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ISSN: 2717-8161 RESEARCH ARTICLE

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Investigation of the Profile Promotor Methylation of the E-Cadherin Gene in Patients with Pterygium

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Abstract: Pterygium is one of the most controversial ocular diseases whose pathology is not yet known. Although it is defined as a benign lesion, it behaves similar to tumor formation due to epithelial and fibrovascular overgrowth on the ocular surface. DNA methylation of specific genes as an epigenetic regulatory mechanism plays critical roles in the pathogenesis, progression, diagnosis and treatment of many cancers. The E-cadherin gene is involved in cell metabolism and is known to be associated with multiple tumor malignancies. In this study, we evaluated the possible relationship of the e-cadherin gene methylation profile with pterygium. E-cadherin gene promoter methylation profile was determined using methylation specific PCR (MSP) method in tissue samples of a total 36 patients with pterygium. According to the data obtained, the percentage of methylation of the E-cadherin gene in tissues with pterygium was statistically significant compared to the control group ($p<0,005$). Methylation in the E-cadherin gene is thought to be related to pterygium formation and development.
Keywords: Pterygium; E-Cadherin; MSP; DNA Methylation.

1. Introduction

Pterygium is defined as the wing-shaped fibrovascular growth of the conjunctiva along the limbus on the cornea (1). The etiology of pterygium, which is a common disease in many parts of the world, is still unknown (2). It is seen that it is more common in sunny, hot, windy, dusty climates and tropical-subtropical regions. These locations are equatorial regions, which have been associated with greater exposure to ultraviolet (UV) radiation (3). Symptoms of pterygium include burning, irritation, eye redness, decreased vision due to the occlusion of the visual axis due to migration of pterygium tissue over the central cornea, and irregular astigmatism (4). Pterygium is a relatively benign formation. However, after removal, aggressive recurrence and locally invasiveness, various degrees of abnormalities ranging from mild dysplasia to cancer, and tumor-like features caused the disease to be referred to as a neoplastic-like growth disorder.

As a matter of fact, as a result of studies conducted by many researchers, it has been shown that preneoplastic lesions may be associated with pterygium (5-6). Pterygium is characterized by inflammation, angiogenesis, cell proliferation, fibrosis, disruptions in the extracellular matrix (ECM) of the conjunctiva, and increasingly corneal invasion (7-8).

E-cadherin, known as epithelial-cadherin, is encoded by the CDH1 gene. CDH1 gene is localized on the 16. chromosome (16q22.1) and covers an area of approximately 100kb (9). E-cadherin is expressed as a well known tumor suppressor gene (10). Abnormal methylations occurring in the promoter regions of tumor suppressor genes and silencing of related genes play crucial roles in elucidating the pathology of most human cancers (11). Hypermethylation of CpG islands in the promoter region of the E-cadherin gene (CDH1) is considered to be one of the factors contributing to the

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inactivation of E-cadherin (12). E-cadherin function as calcium-dependent cell adhesion molecules and mediates the formation of cell connections (13). For this purpose, in our study, we evaluated the methylation of the E-cadherin gene promoter region in terms of pterygium, a common eye condition that exhibits tumor-like behavior.

2. Material and Methods

2.1 Collection of tissues

36 patients (13 females, 23 males) who applied to Tokat Gaziosmanpaşa University Hospital Ophthalmology Outpatient Clinic and were diagnosed with pterygium were included in this study. Conjunctival tissue belonging to the same eye of the same patient was used as the control group. It was ensured that all patients did not experience any corneal discomfort such as glaucoma and uveitis in their medical history. With the permission and knowledge of the participants included in the study, tissue pieces taken during the surgery were appropriately labeled and quickly frozen in liquid nitrogen for DNA isolation and subsequently methylation studies and kept at -80 ° C until the time of study. A portion of tissue was separated for histopathological examination and the diagnosis of pterygium was confirmed.

The necessary permission for the study was obtained by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee at its meeting on 02.04.2019 with the project number 19-KAEK-090.

The demographic data of the patients are shown in Table 1.

Table 1: Demographic data of pterygium patients.

	Pterygium (n = 36)
Age	56±13,29
Gender	13 Women / 23 Men
Right / Left Eye	24 Right / 12 Left Eye

2.2. Genomic DNA Isolation from Tissue

GeneALL Clinic SV Mini (108-101) brand kit was used for DNA isolation from the tissue, and the protocol stipulated by the manufacturer was applied for the isolation steps. Measurements were made using ABP iQuant ™ dsDNA HS Assay Kit for purity and concentration of isolated DNA.

2.3. Methylation-Specific PCR (MSP)

The modification of the isolated and measured DNAs was carried out using the EZ DNA Methylation-Gold Kit (Zymo D5005 & D5006) in accordance with the manufacturer's instructions. From the modified DNAs, the E-cadherin gene promoter region was used with primers suitable for the MSP stage. Primary sequence information used for the study is given in Table 2 below. The applied PZR program was carried out as 2 minutes at 95 °C, 30 seconds at 95 °C, 30 seconds at 55 °C, 30 seconds at 72 ° C, 10 minutes at 72 °C, 45 cycles. PCR samples were run on a 2% agarose gel.

Table 2: E-cadherin gene primer sequences.

CDH1-M-F	TTAGGTTAGAGGGTTATCGCGT
CDH1-M-R	TAACTAAAAATTCACCTACCGAC
CDH1-UM-F	TAATTTTAGGTTAGAGGGTTATTGT
CDH1-UM-R	CACAACCAATCAACAACACA

2.4. Statistical analysis

According to the E-cadherin gene promoter region agarose gel electrophoresis images, the ratings of the tissue samples were expressed as fully methylated, semi-methylated and non-methylated. In determining the methylation percentage, the semi-methylated ones were calculated by adding them to the fully methylated ones. Statistical analysis of methylation data was done with the $\chi 2$ test. The statistical significance level was accepted as 0.05, if $p \leq 0.05$ was significant, and if $p > 0.05$, it was evaluated that there was no difference.

3. Results

3.1. MSP gel images

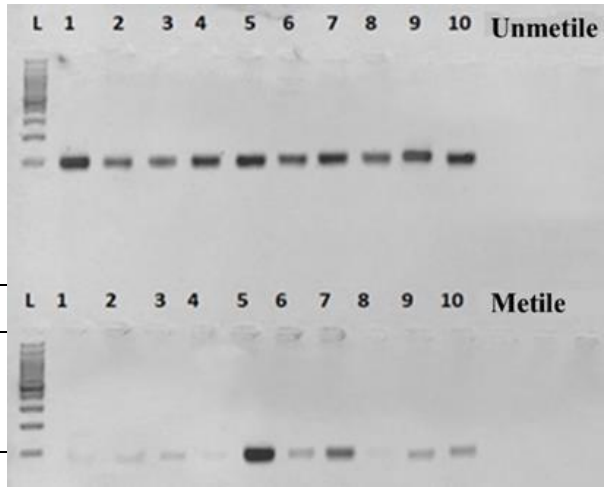

Figure 1: E-cadherin gene agarose gel image after MSP analysis. 2% agarose gel image after MSP analysis of the E-cadherin gene promoter region. L: ladder (100bp), wells 1-5: conjunctival tissue, wells 6-10: pterygium tissue.

When the methylation percentages of E-cadherin gene promoter region were compared with pterygium and control conjunctival tissues, 72.09% of the patients with pterygium have methylation, 27.9% of the patients with pterygium have no methylation. In conjunctival tissue, methylation was not observed in 95.14%, while methylation was observed in 4.85% (p=0.024). According to MSP analysis data, methylation percentage values between pterygium and conjunctival tissues were found to be statistically significant. When both pterygium and conjunctival tissues were compared within themselves, the methylation percentages for both groups were found to

be significantly lower. The percentage of methylation in tissues with pterygium was higher than in conjunctival tissue. Based on this, we see that the rate of methylation increases in tissues with pterygium compared to the conjunctival tissue (Table 3).

Table 3: Methylation percentages and statistical analysis of the E-cadherin gene promoter region.

Tissue	Methylation percentage (%)	
	UM	M
Pterygium	26 (72,09%)	10 (27,9%)
Conjunctiva	34 (95,14%)	2 (4,85%)
P	***0,024***	

The numbers and percentages of methylation degrees are given. Statistically significant data are expressed in bold. M: methylated UM: unmethylated.

4. Discussion

Molecular changes in pterygium are similar to molecular changes occurring in tumor cells. There are even opinions arguing that the mechanism of pterygium and oncogenesis is very similar. Methylation, a well-known epigenetic change, acts as a very important alternative to genetic differences in terms of gene inactivation. Abnormal methylations occurring in promoter regions of tumor suppressor genes and silencing of related genes play crucial roles in elucidating the pathology of most human cancers (11). Hypermethylation of CpG islands in the promoter region of the E-cadherin gene (CDH1) is considered to be one of the factors contributing to the inactivation of E-cadherin (12). E-cadherin promoter hypermethylation is involved in many different types of cancer events (14). In a study conducted by Young et al. In patients with pterygium, it was found that hypermethylation of the promoter region of the E-cadherin gene contributed to the decrease in E-cadherin protein expression (11). This situation causes abnormal promoter hypermethylation of tumor suppressor genes such as E-cadherin to turn the related gene into inactive form, causing the gene to deteriorate its function and thus progress in the process leading to cancer. Altered expression of E-cadherin can be cause loss of contact inhibition and abnormal cell proliferation. In this study, we evaluated the E-cadherin gene promoter region methylation in terms of pterygium, a common eye disorder that behaves similar to tumorigenesis. According to the data obtained, methylation percentage values between pterygium and conjunctival tissues were found to be statistically significant ($p = 0.024$). In other words, E-cadherin gene promoter methylation was observed to increase significantly in tissues with pterygium compared to the control group. Consistent with other cancer and its derivative studies, this increase in the rate of methylation in tissues with pterygium suggests a relationship between the disease and the gene E-cadherin. Because the E-cadherin gene is irregular promoter hypermethylations impair the expression of the gene. Abnormal expression of such a key tumor suppressor gene can lead to disruption of many important biological processes, including cell-cell connections, epithelial-mesenchymal transition (EMT), and cytoskeleton, resulting in cancer.

5. Conclusions

Hypermethylation of the promoter region of E-cadherin, a tumor suppressor gene, has been associated with pterygium disease exhibiting behaviors similar to cancer formation. In this sense, we believe that our study results shed some light on the molecular-based uncertainty of pterygium, whose pathology is still controversial.

Conflict of Interests

The authors declare no conflict of interest.

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Author Contributions

Ensari E, developed the concept and designed the manuscript: 70%; Aateş Ö, provided key information and help revise the manuscript: 30%.

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Blunt Abdominal Trauma Analysis in the Deaths due to Traffic Accidents in Erzurum, Turkey

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Abstract: Traffic accidents are one of the most common causes of death in many countries. Death-causing lesions mostly occur in the head-neck, chest, and abdominal region, respectively. Traffic accidents are an important cause of blunt abdominal trauma. Data on abdominal trauma in fatal traffic accidents are limited in autopsy series. In this study, we aimed to determine the frequency of abdominal trauma, the severity of trauma and its effects on the cause of death in traffic accident deaths. A retrospective analysis of autopsy cases of the Morgue Department of the Council of Forensic Medicine, Erzurum Branch between January 2015-June 2019 was conducted. The sample size was determined using the Epi-Info version 7 statistics program. The mean age of the cases was 41.1 years (sd: 23.4; min: 1-max: 96); 76.1% were male. Pre-hospital death occurred in 53.4%, while in the remaining 46.6%, death occurred during hospitalisation. 31.1% of the cases had abdominal trauma. Most commonly injured solid organ was liver in 57.3% of the abdominal trauma cases. Abdominal traumas and liver injuries were more common in pre-hospital deaths, especially in the accident scene deaths ($p<0.001$). Besides, type A liver injury was more common in pre-hospital deaths ($p=0.002$). Liver trauma is one of the leading causes of pre-hospital deaths due to traffic accidents. Type A liver traumas were more lethal.

