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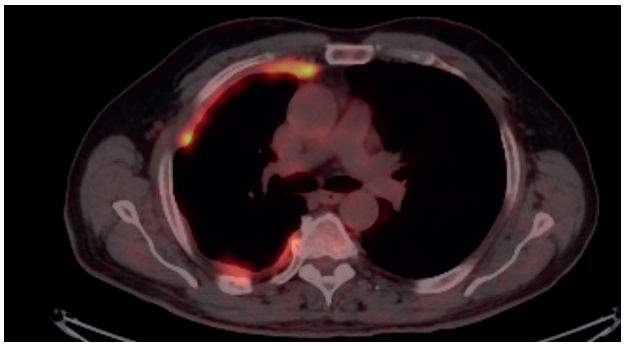

Figure 1: PET CT image.

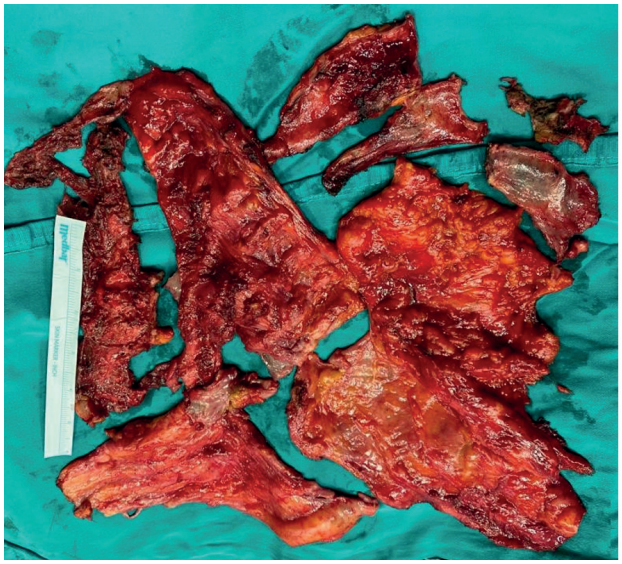

Figure 2: EP/D operation material.

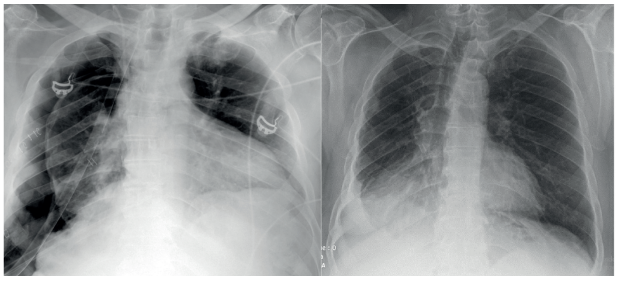

Figure 3: Postoperative 0th day chest X-ray and chest X-ray after chest tube removal.

four cycles induction ChT, we re-evaluted the patient and planned the P/D surgery. During the surgical preperations the SARS-Cov2 qRT-PCR test of patients was positive. Appropriate treatment was started to him and the operation was postponed for 28 days. We performed EP/D procedure to patient after the medical treatment for covid-19 pneumonia (Figure 2). The patient was discharged on the 10th postoperative day with heimlich valve but he was hospitalized again due to empyema detected 5th day after discharging. A second tube thoracostomy was inserted at junction of 7th intercostal space with posterior axillary line for empyema drainage. Antibiotherapy with wide spectrum was started. The first chest tube was removed 7th day of antibiotic therapy. During the treatment period, consecutive pleural cultures became negative and air leak stopped on the 55th postoperative day and the other chest tube was removed and, patient was discharged (Figure 3).

CASE 2

A 65-year-old farmer male referred to us for right pleural effusion and pleural thickening on thorax CT. The pathological increased uptake of 18f-FDG was detected at the pleural thickening areas on PET-CT (SUV-max: 9,4). Results of histopathologic examination of pleural specimen taken by VATS was epitheloid type mesothelioma. Similarly, we planned surgery after induction Cht, we re-evaluted the patient and planned the P/D surgery. Histopathological report indicated epitheloid type mesothelioma, and the patient received 4 cycles of chemotherapy. The SARS-Cov2 qRT-PCR test given by the patient for preoperative preparation after KT was positive. Appropriate treatment was applied and the operation was postponed for 28 days. EP/D was performed to him and he was discharged with a chest tube removed on postoperative 22nd day (Figure 4).