Keywords: Traffic Accident, Blunt Abdominal Trauma, Pre-Hospital Death, Liver Injury.

1. Introduction

Trauma is one of the leading cause of deaths in Turkey and many other countries. (1, 2) Traumatic deaths mainly occur due to road traffic accidents (3, 4). More than 90% of transportation activities are facilitated by the highways and Turkey is among the top ten countries that almost half of the global traffic accident deaths occured (4).

In blunt traumas, head-neck, chest and abdominal injuries most often occur, respectively. Solid organs such as spleen, liver and kidney are frequently injured in blunt abdominal traumas (5). Approximately 70% of blunt liver injuries are caused by traffic accidents and death occur in 54% blunt liver injuries (6). Data on abdominal traumas in fatal traffic accidents are limited in autopsy series. In this study, we aimed to determine abdominal traumas, abdominal solid organ traumas and the effect of solid organ traumas in the cause of death occurred in fatal traffic accidents.

2. Material and Methods

2.1. Sample Size

The sample size was calculated as 309 cases using the

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Epi-Info version 7 program with a confidence interval of 99.99% and the expected prevalence of 5.4% according to TURKSTAT 2018 traffic accident data (7).

2.2. Selection and Evaluation of the Cases

The autopsy records of the Council of Forensic Medicine, Erzurum Branch between January 2015-June 2019 were examined retrospectively. A total of 309 cases who died due to traffic accident were examined in the study. Demographic data of the cases such as age, sex and traumatic lesions causing death were examined. The severity of liver, spleen, kidney injuries was evaluated according to the 'Organ Injury Severity Scale' of American Association of Surgery for Trauma (AAST) (8). Grade I and Grade II injuries of the liver were considered minor, Grade III and higher grade injuries were considered as major injuries (9). Also, injuries on the left side of the falciform ligament of the liver (segments I, II, III, and IV) were classified as Type A liver trauma, and injuries on the right side of the falciform ligament (segments V, VIII) were classified as Type B liver trauma (10).

The deaths were divided into two groups as pre-hospital deaths and deaths during hospitalisation.

2.3. Statistical Analysis

SPSS Windows 20.0 Software program was used for statistical analysis. The normality test of the data was evaluated by values of skewness and kurtosis. The z-scores were obtained by dividing the skew values or excess kurtosis by their standard errors (11). Comparisons between the groups were analysed with *the chi-square test*, and paired comparisons were analysed with *the student's t-test*. A value of $p<0.05$ was considered statistically significant.

3. Results

76.1% (n=235) of the cases were male, 23.9% (n=74) were female and the mean age was 41.1 (sd: 23.4; min: 1-max: 96). 75.4% (n=233) of the cases were injured in in-vehicle traffic accidents, while 24.6% (n=76) were pedestrian traffic accident deaths. 53.4% (n=165) of the cases were pre-hospital deaths, while 46.6% (n=144) died during hospitalisation. 78.6% (n=243) of the cases were <65 years of age and 21.4% (n=66) were ≥65 years of age. Mean age of pre-hospital death group was 39.1 (±22.8) and deaths in hospital group was 43.5 (±23.9). The mean hospitalization time was 14.3 days (sd: 24.9; min: 1-max: 150) in the hospital deaths. There was no significant difference between the case numbers of age groups of pedestrian traffic accidents ($p>0.05$); however, in-vehicle accident cases were mostly under 65 years of age ($p=0.036$), however there was no such significant difference among the age groups of pedestrian traffic accident deaths.

In 38.2% (n=118) of the cases, the lesions causing death were isolatedly located in one anatomical region, while they were multiply located in more than one anatomical region in other cases. The distribution of death-causing lesions according to anatomical regions is shown in Table 1.

Table 1: Distribution of traumatic lesions according to anatomical regions.

	Isolated* % (n)	Multiple** % (n)	Total % (n)
Head and Neck	17.1 (53)	50.2 (155)	67.3 (208)
Face	0.3 (1)	9.4 (29)	9.7 (30)
Thorax	15.5 (48)	53.7 (166)	69.2 (214)
Abdomen	3.2 (10)	27.8 (86)	31.1 (96)
Upper Extremity	0.6 (2)	9.7 (30)	10.3 (32)
Lower Extremity and Pelvis	1.3 (4)	20.1 (62)	21.4 (66)

*: Cases with death-causing lesions in one anatomical region,
**: Cases with death-causing lesions in more than one anatomical region.

Trauma was detected in the abdominal region in 31.1% (n=96) of the cases. Liver damage was detected in 57.3% (n=55), spleen damage was detected in 36.4% (n=35) and kidney damage was detected in 25% (n=24) of the abdominal traumas. Abdominal solid organ injuries according to the 'Organ Injury Severity Scale' of AAST are shown in Table 2.

Table 2: Abdominal solid organ injuries according to ASST 'Organ Injury Severity Scale'.

Grade	Liver % (n)	Spleen % (n)	Kidney % (n)
Grade 1	7.2 (4)	11.4 (4)	33.3 (8)
Grade 2	11.0 (6)	14.2 (5)	25 (6)
Grade 3	18.2 (10)	20 (7)	16.6 (4)
Grade 4	21.8 (12)	25.8 (9)	16.6 (4)
Grade 5	18.2 (10)	28.6 (10)	8.5 (2)
Grade 6	23.6 (13)	-	-

89.1% (n=49) of the liver traumas occurred in in-vehicle traffic accidents while 10.9% (n=6) occurred in pedestrian traffic accidents and liver traumas occurred mostly in the in-vehicle traffic accidents ($p=0.009$).

When the places of death were compared, pre-hospital death cases were found to be higher in cases under 65 years of age ($p=0.004$) and in cases with liver damage ($p<0.001$). 23.6% (n=13) of the cases with liver trauma had only Type A injury, 56.4% (n=31) had only Type B injury, and 20% (n=11) had both Type A and Type B injuries. Pre-hospital deaths were more frequently observed in the cases with Type A liver injury ($p=0.002$). The traumatic characteristics of the cases according to the place of death are shown in Table 3.

4. Discussion

In our study, we examined death-causing traumatic lesions in fatal traffic accidents. According to the WHO Global Status Report on Road Safety, road traffic injury was the leading cause of death for people aged between 5 and 29 years (12). In our study, traffic accident deaths mostly affected the young male population similar to literature. According to Lee et al., head-neck region is the most frequently injured in blunt traumas and abdominal traumas rank third after thoracic injuries (13). Abdominal trauma ranked also third in terms of frequency after head-neck and thoracic traumas. Spleen is the most frequently injured solid organ in blunt abdominal traumas in many clinical studies (3, 14-20). According to the findings of our study, liver, spleen and kidney are the most frequently damaged solid organs in abdominal traumas, respectively. Other organs may have less damage due to anatomical localisation and organ elasticity such as small-large intestines and bladder.

Table 3: Comparison of the pre-hospital and hospital deaths.

		Pre-hospital death, (n=165) n (%)	Deaths in the hospital, (n=144) n (%)	*p* value
Age	65>	140 (57.6)	103 (42.4)	**0.004**
	65≤	25 (37.9)	41 (62.1)	
	Mean (sd)	39.1 (±22.8)	43.5 (±23.9)	0.096
Gender	Male	127 (54)	108 (46)	0.686
	Female	38 (51.4)	36 (48.6)	
Accident Type	In-vehicle accident	131 (56.2)	102 (43.8)	0.081
	Pedestiran	34 (44.7)	42 (55.3)	
Head-Neck Trauma	Yes	111 (53.4)	97 (46.6)	0.987
	No	54 (53.5)	47 (46.5)	
Maxillofacial Trauma	Yes	17 (56.7)	13 (43.3)	0.706
	No	148 (53.0)	131 (47.0)	
Upper Extremity Trauma	Yes	17 (53.1)	15 (46.9)	0.974
	No	148 (53.4)	129 (46.6)	
Thorax Trauma	Yes	115 (53.7)	99 (46.3)	0.857
	No	50 (52.6)	45 (47.4)	
Abdominal Trauma	Yes	57 (59.4)	39 (40.6)	0.157
	No	108 (50.7)	105 (49.3)	
Liver Trauma	Yes	41 (74.5)	14 (25.5)	**<0.001**
	No	124 (48.8)	130 (51.2)	
Liver Trauma Type	Minor trauma	4 (80)	1 (20)	0.769
	Major trauma	37 (74)	13 (26)	
Liver Trauma Type	Only type A	12 (92.3)	1 (7.3)	**0.002**
	Only type B	20 (64.5)	11 (35.5)	
Spleen Trauma	Yes	20 (57.1)	15 (42.9)	0.637
	No	145 (52.9)	129 (47.1)	
Kidney Trauma	Yes	11 (45.8)	13 (54.2)	0.439
	No	154 (54.0)	131 (46.0)	
Lower Extremity and Pelvis	Yes	30 (45.5)	36 (54.5)	0.145
	No	135 (55.6)	108 (44.4)	

We also detected major injuries more frequently in the liver and spleen, and minor injuries in the kidneys. Although ribs protect the liver, its large size cause the liver to be injured more frequently in blunt traumas (3, 21). In our study, one of the most important reasons why we identified the liver as the most frequently injured solid abdominal organ might be that all of the cases were selected from blunt traumas resulted in death. Most of the studies indicating the spleen damage more frequent were carried out on blunt traumas which did not result in death. Therefore, it can be said that the liver was the most frequently injured solid organ in blunt abdominal traumas resulting in death. According to our findings, the third most frequently injured solid organ in blunt abdominal traumas was kidney. Retroperitoneal localisation of the kidney leads to less injury in blunt abdominal traumas (3, 21).

There is a wide range of pre-hospital death ratios in traffic accidents, from 7% to 45% in the literature and these deaths occurred mostly in rural areas and at the accident scene (22-24). In our study, pre-hospital deaths were observed in 53.4% of the cases and these deaths occurred mostly in the under 65-year-old age group.

Gulliver et al. suggested young people were more likely to be involved in traffic accidents compared to adults [25]. More severe traumas occured due to more aggressive driving behaviours of young males [26]. In our study, in-vehicle traffic accident deaths and pre-hospital deaths were more frequent in the cases younger than 65 years of age.

Liver is the largest solid organ in the abdomen. The thin capsule, low elasticity and fixed position between the vertebrae and ribs makes the liver prone to injury in blunt traumas (3, 21). The centre and usually the right lobe of the liver are damaged as it holds a larger surface in a direct impact to the abdomen by a blunt trauma. The steering wheel may compress the drivers who are not wearing seat belts during the deceleration period when the speed of the vehicle decreases suddenly, and the risk of liver injury may increase significantly in traffic accidents (27). In our study, liver trauma was mostly seen in in-vehicle traffic accidents. Trauma was detected in the right lobe of the liver in the majority of the cases. However, Type A liver injury (left lobe injury) was more common in pre-hospital deaths compared to hospital deaths.

Anatomically, the area on the left side of the falciform ligament is smaller than the area on the right side of the falciform ligament. The anatomical formation of the falciform ligament can control bleeding on the right side of the liver. However, it can not control such bleeding on the left side of the liver. (19). One of the most important causes of the high mortality in liver trauma is uncontrolled bleeding that can cause severe hemorrhagic shock (6, 10). The reason why Type B liver injury (right lobe injury) was more frequently observed may be the anatomically larger right side of the liver. However, higher mortality of Type A liver injury may be due to the lack of the ability of the falciform ligament to control bleedings on the left side of the liver (6, 19). This also explains why Type A liver injury was more common in pre-hospital deaths.

5. Conclusions

The liver is the most frequently injured solid organ in fatal traffic accidents with abdominal trauma. Although Type B liver trauma was more common, Type A liver trauma was more lethal and one of the major causes of pre-hospital deaths due to traffic accidents.

Conflict of Interests

The authors declare that they have no conflict of interest.

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Author Contributions

Şener MT; Conceptualization, investigation, methodology, writing-original draft, writing-review and editing. Vural T: Conceptualization, methodology, formal analysis, investigation, writing-review and editing. Sezer Y: Investigation, writing-original draft, Writing-review and editing. Kok AN: supervision, writing-review and editing.

Ethical Approval

This study was conducted in accordance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. Ethics committee approval was obtained for this study (Ethics No: 07.11.2019/01).

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Evaluation of *Staphylococcus Aureus* Presence and Meticillin Resistance in Nasal Swab Samples in Erzincan Mengucek Gazi Training and Research Hospital

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Abstract: To investigate the *Staphylococcus aureus* strains and methicillin resistance rates retrospectively isolated from nose swab samples sent from various clinics to our laboratory during the study period. 3002 nose swab samples were sent to Erzincan Mengücek Gazi Training and Research Hospital Microbiology Laboratory between January 2015 and January 2020. Samples were inoculated in 5% sheep blood and chromogenic MRSA medium. The media were incubated at 35 ºC for 24 hours. Colonies compatible with *S. aureus* in 5% sheep blood medium and forming pink color in MRSA medium were evaluated. Methicillin susceptibilities of isolates identified as *S. aureus* by conventional methods and subsequently by MALDI-TOF MS system were determined by Kirby-Bauer disc diffusion method in Mueller Hinton agar. Sensitivity results were interpreted according to EUCAST guidelines. *S. aureus* was isolated in 536 (17.8%) of the samples. 491 (91.6%) of the patients with reproduction were male and 45 (8.4%) were female. 504 (94%) of the 536 isolated *S. aureus* strains were found to be methicillin-sensitive *S.aureus* (MSSA) and 32 (6%) were methicillin-resistant *S.aureus* (MRSA). 31 of the MRSA isolated patients were male and the mean age was 26.3. The lowest number of MRSA cases was determined in 2015 and the highest in 2019. All MRSA isolates were isolated from outpatients. In our study, the nasal S. aureus carrier rate was 17.8%, and nasal MRSA carrier rate was 6%. Investigation of the nasal presence of S. aureus, an important pathogen for hospital and community-acquired infections, and determination of antibiotic sensitivity will be effective in preventing these infections. Especially for the prevention and control of MRSA transmission; It is necessary to reduce uncontrolled and excessive use of antibiotics, to comply with hand hygiene rules, to screen the carriers periodically and to pay attention to contact measures.

Keywords: *Staphylococcus Aureus*, Methicillin Resistance, Nasal Swab, Infection Control.

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1. Introduction

Staphylococcus aureus species, especially those which are resistant to methicillin (MRSA), have an important place among factors that cause both hospital-originated infections and society-acquired infections. *S. aureus* carriage that is usually in the form of nasal carriage plays a role in the epidemiology of staphylococcus infections. Nasal *S.aureus* colonization leads to certain problems such as high rates of resistance, predisposition to infections and high treatment costs (1, 2).

It was found that MRSAs are responsible for 30-38% of hospital-originated infections in Turkey (3). *S. aureus*, which can be an element of normal flora on the skin and mucosal surfaces, is consistently found in the nasal flora in 15% of healthy adults. However, 60% of the population carries *S. aureus* in their nasal flora at certain periods. The rate of infections caused by these factors is higher in healthcare professionals, hospitalized patients, patients with diabetes mellitus, patients with chronic renal failure, hemodialysis patients, those with eczema and drug addicts, compared to the normal population (4-6).

This study aimed to determine the presence of *Staphylococcus aureus* in the nasal swab samples sent by various clinics to our laboratories during the 5-year period, and to investigate the methicillin resistance rates of the strains retrospectively.

2. Material and Methods

The study was started after the approval was taken from the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (access number: E.25464-06/07) and included 3002 nasal swab samples which were sent to the Microbiology Laboratory of Erzincan Mengücek Gazi Training and Research Hospital between January 2015 and January 2020. The samples, which were assumed to have been properly taken from patients who applied to various clinics and had different pre-diagnoses, were inoculated in chromogenic MRSA Medium (bioMerieux ®, France) with 5% sheep blood. The mediums were incubated at 35 ºC for 24 hours, and then colonies which were mostly beta-hemolytic and golden-yellow and other suspicious colonies were evaluated. The colonies which had gram-positive coke morphology and positive catalase and coagulant test results and produced pink color in the MRSA Medium were defined as *S. aureus* by MALDI-TOF MS (Vitek MS system, bioMerieux, France) system. The methicillin susceptibilities of the isolates were determined using Mueller Hinton agar (bioMerieux ®, France) and 30 µg cefoxitin disk (bioMerieux ®, France) based on the Kirby-Bauer disk diffusion method. The susceptibility results were interpreted as susceptible/resistant per the EUCAST guidelines (7). *S. aureus* ATCC 25923 was used as a standard strain for quality control.

2.1. Statistical Analysis

Statistical analysis was performed using the Statistical package for Social ScienceS (SPSS) software package version 21.0 (IBM Corp. Armonk, NY, USA). Descriptive statistics as number and percentage parameters were calculated.

3. Results

During the study period, 3002 nasal swab samples were sent by various clinics to our laboratory. The samples which belonged to the same patient and had the identical susceptibility result were considered as a single sample. *S. aureus* was isolated in 536 (17.8%) of these samples. Of the patients with a positive culture, 491 (91.6%) were male and 45 (8.4%) were female. It was found that of the 536 isolated *S. aureus* strains, 504 (94%) were cefoxitin-susceptible (MSSA) and 32 (6%) were cefoxitin-resistant (MRSA). 31 of the MRSA isolated patients were male and the average age was 26.3 years. One of the MRSA isolates was isolated from a 61-years old female patient. The distribution of the patients with positive *S. aureus* culture by age, sex and methicillin susceptibility result was given in Table1.

Table 1: Distribution of patients with positive *S. aureus* culture by sex and methicillin susceptibility.

	n (average age)		Total
	MRSA	**MSSA**	
Men	31 (26.3)	460 (29.3)	491 (29.1)
Women	1 (61)	44 (39.5)	45 (40)
Total	**32 (27.4)**	**504 (30.2)**	**536 (30)**

MRSA: Methicillin-Resistant *S.aureus*, MSSA: Methicillin Susceptible *S.aureus*

When the distribution of MRSA strains by years was examined, it was found that the number of cases was directly proportional to time, the lowest number of cases was in 2015, while the highest number of cases was in 2019. The distribution of MRSA isolates by years is given in Figure 1.

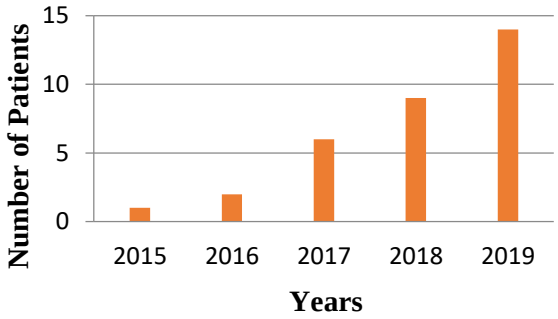

Figure 1. Distribution of MRSA strains by years.

Of 3002 samples included in the study, 14 (0.5%) were taken from hospitalized patients and *S.aureus* did not grow in any of these samples. Of MRSA isolates which were all isolated from patients applied to infectious diseases (n=29), nephrology (n=2) and family medicine (n=1) departments, 28 (87.4%) were detected in samples sent for general medical examination, 2 (6.3 %) in samples sent due to a bacterial infection such as abscess, furuncle, carbuncle, and 2 (6.3%) in samples sent with a diagnosis of chronic renal failure (CRF). The diagnoses of the majority of MRSA-colonized patients were general medical examination; however, when the results were examined in terms of proportion, it was found that the patient group where the probability of MRSA isolation was the highest was the CRF patients. The relationship between diagnosis and methicillin resistance rates is given in Table 2.

4. Discussion

S. aureus is among the leading causes of nosocomial and society-originated bacterial infections around the world. his pathogen is commensally present in approximately 10-40 % of healthy adults (8). MRSA is an important pathogen associated with both hospital- and society-originated infections. Determination of MRSA isolates in a timely and accurate manner is important for controlling the infection and preventing the nosocomial spread of bacteria. The increasing incidence of MRSA-related infections makes the treatment of such infections further difficult (2, 9, 10). MRSA isolates are highly resistant to many common antibiotics such as aminoglycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones (11). In our country, many studies to investigate the nasal carriage of *S. aureus* have been conducted, and it was found that there were differences in carriage and methicillin resistance rates (12).

A study performed in Denizli with 466 adult patients reported that *S. aureus* was isolated in 204 (43.8%) of the patients and 34 (16.7%) of them were resistant to methicillin. The rate of MRSA-colonized patients was found to be 7.3% (34/466) (13).

Table 2: Methicillin resistance rates of *S.aureus* strains by diagnoses.

Diagnosis	**n (%)**		
	MRSA	**MSSA**	**Total**
General Medical Examination	28 (5.5)	479 (94.5)	507 (100)
Bacterial Infection	2 (8.3)	22 (91.7)	24 (100)
CRF	2 (40)	3 (60)	5 (100)
Total	**32 (6)**	**504 (94)**	**536 (100)**

In another study conducted in İzmir Tepecik Training and Research Hospital, 1373 nasal swab samples from various polyclinics and wards were analyzed and *S. aureus* was detected in 112 (8.2%) samples, and 11 (9.8%) of them were found to be methicillin-resistant. 6 of the MRSA isolates were isolated from hospitalized patients, and 5 of them were isolated from outpatients (3).

In a study conducted in Antalya, a total of 15,600 nasal swab samples from individuals who were working in different food sectors and administered for a health check to the Antalya Hygiene Institute (ANHEM) between September 2009 and April 2010 were investigated. *S. aureus* was isolated in 526 (3.37%) of the samples and 28 (5.3%) of them were defined as MRSA (14).

In another study in Ankara, MSSA was isolated in 601 (16.6%) of 3,599 nasal cultures and MRSA was isolated in three (0.08%) (15). In a study conducted with the Kayseri Maternity Hospital staff, the nasal swab samples from 203 individuals were scanned. *S. aureus* was detected in 43 (21.2%) of the nasal cultures, and MSSA and MRSA were detected in 41 (95.3%) and 2 (4.7%) of them, respectively (12).

In a study conducted in Ethiopia, S. aureus was isolated in 52 (13%) of 400 nasal swab samples from pre-school children, and MSSA was detected in all the isolates (16). 299 patients were included in a study conducted in Germany. The MRSA rate was found to be 2.1% (17).

In our study, 3002 nasal swab samples from various polyclinics and wards to our laboratory were scanned, and *S. aureus* was isolated in 536 (17.8%) of the samples. It was found that of the 536 isolated *S. aureus* strains, 504 (94%) were MSSA and 32 (6%) were MRSA. This rate was similar to the local studies but found to be high compared to the foreign studies.