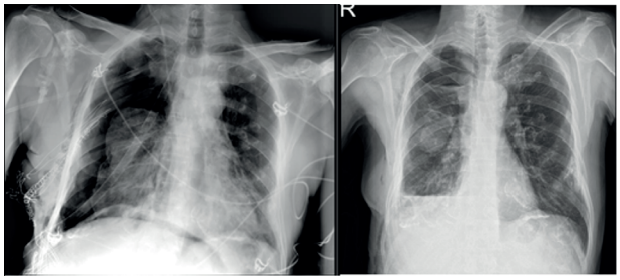

Figure 4: Postoperative 0th day chest X-ray and chest X-ray after chest tube removal.

DISCUSSION

Here, we aimed presenting the prolonged air leak that developed after EP/D surgery for MPM in a patient with intense pleural inflammation caused by Covid-19 pneumonia. The most common surgical morbidity associated with P/D is prolonged air leak and duration of tube thoracostomy. The known reason of that situation is the absence of a surgical dissection plan between the visceral pleura and the alveoli. The air leak problem is generally managed by conservatively such as suction, pleurodesis etc., until the air leak stops and lungs expand. The mean hospital duration was reported as 18.3 days in a case series including 90 cases of EP/D (9). Hashimoto et al. reported median duration of hospital stay was 21 days in patients with MPM treated by P/D and EP/D (10). P/D and EP/D were performed to total of 41 patients between March 2010 and May 2021 in our department and the median duration of hospitalization was 19 days. However, prolonged hospitalization occurred in these two patients with COVID-19. Hameed et al. reported that an exaggerated inflammatory response, increased pleural adhesion and increased tendency of alveoli to tearing occurred in COVID-19 pneumonia and they claimed there was a prolonged air leak for these reasons (8). Prolonged air leak leads to both prolongation of tube thoracostomy and hospital stay and increases complication rates. The most important complication is pleural empyema, and mortality and morbidity increase significantly in this case (11). In Case-1, a pleural empyema due to prolonged TT occurred and a second TT was required for loculated empyema.

CONCLUSION

Prolonged air leak and empyema complications should be keep in mind in patients with COVID-19 pneumonia when required P/D or EP/D surgeries for MPM. Multicenter studies including larger numbers of patients are needed to define measures such as timing of surgery, necessary immunosuppression, etc. to prevent these complications.

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CASE REPORT

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Adrenal Ganglioneuroma with Lymph Node Metastasis: A Rare Case Report

Lenf Nodu Metastazı Gösteren Adrenal Ganglionörom: Nadir Bir Olgu Sunumu

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ABSTRACT

Ganglioneuroma (GN) is a rare benign, well-differentiated neoplasia originating from the neural crest. Although it is most commonly seen in the posterior mediastinum, it can be observed in many areas including the adrenal gland. Lesions located in the posterior mediastinum and retroperitoneum are mostly seen in the pediatric population and adrenal ganglioneuromas are more common in the 4-5. decade. GN is a benign neoplasia but very rarely lymph node and distant organ metastases have been reported. In this study, a case of adrenal gland ganglioneuroma showing lymph node metastasis in a 3-year-old male patient is presented.

ÖZET

Ganglionöroma (GN) nöral krestten köken alan nadir görülen benign, iyi diferansiye bir neoplazidir. En sık posterior mediastende görülmekle birlikte adrenal bez de dahil olmak üzere birçok alanda izlenebilir. Posterior mediasten ve retroperiton yerleşimli lezyonlar daha çok çocuk popülasyonda, adrenal ganglionöromalar ise daha çok 4-5. dekadta izlenmektedir. Benign olmalarına rağmen çok nadir lenf nodu ve uzak organ metastazı bildirilmiştir. Bu çalışmada 3 yaşında erkek bir hastada lenf nodu metastazı gösteren, adrenal bez yerleşimli bir ganglionöroma olgusu sunulmuştur.

Keywords:
Ganglioneuroma
Adrenal
Lymph node
Metastasis

Anahtar Kelimeler:
Ganglinörom
Adrenal
Lenf nodu
Metastaz

INTRODUCTION

Ganglioneuromas are rare benign neoplasms that represent less than 5% of adrenal masses and develop from the neural crest. It consists of ganglion cells, mature Schwann cells, and neural fibers (1). Ganglioneuromas may develop spontaneously or by maturation of more immature neuroblastic tumors (2). It develops mainly from the posterior mediastinum and retroperitoneum. Adrenal ganglioneuromas are rare tumors that make up 20% of all ganglioneuromas (3). The median age at diagnosis is 35.3 (13-59)(4). Adrenal ganglioneuromas are usually asymptomatic and hormonally silent. Although it is discovered incidentally in 66%, they may present with abdominal discomfort, hypertension, headache, palpitation, menstrual irregularity (4).