In a study with hemodialysis patients in Kırıkkale province, *S. aureus* was isolated in the nasal culture of 18 (15.2%) of 118 patients, and 13 (11%) of them was found to be MRSA (18). Karapınar et al. isolated MRSA from the lesion of one patient and from the nasal culture of one patient among 38 patients with pyodermia, and stated that the rate of MRSA incidence was lower in the patients with pyodermia (19). In our study. MRSA was isolated in 2 of 5 samples taken from hemodialysis patients and 2 of 24 samples from patients with the diagnosis of bacterial infection. The diagnoses of the majority of MRSA-colonised patients were

general medical examination; however, when the results were examined in terms of proportion, it was found that the patient group where the probability of MRSA isolation was the highest was the CRF patients. Many well-known risk factors such as advanced age, male sex, alcohol use, chronic lung diseases, cancer, diabetes, chronic kidney failure were defined for society-originated staphylococcus infection and nasal carriage. Considering the region where our hospital is located, it was thought that social living conditions and poor hygiene conditions have played a role in the high MRSA rates.

5. Conclusions

Consequently, it is important to determine the presence of *S.aureus* and the antibiotic susceptibility of strains as well as to determine the methicillin resistance in isolates and to raise awareness. Nasal staphylococcus carriage is a serious hazard not only for carriers but also for community health. To prevent and control the MRSA infection, the uncontrolled and unnecessary use of antibiotics should be reduced, the hand hygiene rules should be followed, the carriers should be scanned periodically and the contact measures should be followed.

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Thymoquinone Ameliorates Indomethacin-Induced Gastric Ulcers in Rats: A Dose Response Study

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Abstract: A peptic ulcer is painful sores in the lining of the stomach. Acute and chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) is considered a significant cause of peptic ulcers. Different doses (20-60 mg/kg body weight) of thymoquinone (TQ), the main constituent of *Nigella sativa* L. essential oil, have been shown to protect gastric tissue from NSAID-induced peptic ulcers. Researchers attributed the protective effect of TQ to its antioxidant properties. This study aimed to determine effective antiulcer dose range of TQ. In this study, we conducted a series of experiments to determine the optimal dosage of TQ in indomethacin-induced gastric ulcers in rats. Additionally, we investigated the effect of TQ on superoxide dismutase (SOD) activity, glutathione (GSH) levels, and malondialdehyde (MDA) levels. Our results showed that, when administered at 40 and 20 mg/kg body weight, TQ was ineffective in preventing indomethacin-induced gastric ulcers. Moreover, TQ itself induced gastric ulcers at 40 mg/kg dose. As the doses of TQ decreased, the protective effect increased. 0.5 mg/kg TQ provided the best protection in terms of gastric ulcer area and antioxidant parameters, having statistically the same result with famotidine. Low dose TQ is an efficient protector of indomethacin-induced gastric damage, and it significantly enhances antioxidant parameters of gastric tissue. High dose TQ administration did not produce any desirable effects, but increased ulcer index.
Keywords: Thymoquinone, Rats, Indomethacin, SOD, GSH, MDA.

1. Introduction

Peptic ulcer disease shows a great deal of variation among societies and regions (1). In the past, investigators estimated the lifetime prevalence of peptic ulcer disease to be about 5-10% in the general population, with an incidence of 0.1-0.3% per year. Although researchers noted a worldwide decline in uncomplicated peptic ulcer disease, it remains a common and costly condition (2, 3).

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Helicobacter pylori are the principal cause of peptic ulcers. A potentiating factor for gastric ulcers, mucosal ischemia, is induced by free radical generation, which is the result of lifestyle factors, such as diet, smoking, alcohol, stress, and consumption of fatty foods. Excess secretion of hydrochloric acid, which decreases local blood flow and mucus, is also implied in the etiology of peptic ulcers. The constant use of drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), anticoagulants, corticosteroids, and chemotherapeutic agents are also related to the formation of peptic ulcers (4).

NSAIDs induce gastric damage through inhibition of COX-1, which leads to a reduction of prostaglandin secretion in the gastrointestinal tract and its cytoprotective effects in gastric mucosa. COX-2 inhibition may also play a role in the mucosal injury (5). Increase in mucosal permeability, neutrophil infiltration, production of oxyradicals, and finally, lesions in the gastric mucosa follow the mentioned inhibition (6).

Throughout history, people have been trying various plants for the treatment of ulcers (4, 7, 8). One of the most frequently used plants for the treatment of ulcers is Nigella sativa L., which has been utilized for many diseases (9). Nigella sativa L. is a member of the Ranunculaceae family and is cultured in tropical and subtropical areas. Its seeds are known as black seeds, and the essential oil these seeds possess is considered to be the pharmacologically active part. The most abundant compound present in the essential oil is thymoquinone (TQ), with reported proportions ranging between 18 and 57% (10).

Numerous studies were conducted regarding the gastroprotective effects of Nigella sativa L. essential oil and TQ in different animal models (11-13). Researchers used different doses of TQ (up to 60 mg/kg body weight), and several vehicles (corn oil, water, Carboxymethyl cellulose). Earlier studies (14, 15) reported that TQ exerts its therapeutic actions by scavenging free radicals, however, recent reports suggest that TQ is anti-oxidant in low doses and pro-oxidant at high doses (16, 17).

Our aims in this study were to evaluate the effects of TQ in indomethacin (IND)-induced gastric ulcer in rats, to determine the effective dosage of TQ, and to assess antioxidant parameters, namely superoxide dismutase (SOD) activity, glutathione (GSH) amount and malondialdehyde (MDA) levels in ulcerous tissues.

2. Material and Methods

2.1. Animals

In this study, we used 84 male albino *Wistar* rats obtained from Ataturk University Medical Experimental Research Center (ATADEM). The animals weighed between 180 and 220 g. They were fed standard rat chow under normal temperature conditions (22±2 °C) and kept in separate groups before the experiment. Animal care and experiment protocols were approved by the Experimental Animal Ethics Committee, Ataturk University, Erzurum, Turkey (27.11.2014/1307).

2.2. Chemicals

Indomethacin was purchased from Deva Holding A.S. (Istanbul, Turkey) and famotidine from Sandoz a Novartis Drug Company (Turkey). We obtained thiopental sodium from IE Ulagay A.S. (Istanbul, Turkey) and TQ ($C_{10}H_{12}O_2$, 2-Isopropyl-5-methylbenzo-1,4-quinone, purity≥98%) from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Study Design

We performed three experiments to assess the influence of TQ in IND-induced gastric ulcer in rats (18, 19).

In the first experiment, 24 rats were randomly assigned to 4 groups (n=6) and fasted for 24 hours. The first group received water, the second group 40 mg/kg TQ, the third group 20 mg/kg TQ, and the fourth group 40 mg/kg TQ. After five minutes, the first, third and fourth groups received 25 mg/kg IND. TQ was suspended in water since it did not dissolve. Six hours later, the rats were sacrificed using high dose anesthesia. Their stomachs were removed and evaluated for damaged areas. This experiment was designated EXP1.

Results of EXP1 led us to perform a second experiment. Twenty-four rats were randomly assigned to 4 groups (n=6). The first group received water, the second 10 mg/kg TQ, the third 5 mg/kg TQ, and the fourth 3 mg/kg TQ. Five minutes later, all groups received 25 mg/kg IND. Six hours later, the rats were sacrificed with a high dose of thiopental sodium. The rats' stomachs were removed and evaluated for damaged areas. This experiment was designated EXP2.

After observing a substantial decrease in ulcer areas in EXP2, a third experiment was performed using 36 rats. The animals were fasted for 24 hours and randomly assigned to 6 groups (n=6). The first and second groups received water. The third group received 40 mg/kg famotidine (FAM), the fourth group 2 mg/kg TQ, the fifth group 1 mg/kg TQ, and the sixth group 0.5 mg/kg TQ. Five minutes later, all groups except the first received 25 mg/kg IND. Six hours later, rats were sacrificed with high dose anesthesia. Stomachs were removed and assessed for ulcer areas. This experiment was designated EXP3. The stomachs obtained in this experiment were used to assess SOD activity and GSH and MDA levels.

2.4. Macroscopic analyses of stomach tissues

The rat stomachs were opened along the greater curvature and washed with serum physiologic. The ulcer areas were determined using a magnifier and a millimeter paper. The sum of the ulcerous areas was expressed in square millimeters (mm^2) as the ulcer score. Antiulcer effects were calculated using the following formula:

$$\text{Antiulcer effect}=\%\ \text{protection}=\left(1-\frac{\text{ulcer score of the treatment group}}{\text{ulcer score of control group (indomethacin)}}\right)\times 100$$

After macroscopic analyses, all rat tissues from EXP3 were kept at -80 °C and further used in biochemical assays. All other tissues were discarded.

2.5. Biochemical investigation of stomach tissues

All stomach tissue samples were first perfused with PBS/heparin and then ground in liquid nitrogen using a tissue lyser II grinding jar set (Qiagen, Hilden, Germany). For each sample, 100 mg was weighed and then treated with 1 mL of PBS buffer. This mixture was homogenized on ice in an Eppendorf tube, using a tissue lyser II adapter sets 2x24 homogenizer. Homogenates were centrifuged at 4 °C using a refrigerator centrifuge. SOD (20), GSH (21) and MDA (22) levels from the samples' supernatants and standards were measured at room temperature, in duplicate, according to the modified methods of the ELISA reader as previously described. The average absorbance of each sample and standard were measured. A standard curve was plotted, and an equation obtained. This equation was employed to calculate SOD activity and GSH and MDA concentrations. The SOD activity was expressed as U/mg protein, while GSH and MDA levels as nmol/mg protein.

2.6. Protein determination

Protein concentrations were determined using a commercial total protein kit (Sigma Aldrich, St. Louis, MO), according to the Lowry method (Peterson's modification).

2.7. Statistical analyses

IBM SPSS program (version 19.00) was used to conduct statistic comparisons between groups. The results are presented as Mean±SD. Group comparisons were performed using one-way ANOVA and Duncan multiple comparison tests. $P<0.05$ was considered significant.

3. Results

3.1. Indomethacin-induced gastric ulcers

Results of EXP1 and EXP2 are summarized in figure 1 The indomethacin-induced gastric ulcer model is well established, as can be seen from the ulcer areas of IND groups in EXP1 and EXP2. The amount of ulcer area in the TQ groups decreased as the amount of TQ decreased.

Figure 2 presents the results of the EXP3. Famotidine (40 mg/kg) produced 96.15% protection from the ulcer, while 0.5 mg/kg TQ decreased the ulcer area by 85.48%. 1 and 2 mg/kg doses of TQ ameliorated the gastric damage by 77.35 and 47.17%, respectively.

Figure 3 presents the macroscopic evaluation of tissues. 40 mg/kg TQ induced hemorrhages in gastric tissue. IND induced gastric ulcer model was successfully established.

3.2. Biochemical investigation of stomach tissues

The antioxidant levels (SOD, GSH, and MDA) in the stomach tissues obtained from EXP3 were evaluated to investigate the influence of antioxidant defenses on the ulceration process. Figure 4 presents the results. These results show that indomethacin reduced the activity of the SOD enzyme, decreased the GSH concentration, and increased the MDA concentration. Famotidine and all doses of TQ significantly ($P<0.05$) improved the SOD activity, increased GSH levels, and decreased MDA levels when compared with the IND group. 0.5 mg/kg dose TQ performed best in all three parameter tests amongst TQ doses.

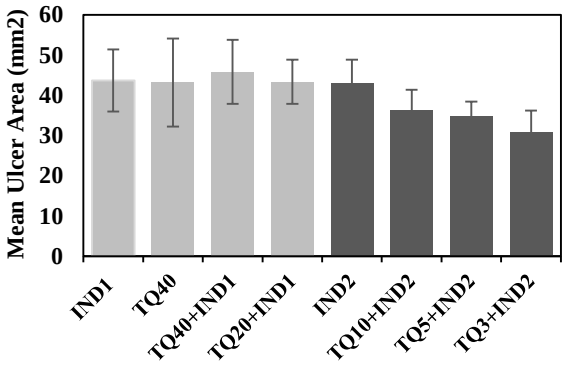

Figure 1: Results of EXP1 and EXP2. Light bars represent EXP1 and dark bars represent EXP2. IND1 and IND2: indomethacin (25 mg/kg), TQ40: thymoquinone (40 mg/kg). The numbers following TQ are the doses in mg/kg body weight. Error bars indicate±standard deviation.

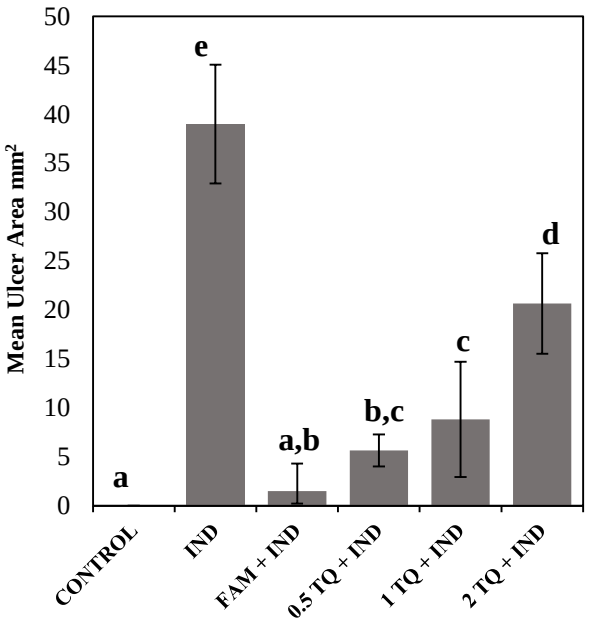

Figure 2: Results of EXP3. Effects of TQ and famotidine on indometacin induced gastric damage in rats. TQ, thymoquinone; FAM, famotidine; IND, indomethacin. Error bars indicate±Standard Deviation. Means in the same column with the same letter do not differ significantly (oneway ANOVA followed by Duncan test ($p<0.05$)).

4. Discussion

In the present study, the indomethacin-induced gastric ulcer model in rats was used for the first time to investigate the gastroprotective effect of TQ. Additionally, the antioxidant effect of TQ was examined in ulcerative gastric tissues.

Induction of gastric ulcers in rats by indomethacin is an established model of NSAID-induced ulcers in humans (23). NSAID usage deteriorates stomach mucosal integrity, which results in erosions, ulcers, hemorrhages, and perforations in gastric mucosa. This effect on gastric mucosa reduces the production of bicarbonate, mucus, and cytoprotective prostaglandins. It also causes the formation of free radicals (ROS) and lipid peroxidation (24). Though several factors are involved in gastric mucosal damage, oxygen-derived free radicals play a notable role in the pathogenesis of NSAID damage. ROS are continuously generated during typical physiologic incidents and removed by antioxidant defense mechanisms. The imbalance between ROS and antioxidant defense mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules (25).

We started by testing 20 and 40 mg/kg body weight doses of TQ in indomethacin-induced gastric ulcers. We were unable to dissolve TQ in water and had to prepare a homogenous suspension of it in water. This observation is in accordance with recent findings that aqueous solubility of thymoquinone is in the range of 549-669 μg/mL (26). Unexpectedly, pretreatment with TQ did not produce any improvement. Moreover, the application of 40 mg/kg TQ alone resulted in hemorrhage in rat stomachs (Figure 3, C and D), and ulcer area of the TQ40+IND1 group increased when compared with the IND1 group. 20 mg/kg TQ exhibited no improvement in terms of ulcer formation (Figure 1, light bars).

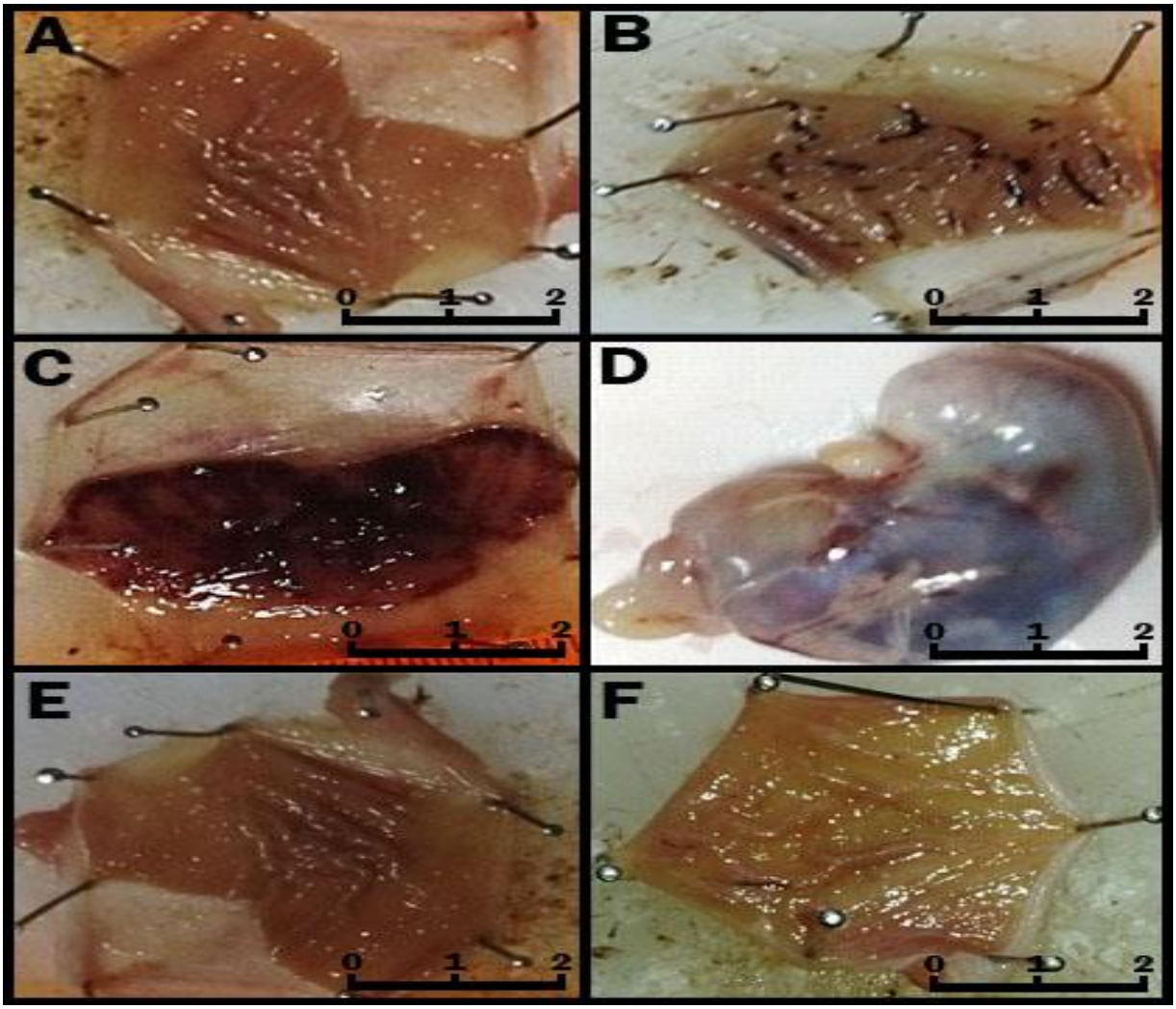

Figure 3: Ulcerous areas in the gastric tissues of indomethacin (IND)-induced ulcer model. Sections of the gastric tissues after IND administration were obtained from experimental groups. A: control group, B: IND, 25 mg/kg body wt., C: 40 mg/kg TQ alone, D: stomach before opening 40 mg/kg TQ, E: FAM 40 mg/kg, and F: 0.5 mg/kg TQ.

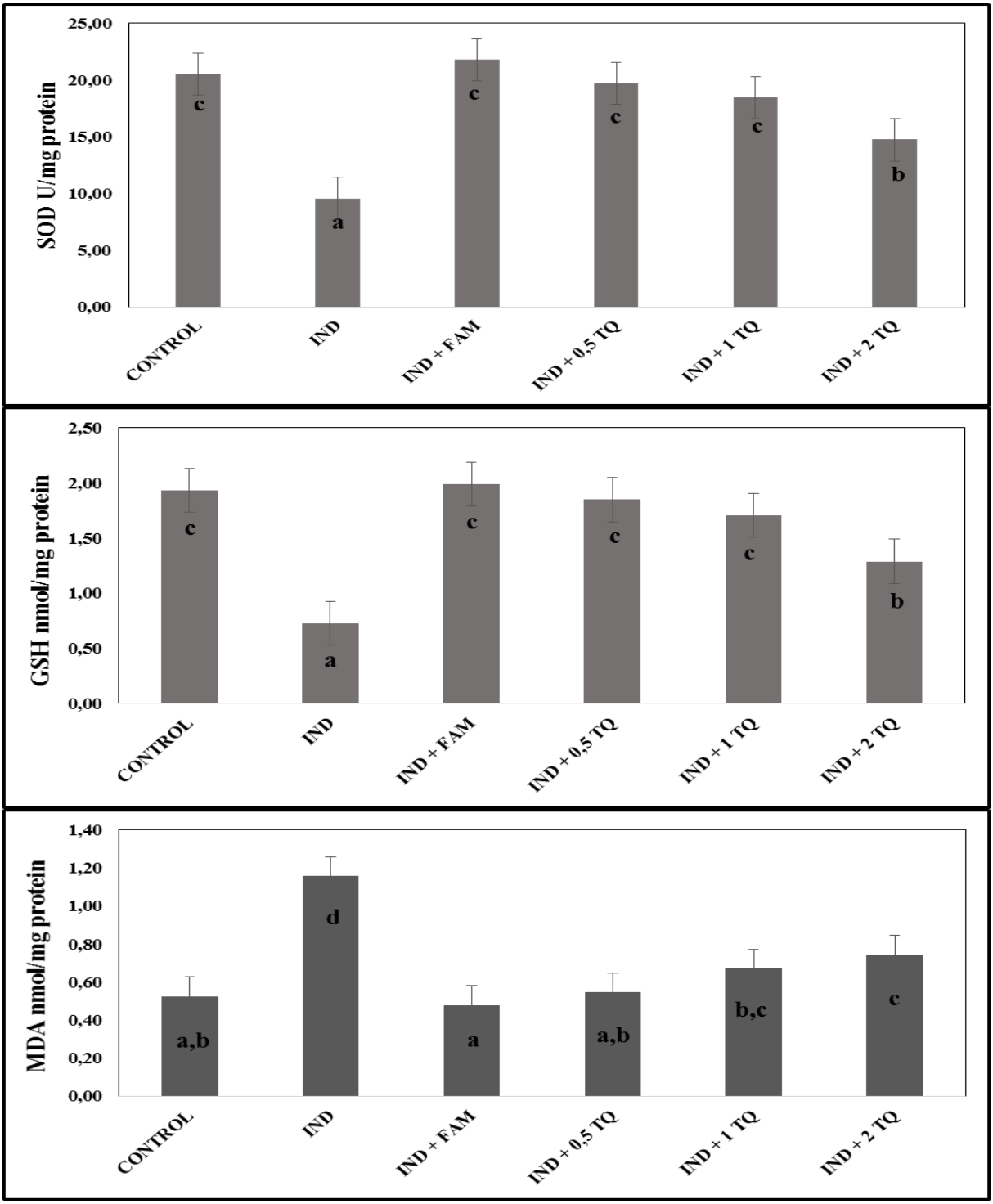

Figure 4: Effects of thymoquinone and famotidine pretreatment on changes in SOD activity GSH and MDA levels induced by indomethacin for EXP 3. TQ, thymoquinone; FAM, famotidine; SOD, superoxide dismutase; GSH, glutathione; MDA, lipid peroxidation. Error bars indicate±Standard Deviation Means in the same column with the same letter do not differ significantly (Oneway ANOVA followed by Duncan test ($p<0.05$).