Despite the fact that these tumors are benign, lymph node and distant organ metastases have been reported very rarely (3,5,6,7,9,11-14).

CASE

A mass in the left adrenal gland was detected in the computed tomography (CT) imaging of a 3-year-old male patient who had no previous known disease and applied to an external center due to bloody diarrhea caused by rotavirus infection. The case was referred to the pediatric oncology department with a preliminary diagnosis of neuroblastoma. On magnetic resonance imaging, a smooth-contoured solid-weighted mass containing millimetric cystic areas was observed in the left adrenal tract, measuring 41x33x52 mm (Figure 1).

There was no significant restriction in diffusion in the homogeneously enhanced mass after intravenous contrast

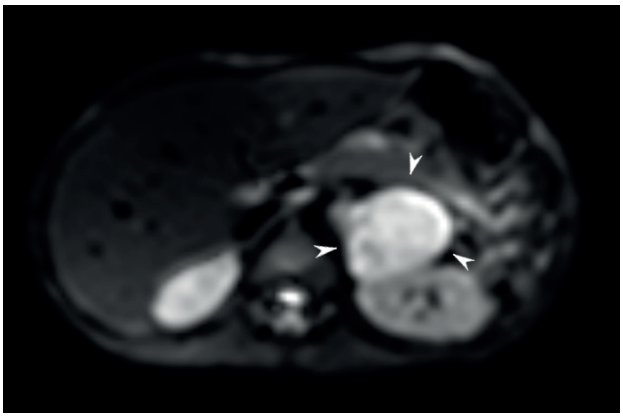

Figure 1: Trace diffusion-weighted transverse MRI shows a large hyperintense mass in the left adrenal tract (arrowheads).

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agent. Therefore, it was evaluated primarily in favor of benign/mature neurogenic tumor (ganglioneuroma, ganglioglioma, etc.). In the operation performed on the patient with normal laboratory values, samples were taken from the paraaortic lymph nodes near the renal vein for staging and the total excision of the well-circumscribed mass.

Macroscopically, 2 different mass excision materials, the largest 5.5x3x3.5 cm and the smallest 3.5x2x0.5 cm, and 2 lymph node-registered tissues measuring 1.5x1.2x0.6 cm and 0.6x0.5x0.6 cm were observed.

Histopathological examination revealed tumoral infiltration surrounded by a connective tissue capsule. The tumor consisted of schwann cells and ganglion cells in the background containing varying degrees of collagen and myxoid areas (Figure 2). Schwann cells are separated by small fascicles and loose myxoid stroma. Interspersed small and large groups of ganglion cells were observed. All of the ganglion cells were mature with compact eosinophilic cytoplasm, single, eccentric nuclei and prominent nucleoli (Figure 3). In between, lymphoid cells forming lymphoid follicle structures were observed. No blastomatous component was observed. One lymph node adjacent to the tumor in an area near the tumor areas was observed to be metastatic (Figure 4). 2 lymph nodes sent separately were found to be reactive.

Immunohistochemical studies showed positive staining with S100, NSE, synaptophysin, neurofilament in Schwann cells and stroma. Positive staining with S100, synaptophysin, NSE, chromogranin A was obtained in ganglion cells. Low proliferation was observed with