We tested pretreatment with 10, 5, and 3 mg/kg TQ in EXP2. Although the mean ulcer area exhibited a steady decrease with the decreased amounts of TQ (Figure 1, dark bars), the final result was far from our expectations and reported results (13, 25). Therefore, we decided to use even lower doses (2, 1, and 0.5 mg/kg) of TQ in EXP3.

Results of EXP3 (Figure 2) reveals that TQ has a potent influence on the development of indomethacin-induced gastric ulcers in rats. 0.5 mg/kg TQ decreased mean ulcer area by 85.48% while 1 and 2 mg/kg TQ decreases mean ulcer area by 77.35 and 47.17%, respectively. 40 mg/kg famotidine, as expected, decreased ulcer area by 96.15%.

These data put forward a new argument that pretreatment with low-dose TQ inhibits indomethacin-induced gastric ulcers in rats while high doses are ineffective or toxic.

Previous studies claimed that much higher doses of TQ (up to 60 mg/kg) had antiulcer effects (11, 12). It is safe to assume that the TQ amount will be significantly lower in Nigella sativa seeds. Therefore, it may be reasonable and efficient to use high doses of Nigella sativa raw products such as extracts and oils. High doses of TQ or TQ-enriched fractions, however, are irrational when compared with our results.

The mechanisms underlying this phenomenon may be the fact that TQ inhibits COX-1 derived PGE_2 production in vitro, with an IC_{50} value of 2.6 μM, and COX-2 derived PGE2 production with an IC_{50} value of 0.3 μM (27). Also, TQ was declared to dose-dependently induce oxidative damage in mitochondria (16). Zubair, Khan (17) demonstrated that TQ was antioxidant in low doses but pro-oxidant in high doses. Putting it all together, we can speculate that high doses of TQ act as a pro-oxidant and have the potential to inhibit COX-1 and COX-2 significantly. This inhibition can reduce the production of PGE_2, leaving gastric tissue open to acid damage. Also worth mentioning is the severe hemorrhage caused by 40 mg/kg TQ in intact rats, which suggests that TQ may be directly acting on gastric tissue and damaging the integrity of it. We cannot speculate anything about the mechanism of this suggestion yet, due to lack of data. However, new studies may shed light on the subject.

TQ has become popular because of its proven therapeutic properties, and it is a common substance of not only *Nigella sativa* but also lots of other medical plants (28). Studies have shown that compounds found in seeds of *Nigella sativa* have anticancer (29), antitumor (30), anti-inflammatory and analgesic (31), antioxidant (32) and immuno-enhancing (33) effects. Studies about the gastro-protective effect of TQ are also available (11-13, 15, 25, 34). These studies were about ethanol-induced gastric damage and gastric ischemia/reperfusion damage.

Among TQ doses, 0.5 mg/kg TQ had the highest efficiency in all tests. Additionally, when compared to the FAM group (positive control group), the gastro-protective effect of 0.5 TQ group was statistically the same. There is a linear relationship between the dose increase in TQ and damage increase in gastric tissues. In a previous study, the gastroprotective activity of Nigella sativa oil and its constituent, thymoquinone, was studied against gastric mucosal injury induced by ischemia/ reperfusion in rats. In this study, similar to our results, it was shown that when the dose increased, the damage increased (13).

One of the most significant markers in ulcer physio-pathogenesis is the increase in free oxygen radicals induced by oxidative stress. The role of free oxygen radicals and oxidative stress was defined in IND induced gastric ulcers (35-38). Numerous researchers investigated TQ and other *Nigella sativa* products in oxidative stress-linked diseases, such as ulcers, in clinical and experimental studies, because of their antioxidant properties (28). TQ was tested routinely in numerous experiments (25) and its radical scavenging properties emerged as partly the source of its protective effects. Quinone structure in TQ shows excellent redox property, can penetrate easily from the cell membrane, and scavenge free oxygen radicals (39). In another metabolic pathway, TQ decreased oxidative stress by inducing cellular glutathione (40) and acted as an antioxidant (41).

Enzymatic (CAT, SOD, GPx) and non-enzymatic (vitamins, GSH) antioxidant defense systems are available to prevent oxidative damage (42). The cells' natural protection system against the destructive actions of free radicals contains the protection enzyme SOD and the antioxidant molecule, GSH. SOD, GSH, and MDA are all established indicators of the antioxidant capacity of the body (the protection against the damage caused by oxidative stress). An increase in mucosal levels of MDA and a decrease in SOD and GSH levels accompanied the gastric lesions observed in this study. Cell membrane damage caused by indomethacin may contribute to elevated MDA levels. The decreased SOD and GSH levels and the increased MDA levels in our study were in accordance with previous research studying indomethacin-induced nephrotoxicity (43-45). These results suggest that low doses of TQ reduce oxidative stress in stomach. We can say that TQ prevented the depletion of antioxidant enzymes, including GSH, in the present study. This suggestion is supported by macroscopic analysis findings, indicating that the administration of the TQ significantly prevented ulceration damage. Previous studies suggesting potential antioxidant capacity of TQ support our findings (46, 47). Also, TQ supplementation reverses lead-induced oxidative stress (14, 39).

5. Conclusions

The cytoprotective role of antioxidants in the prevention and treatment of gastric lesions has been investigated comprehensively in several studies. As far as the authors know, this is the first study to demonstrate that TQ might be a potential inhibitor of indomethacin-induced gastric ulcers. Biochemical analysis and macroscopic investigations revealed that low-dose TQ has protective effects. Pretreatment with 0.5 and 1 mg/kg doses of TQ can decrease the ulcer index and boost the recovery of gastric lesions induced by indomethacin in rats. The fact that the administration of low-dose TQ enhances SOD, GSH and MDA parameters in indomethacin-induced gastric ulcer supports this conclusion. The strong redox potential of quinone in the TQ structure may have scavenged the free radicals caused by IND, leading to a decrease in oxidative stress and protection of gastric tissue from ulceration.

In contrast, pretreatment with high-dose TQ was ineffective in protecting gastric tissue from indomethacin-induced damage. Moreover, high-dose TQ can disintegrate gastric tissue and cause hemorrhage. This may be attributed to TQ's ability to inhibit COX-1 and COX-2, and act as pro-oxidant in high doses.

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Conflict of interest statement
None of the authors has a commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies, and/or medical devices or with commercial providers of medically related services. All the listed authors have read and approved the submitted manuscript.

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Author Contributions
Turan C: Managing the in-vitro and in-vivo process of experiments. Bayir Y: Constructing an idea or hypothesis for manuscript Karagoz Y: Taking responsibility in the construction of the whole or body of the manuscript Albayrak A, Erkayman B: Managing the in-vivo process of experiments Duysak L: Managing the in-vitro process of experiments.

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The Relationship Between Mortality and Hospital-Acquired Infections in Patients Followed-up with Neurological Complaints in the Third Level Intensive Care Unit

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Abstract: We aimed to evaluate the demographic characteristics of patients with neurological complaints in Level III Intensive Care Unit (ICU) and hospital-acquired infection rate, isolated pathogen factors and regional distribution of them, relationship with invasive device use, and mortality rates. 176 patients who were followed up in ICU within 12 months were included in the study. The demographic information, analysis, culture results of the patients, consultation notes of the infection committee doctors, and nurse observation charts were examined and the patient's data were collected. 46 of the 176 patients died included in the present study. The mortality rate was determined at 26.13%. A total of 38 hospital-acquired infection (HAI) attacks were detected in 33 of all patients due to some of the patients having more than one attack. In addition, the rate of HAI was found at 21.59%. Central venous catheter-related bloodstream infections (CRBSI) in 2 patients (6.06%), mechanical ventilator independent pneumonia in 6 patients (18.1%), mechanical ventilator-associated pneumonia (VAP) or ventilator-associated tracheobronchitis (VAT) were detected in 11 patients (33.3%), and urinary tract infection (UTI) was found in 14 patients (42.4%). Whereas the mortality rate was found as 60.60% (20 of 33) in patients with HAI attack, this ratio was found as 18.18% (26 of 143) in patients without HAI attack. In patients who followed up with neurological complaints in the ICU, it was determined that having an HAI, recurrent HAI attacks, and ventilator-associated pneumonia (VAP) or ventilator-associated tracheobronchitis (VAT) increased mortality. By knowing infection surveillance data in ICUs, effective empirical treatment can be provided until the exact HAI pathogen is known. Understanding and effectively treating HAI data in ICU affects the prognosis, cost, and mortality rate of primary neurological disease by lowering HAI rates.

Keywords: Neurology Intensive Care Unit, Hospital-Acquired Infection, Infection Control, Mortality.

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1. Introduction

Infections occurring up to 1 year in case of permanent device use are hospital-acquired infections (HAI), which occur not in the incubation period before applying to a hospital but in 48-72 hours after applying to a hospital, and despite the development in the hospital in the first 10 days after hospital discharge, and 30-90 days after surgery (1). Intensive care units (ICU) are among the most common HAI areas compared to other units, services of the hospital. While only 5-10% of all hospitalized patients are treated in ICUs, 25% of all nosocomial infections are found, and the prevalence of nosocomial infections in ICUs is 5-10 times higher than general hospital wards (2).

Patients hospitalized in neurological intensive care units are the most common patients with cerebrovascular disease. These patients could be need intubation due to cerebral edema, and if they are not fed, they may need to be fed with a nasogastric tube. The patient's changes of the are followed by regular blood tests, close consciousness monitoring and clinical examination due to invasive procedure increases, whiches increases hospital-acquired infection rates. Prolonged hospitalization, clinical severity of primary disease (decrease in consciousness caused by the disease, difficulty in swallowing), multi-organ failure, decreased immune respond resistance, presence of comorbid disease, presence of metabolic disorder, increased invasive interventions (urinary catheter, central venous catheter, mechanical ventilation applications), increased antibiotic use and resistance, non-compliance with hygiene conditions, and advanced age can be considered as the reasons for more frequently development of HAI in ICUs (3-5). HAIs prolong hospital stay, increase mortality, impair quality of life, cause loss of labor, and synonym hospital costs (2, 6). In this study, we evaluated the demographic characteristics of patients who were followed up in our intensive care unit with neurological complaints, pathogenic factors, and regional distribution of HAI, the relationship between invasive device use, mortality, and HAI-related mortality rates.

2. Material and Methods

Among the neurology patients hospitalized in the neuro ICU between August 2018 and August 2019, 176 were included in the study. Patient data were scanned retrospectively between these dates, and the data of the patients were collected using the analysis of the patients, culture results, consultation notes of the infection committee doctors, and nurse observation charts. Patients between 17-90 years old were included in the study (patients under 16 years of age were not followed up in adult intensive care units). Patients who were positive infection on the day of admission to the intensive care unit and patients who died within the first 2 days (48-72 hours after applying to a hospital) after their arrival or were discharged from the intensive care unit were excluded from the study. HAI diagnosis was established according to the 'Disease control and prevention center criteria (7). In addition to the culture samples studied, patients' fever follow-up in the nurse registration forms and daily progress held by intensive care physicians, clinical examination findings, and general conditions of the patients were taken into consideration. While calculating the infection rate, 'the rate of infection=the number of patients detected with infection and hospitalized in the ICU/ the total number of patients hospitalized in the ICU x100' formula is used. The ICU's infection rate was calculated by 'the number of infections detected in the intensive care unit/the number of infections detected in the entire hospital x100.' Other calculations were as listed: VAP rate=VAP number/total ventilator day x1000, catheter-related urinary system infection rate=catheter-related urinary system infection number/urinary system catheter day x1000 and central venous catheter-related bloodstream infection (CRBSI) rate=central venous catheter-associated number of bloodstream infections/central venous catheter day x1000 (8).

Blood, tracheal aspirate, wound site, urine, catheter examinations were taken from every patient with a fever of 38 °C and above. If the patients with catheters have symptoms, catheter tip culture, hematological, biochemical tests and, if required, radiological examinations were performed. Peripheral blood samples were sown in blood culture bottles, eosin methylene blue agar, and bloody agar. The presence of the signal was evaluated by monitoring the culture bottles daily for up to 7 days in terms of reproduction. Wound site, discharge, sputum, and catheter samples were evaluated for the presence of leukocytes. It was defined according to the colony and Gram-staining characteristics of breeding pathogens. In addition to the standard medium, culture samples taken were planted in special nutrients such as motion medium, indole medium, Christensen urea agar, and examined according to gram staining feature, colony characteristics, gram staining properties, coagulase, catalase, pyrrolidinyl arylamidase, esculin hydrolysis. Additional properties such as reproduction in 6.5% sodium chloride, oxidase test were also examined. Tracheal aspirates were taken by aspiration after giving physiological serum with a deep aspiration of the endotracheal tube, following the disinfection rules with sterile catheters. Since this study was retrospective, no consent was obtained from patients. Local ethics committee approval was received (KAEK 2019/16-155). The current study was followed by Helsinky critiques.

2.1. Statistical Analysis

SPSS 20.0 package program was used to evaluate patient data. Frequency, calculations of percentage distribution, and descriptive statistical evaluation were made using the package program. The Mann-Whitney Test, one of the non-parametric tests, was used to analyze the number of deaths in patients with and

without HAI. Kruskal Wallis test, one of the non-parametric tests, was used to analyze death by infection region among those who had HAI, and Mann-Whitney test was used in paired comparisons. SPSS package program was used for all statistical analyzes.

3. Results

176 patients who were hospitalized in ICU for one year were included in the study. The mean age was 66.25±13.66 years (age range 17-90). The patients were female 51.7% (n=91) and were male 48.3% (n=85). The patients were ischemic stroke 67.6% (n=119), were epilepsy 11.3% (n=20), were dementia 6.8% (n= 12), were hemorrhagic stroke 5.6% (n=10), were Parkinson's disease (PD) 5.1% (n=9), subarachnoid hemorrhage (SAH) 1.7% (n=3), Guillain Barre syndrome (GBS) 1.1% (n=2), and amyotrophic lateral sclerosis (ALS) 0.5% (n=1). According to the patients' medical history, the patients were hypertension 27.8% (n=49), diabetes mellitus 18.1% (n=32), chronic heart failure 11.9% (n=21), chronic obstructive pulmonary disease (COPD) 2.2% (n=4), 3 malignant brain tumors (1.7%), chronic kidney failure (CKF) 1.7% (n=3), lung adenocarcinoma 0.5% (n=1), malignant skin tumor 0.5% (n=1), shunt operation 0.5% (n=1), gastric adenocarcinoma 0.5% (n=1), 1 operated larynx malignancy 0.5% (n=1) (Table 1).

Table 1: Demographic Characteristics of Patients in A One-Year Period in The Neuro-ICU.

Sex	
Female	91 (51.7%)
Male	85 (48.3%)
Medical Diagnosis	
Ischemic stroke	119 (67.6%)
Epilepsy	20 (11.3%)
Dementia	12 (6.8%)
Hemorrhagic stroke	10 (5.6%)
Parkinson's disease	9 (5.1%)
Guillain Barre Syndrome	2 (1.1%)
Subarachnoid hemorrhage	3 (1.7%)
Motor Neuron Disease	1 (0.5%)
Concomitant diseases	
Hypertension	49 (27.8%)
Diabetes mellitus	32 (18.1%)
Congestive heart failure	21 (11.9%)
COPD	4 (2.2%)
Chronic renal failure	3 (1.7%)
Malignant Brain Tumor	3 (1.7%)
Lung Cancer	1 (0.5%)
Malignant Skin Tumor	1 (0.5%)
Previous Shunt Operation	1 (0.5%)
Gastric Cancer	1 (0.5%)
Opere Larynx Cancer	1 (0.5%)

The HAI developed in 33 (18.75%) of all patients included in the study. A total of 38 HAI attacks were detected in 33 patients diagnosed with HAI. The HAI rate was calculated as 21.59%. 1 episode in 29 patients (76.31%), 2 episodes in 3 patients (7.89%), 3 HAI episodes in 1 patient (2.63%) were observed. 17 were female (51.5%) and 16 were male (48.5%) of the 33 patients with HAI. HAI was detected in 20 patients with ischemic strokes (60.60%), 5 hemorrhagic strokes (15.15%), 3 epilepsy (9.09%), 2 Parkinson's disease PD (6.06%), 1 Subarachnoid hemorrhage SAH (3.03%), 1 Guillain-Barré syndrome GBS (3.03%), 1 Amyotrophe Lateralsklerose ALS (3.03%) (Table 2).

Table 2: Demographic Characteristics of Patients with a Hospital-Acquired Infection in The Neuro-ICU For A Year.

Total Number of Patients	**n=33**
Sex	
Female	17 (51.5%)
Male	16 (48.5%)
Medical Diagnosis	
Ischemic stroke	20 (60.6%)
Hemorrhagic stroke	5 (15.15%)
Epilepsy	3(9.09%)
Parkinson's Disease	2(6.06%)
Guillain Barre Syndrome	1(3.03%)
Subarachnoid hemorrhage	1(3.03%)
ALS	1(3.03%)

46 of the patients who were hospitalized in the neuro ICU died. The mortality rate was found as 60.60% (20 of 33) in patients with HAI attack, and this ratio was found as 18.18% (26 of 143) in patients without HAI attack (p=0.024) (Table 3).

Table 3: Mortality Rates of Hospital-Acquired Infections in The Neuro-ICU For One Year.

Total Inpatient	176
Patient not having a hospital-acquired infection	143
Patient having hospital-acquired infection	33
Total deceased patient	46 (26.1%)
Decased patients who did not have a hospital-acquired infection	26 (18.1%)
Deceased patients who had a hospital-acquired infection	20 (60.6%)
Deceased patient who had pneumonia as a hospital-acquired infection	14 (70%)
Deceased patients who had UTI as a hospital-acquired infection	6 (30%)

14 of the deceased HAI patients were pneumonia (70%) and 6 of them were patients with UTIs (30%). All of the 4 patients (100%) who had more than one HAI attack were patients with pneumonia, and all of these patients died. Distribution of patients with HAI according to localization was 17 pneumonia (51.5%), 14 urinary tract infections (42.4%), and 2 central venous catheter-related bloodstream infections (p=0.018, p=0.024) (Table 4).

Table 4: Regional Percentage Distribution of Hospital-Acquired Infections in The Neuro-ICU For A Year.

Pneumonia	17 (51.5%)
Mechanical ventilator-associated lung infection	11 (64.7%)
Mechanical ventilator unrelated pneumonia	6 (35.3%)
Urinary system infection	14 (42.4%)
Bloodstream infection	2 (6.06%)
Central venous catheter-associated bloodstream infection	2 (6.06%)

The total hospitalization day of the ICU was calculated as 837 days. The day of hospitalization in the total ventilator in the intensive care unit was 309 days, the ventilated patient follow-up rate was 36.9%, and the VAP rate was calculated as 19.2%. Total central venous catheter use day was calculated as 202, and the catheter-related bloodstream infection rate was calculated as 4.9% (Table 5).

Table 5: The Use of Mechanical Ventilators, Urinary Catheters and Central Venous Catheters ın The Neuro-ICU For One Year and Associated Infection Rates.

	Day	**%**
Mechanical ventilator usage	309	36.9
Ventilator-associated pneumonia rate	-	19.2
Central venous catheter usage	202	24.13
Central venous catheter-associated bloodstream infection rate	-	4.9

22 HAI attacks were detected in 17 patients with pneumonia. 11 of these patients were defined as VAP or ventilator-associated tracheobronchitis (VAT) (64.7%), and 6 of these were ventilator-unrelated pneumonia (35.3%). When looking at the distribution factors of 16 HAI attacks of 11 patients diagnosed with VAP-VAT, there were 5 HAI attacks of *Acinetobacter baumannii*, 4 HAI attacks of *Pseudomonas aeruginosa*, 3 HAI attacks of *Escherichia coli*, 2 HAI attacks of methicillin -resistant *Staphylococcus aureus*, 2 HAI attacks of *Klebsiella pneumonia*. In the distribution factors of 6 HAI attacks of patients suffered from mechanical unrelated ventilator pneumonia, it was observed that 2 HAI attacks of methicillin-resistant *Staphylococcus aureus*, 1 HAI attack of *Acinetobacter baumannii*, 1 HAI attack of *Pseudomonas aeruginosa*, 1 HAI attack of Klebsiella Pneumonia, and 1 HAI attack of *Burkholderia cepacia* (Table 6).

Table 6: The Factors of The Hospital-Acquired Pneumonia in The Neuro-ICU For A Year and The Percentage Distribution of These Factors.

Ventilator-associated	**11(64.7%)**
Acinetobacter Baumannii	5 (45.4%)
Pseudomonas aeruginos	4 (36.3%)
E. coli	3 (27.2%)
Meticillin-resistant Staphylococcus Aureus	2 (18.1%)
Klebsiella pneumoniae	2 (18.1%)
Ventilator unrelated	**6 (35.6%)**
Meticillin-sensitive staphylococcus	2 (33.3%)
Acinetobacter	1 (16.6%)
Pseudomonas aeruginosa	1 (16.6%)
Klebsiella pneumoniae	1 (16.6%)
Burkholderia cepacia	1 (16.6%)

In our intensive care unit, 12 different types of pathogens causing HAI were detected. The most frequently isolated microorganism was *Acinetobacter baumannii*. The distribution areas of these microorganisms are given in Table 5 and Table 6.
Urinary catheters were used in all (100%) of 14 HAI patients with UTI. The distribution factors in patients with UTI was *Escherichia coli* in 4 patients (28.5%), *Klebsiella* in 3 patients (21.4%), and *Candida spp*. in 2 patients (7.1%), *Candida glabrata* in 1 patient (7.1%), *Enterococcus faecali* in 1 patient (7.1%), methicillin-resistant coagulase-negative staphylococci in 1 patient (7.1%), and pseudomonas in 1 patient (7.1%), and *Enterococcus faecali* in 1 patient (7.1%) (Table 7).

Table 7: The Factors of The Hospital-Acquired Urinary System Infections in The Neuro-ICU For A Year and The Percentage Distribution of These Factors.

Escherichia coli	4 (28.5%)
Klebsiella pneumonia	3 (21.4%)
Candida spp.	2 (14.2%)
Candida glabrata	1 (7.1%)
Enterococcus faecalis	1 (7.1%)
Coagulase negative staphylococcus	1 (7.1%)
Pseudomonas aeruginosa	1 (7.1%)
Enterococus faecium	1 (7.1%)

CRBSI was detected in 2 patients. Coagulase-negative *Staphylococcus aureus* was produced in the blood culture of 2 patients. Both patients with reproduction in blood culture were evaluated and treated as sepsis.