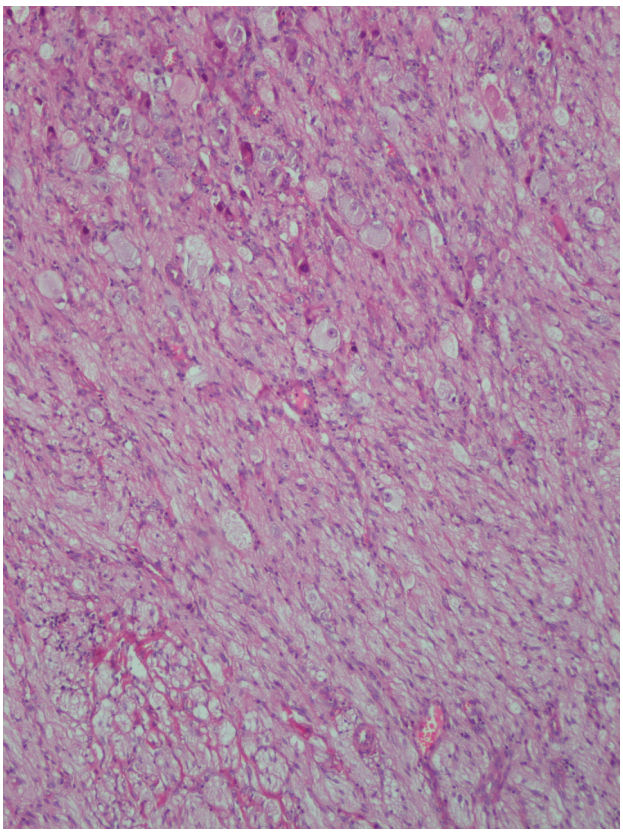

Figure 3: Mature ganglion cells with eosinophilic cytoplasm, eccentric nuclei and prominent nucleoli (HEx100).

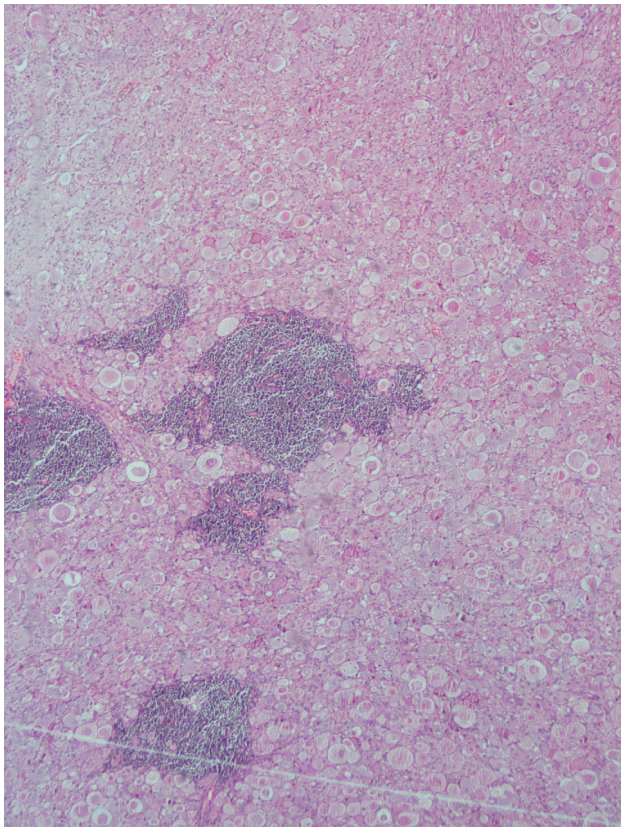

Figure 2: Tumoral infiltration consisting of schwann cells and ganglion cells (HEx40).

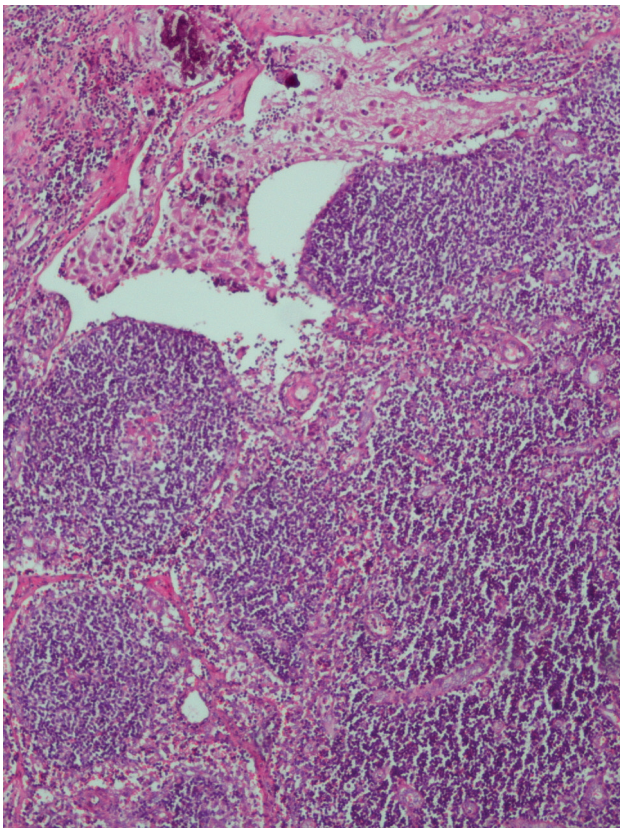

Figure 4: Metastatic lymph node (HEx100).

Table 1: Metastatic ganglioneuromas in the literature.

Patient	Age	Gender	Site	Symptoms	Size	Location of Metastasis
1	31	Male	Adrenal	Incidental	10 cm	Liver
2	27	Male	Retroperitoneum	Left upper qadrant and epıgastrıc pain	Not reported	Lymph node
3	43	Male	Retroperitoneum	Upper abdominal pain	25 cm	Liver (PNST)
4	3 yrs 10 mo	Female	Toracic	Tachypnea and left hemithorax	Not reported	Lymph node
5	30	Male	Adrenal	Abdominal pain, early satiety, nausea	30x18x13 cm	Retroperitoneum (PNST)
6	8	Male	Adrenal	Autopsy case	Not reported	Liver and spleen
7	52	Female	Retroperitoneum	Dyspeptic symptoms	Not reported	Lymph node
8	13	Female	Parafarengeal	Swelling on the left side of the neck	3.5x4x8 cm	Multifocal bone metastases
9	2 yrs 1 mo	Male	Adrenal	Swelling of legs	243 ml	Soft tissue metastases in buttocks and legs
10	3 yrs 8 mo	Female	Toracic	Fever and cough	173 ml	Lymph node
11	5	Female	Abdominal	Abdominal paın	480 ml	Lymph node

PNST: peripheral nerve sheath tumor

Ki67. Histochemically, positive staining was observed in myxoid areas. The case was diagnosed as Schwannian stroma rich mature ganglioneuroma.

No residue-recurrence was detected in the follow-up of the patient who underwent total resection.

DISCUSSION

Due to the frequency of using medical imaging methods, there has been an increase in the number of adrenal incidentilomas (8). Adrenal ganglioneuromas should be kept in mind in the differential diagnosis of adrenal masses. Ganglioneuromas most commonly develop in the thoracic (41.5%), abdominal non-adrenal (37.5%) and 21% of the adrenal regions (3). Primary GN occurs at a slightly older age than neuroblastomas.

In a study of 42 cases, the median age of ganglioneuromas located in the adrenal region was 35.3 (13-59)(4). In another study, the median age was 35 (19-73)(1). In this case, we presented an incidentally detected adrenal ganglioneuroma in a 3-year-old male patient. Although GN in adrenal localization is seen at an older age, there are cases reported in the pediatric population (3,13). In the literature, In a study of 49 cases conducted by Goerger et al. ; 9 out of 10 adrenal localized cases were children (<10 years old) (3). GN are benign neoplasms and are usually asymptomatic. In cases with symptoms, the findings depend on the compression effect of the tumor. Rarely, it depends on the vasoactive peptides secreted by the tumor. Although our case was detected incidentally, the patient did not have any tumor-related symptoms. Ganglioneuromas are benign neoplasms but distant organ metastases (7,13,14) and lymph node metastases (3,5,6,9) have been reported rarely.

In the literature, 12 GN cases together with our case have metastasized (Table 1). Five of them are metastatic GN cases with adrenal localization. Seven of these cases showed lymph node metastasis. Five (5/12) of the cases in the literature are female and 7 cases are male. Since lymph node metastasis was also observed in our case, it should be kept in mind that although ganglioneuromas are benign lesions, they may rarely present with metastasis.

The prognosis after total excision in ganglioneuromas is excellent (3,7). However, local recurrence and malignant peripheral nerve sheath tumor (PNST) transformations have been reported (11,12). Therefore, long-term follow-up of the case after total excision of the lesion is important. In the latest World Health Organization (WHO) classification of ganglioneuromas, there are two histological subtypes, mature GN and maturing GN. Our case was a mature ganglioneuroma rich in stroma.

CONCLUSION

In conclusion, ganglioneuromas should be considered in childhood adrenal tumors. Although GN is benign neoplasms, it should be kept in mind that very rarely, they have the potential for malignant transformation and metastasis to localizations such as lymph nodes, liver, spleen, and soft tissue.

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Approval of final manuscript: All authors.
Presentsation: This case was presented as a poster presentation at the National Pathology Congress on 25-29 October 2023.

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