4. Discussion

In current study, the rate of HAI was found as 21.59%, the mortality rate of all patients was 26.13%, and the mortality rate of patients who suffered from HAI was found to be higher than patients without HAI (60.60%, 18.18%, respectively). It was observed that the development of HAI is an important factor that increases the mortality rate. In studies conducted in the literature, it was observed that suffering from HAI increases the mortality rate (2). The majority of patients in this study were stroke patients. In a meta-analysis examining the effect of post-stroke infection development on mortality rate, the mortality rate was 48% in stroke patients who developed an infection and 18% in stroke patients who did not develop infection (9).

Localization of infection and pathogens of HAIs seen in intensive care units may differ according to each other (10). In a study conducted by Eren et al. in our country, it was found that the most common HAI in the neurological ICU was UTI, with 71.88% over a period of one year (11). Again, in our country, in a study conducted by Şahin et al., it was stated that the most common HAI in the neuro ICU was pneumonia with 43.3% (12). In a multicenter study conducted with 6-year data abroad, it was found that 102 of 227 HAI attacks diagnosed in neuro ICUs were pneumonia and 78 of them were UTI attacks (13). In this study, the most common localization of HAI in the ICU and the number of HAI attacks were pneumonia as the lower respiratory tract infection, and patients who had HAI in the form of pneumonia had higher mortality. 14 of the 20 patients, who died with the diagnosis of HAI, were patients who had pneumonia (70%). Following in this study here, studies in the literature have shown that having pneumonia increases the risk of developing mortality (9, 14).

In this study, here all patients who had recurrent HAI also had VAP / VAT and all 4 patients (100%), who had recurrent HAI, died. It was seen that having a recurrent HAI attack increased the mortality rate (9,15). In this study, it was observed that the second most common cause of HAI was UTI after pneumonia in the ICU. All patients who had UTI attacks had urinary catheterization. UTI attacks were detected in 14 patients. 6 of the 20 patients who died with the diagnosis of HAI were patients with UTI (30%). Before the intervention to patients to reduce UTIs, UC should not be worn except for compulsory situations. Infection control measures such as hand washing, changing gloves and paying attention to isolation rules must be followed (16).

Thanks to the fact that ICUs are divided into branches so that the intensive care of neurology and neurosurgery became frequent, a recuperation in the prognosis of patients, who have been followed by physicians who have mastered the anatomy and physiology of the central nervous system, and a decrease in the cost of inpatient treatment are detected (17, 18). The introduction of new antibiotics every day, the rapid increase in the technology of medical devices and equipment used in intensive care units, the planned work of hospital infection committees, and regular training of employees provide the opportunity to survive in the intensive care units of many patients. There are advantages of the frequent presence of ICUs, and there are disadvantages such as the risk of developing HAI as well. Intensive care units are areas with a high risk of infection where changes in consciousness, dysphagia, and comorbidity are common, and patients with multiple organ damage and low immune resistance are treated. When one or more of these conditions coexist, colonization and pathogen transmission become easier. Long hospitalization, usage of the central and peripheral venous catheter, follow-up with endotracheal intubation, and urinary catheterization cause an increase in HAI (16, 19, 20). Early diagnosis of HAI, early initiation of treatment, determination of pathogen type and antibiotic susceptibility affects the prognosis of primary neurological disease, reduces mortality and morbidity rate, and reduces antibiotic resistance and cost of expense spent (20-22).

Acinetobacter baumannii was seen as the most frequently isolated pathogen in this study, and it was also the most common factor of pneumonia. In Ciraligil's study, which examined antimicrobial resistance in ICUs at the national level, it was emphasized that *Acinetobacter baumannii* infections are an important problem for ICUs (23). In current study, the most common factor of UTI was found to be *Escherichia coli*. In a study in which catheter-related UTIs were evaluated in ICUs, the most common pathogen was reported to be *Escherichia coli*.

5. Conclusion

The limitations of in current study are that it is a single-center study, the number of patients is limited, and that consciousness of patients' and disease severities, antibiotics, and resistance profiles given to the patients, pediatric patients are not included in the study.

Providing hand hygiene practice, trying to avoid insertion of a urinary catheter, if a urinary catheter is mandatory, compliance with hygiene conditions when inserting a urinary catheter, paying attention to sterilization while performing aspiration, receiving regular infection control training, increasing the number of health personnel per patient, paying attention to the isolation rules and regulating the conditions of the intensive care units are important to reduce HAI. (16, 19, 24, 25)

The infection rate and ratio of each intensive care unit should be calculated periodically, microorganisms seen in intensive care should be evaluated, and ICU-specific pathogen profiles should be determined frequently. To reduce the antibiotic resistance increasing day by day, unnecessary antimicrobial therapy should be avoided by periodically reviewing the resistance profiles of HAI

pathogens in ICUs. Knowing the factors of HAI specific for ICU increases the effectiveness of the empirical treatment to be started. In each ICU, HAI data should be regularly compared with the literature and included in surveillance studies. Prospective surveillance studies involving multicentre, broad patient participation, and pediatric and adult age groups are needed to reduce and control HAIs. In the present study, the indomethacin-induced gastric ulcer model in rats was used for the first time to investigate the gastroprotective effect of TQ. Additionally, the antioxidant effect of TQ was examined in ulcerative gastric tissues.

Conflict of Interests
None
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Author Contributions
Yardım A and Çelik H contributed to the conception and design of the study. Yardım A and Yeşildağ K contributed to the collection of the data and statistical analysis and evulation of the results. Yardım A and Doğan M contributed to the creating and writing of manuscript. Yardım A and Yıldız O contributed to revising the work and final approval of the version

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Comparison of Chloral Hydrate and Hydroxyzine in Pediatric Electroencephalogram Recording; Sedation Successes and changes in Vital Signs

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Abstract: During electroencephalogram (EEG) recording of children, sedatives are frequently needed. It's reported that Chloral hydrate (CH) and Hydroxyzine (H) have negligible negative effect on EEG and are safe. The effect of CH and H on vital signs has not been studied in detail previously. We compared the sedation success, side effects, and effects on vital signs of CH and H during pediatric EEG recording. A total of 60 children with a mean age of 43.3±33 months (CH) and 39.7±29 months (H) were involved in the study. Oral CH (50 mg/kg) was given to thirty children, and oral H (1 mg/kg) to another 30. Vital signs were recorded during the procedure. Sedation success of CH (96.6%) was higher than H (76.6%) (p=0.023). Mean Ramsay Sedation Score (RSS) of CH (4.8±0.7) was higher than H (3.5±1.6) (p=0.00). The mean procedure time in CH group was significantly longer (p=0.000). The CH significantly reduced the mean systolic and diastolic blood pressure compared to H (p=0.007, p=0.003, respectively). SO_2 of a patient from CH group decreased to 87%, and vomiting (6.6%) and agitation (3.3%) were observed in two patients. Our findings indicate that CH, due to it's higher success rate, can be preferred in children who need sedation for EEG. However, in patients who have limited time, H with a shorter total procedure time can be preferred. More comprehensive studies are required about the effects of decreased blood pressure on systems, caused by CH. © 2021 NTMS.

Keywords: Child, Chloral Hydrate, Electroencephalogram, Hydroxyzine, Sedation.

1. Introduction

Electroencephalogram (EEG) is an auxiliary method used to detect abnormal electrical activities in the brain or to diagnose epilepsy.

In order to make the abnormality prominent during standard EEG, provocation methods such as hyperventilation and photic stimulation are applied (1).

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Another provocation method is sleep EEG (1-3). It is generally required to administer sedative agents to children who cannot be put to sleep or are unable to communicate in order to reduce anxiety and muscle movements for EEG (2-6).

Chloral hydrate (CH) and hydroxyzine (H) have been used for a long time in short-term sedation procedures, and current publications indicate that they have a negligible effect on EEG and are relatively safe at recommended doses (3-7). Although there are studies about the effects of sedation successes of CH and H on vital signs and side effects in EEG recording (3-8), in most of these studies there is no information about the detailed monitoring results of vital signs during sedation.

In this study, we aimed to compare the adequacy of the sedation level provided by these two drugs in children during EEG recording, their side effects, and especially their effects on vital signs.

2. Material and Methods

Pediatric patients who underwent EEG at Atatürk University, Faculty of Medicine, Department of Pediatrics between April and May 2008 were included in the prospective study. Informed consent was obtained from the families of the patients. Patients in whom an optimal recording could not be obtained due to incompatibility, and who could not sleep spontaneously after 20 minutes of waiting time for sleep EEG were included in the study. Patients who could be communicated with were ensured to defecate before sedation. Attention was paid to ensure that patients had at least four-hour fasting and vigil periods before sedation.

The medical requirements recommended by the American Society of Anesthesiologists (ASA) and the American Academy of Pediatrics (AAP) are established before sedation (9, 10). Patients with a history of drug allergy, signs of diseases in that sedation is contraindicated, and signs of obstruction in the upper respiratory tracts were excluded. The patients were taken to the quiet and dark sedation room near by the EEG room with their mothers, and were waited to sleep spontaneously. ASA class-I-II patients (9), who could not sleep spontaneously within 20 minutes (min) were sedated.

Body temperature was measured by the axillary method with a digital thermometer. Vital signs (blood pressure, heart rate, respiratory rate, and oxygen saturation) were continuously followed via a monitor (NIHON KOHDEN BSM-2301 K, Tokyo, Japan). The values measured while the patient was calm before sedation were recorded as basal values. The patients were grouped as Seizure, Seizure (Neuromotor developmental retardation is present), other causes (acute encephalopathy, ataxia, speech disorder, etc.). The chloral hydrate (CH) and hydroxyzine (H) group was formed from similarly diagnosed patient groups (Table 1).

During the study, we administered CH to approximately half of the patients whom we decided to include in the study and H to the remaining half in daily program. The study was terminated when the number of patients in each group reached 30. A 50 mg/kg CH was administered orally by dissolving it in 2-3 cc juice. It was re-administered with a dose of 25 mg/kg to patients who were not sedated within one hour after the first administration. H, in suspension form, was administered orally at a dose of 1 mg/kg. It was re-administered to patients who were not sedated within one hour at a dose of 1 mg/kg. If sedation could not be achieved within one hour after the second dose for both drugs, the sedation procedure was deemed to be unsuccessful.

The sedated patients were taken to the EEG recording table were monitorised. Electroencephalogram recordings were performed with the same device in all patients (NIHON KOHDEN-Neurofaks, Tokyo, Japan). During the recording, vital signs of all patients were monitored, and data were recorded every 10 minutes. Non-sedated patients were monitored for 20-90 minutes to observe the side effects of the drugs. While evaluating the data, the following definitions were used.

Transition time to sedation (sleep): The time from drug administration until the patient closes his eyes and becomes irrelevant to the environment. Sedation time: The time until he wakes up spontaneously after sleeping. Successful sedation: Providing sedation at a level that allows successful electroencephalogram recording. Prolonged sedation: A situation in which the patient does not wake up after two hours have passed since the beginning of sedation. Procedure time: Time from drug administration to discharge. Minimal hypoxia: Oxygen saturation between 90-95%. Desaturation: Oxygen saturation below 90%. Hypothermia: Body temperature of <36 C^{o}. Bradycardia: Heart rate below the average values for age. Tachycardia: Heart rate above the average values for age. Hypotension: Systolic blood pressure values below 50 percentile (p) for age and gender or systolic blood pressure at least 20 mmHg reduction. Diastolic blood pressure at least 10 mmHg reduction. Hypertension: Systolic or diastolic blood pressure values above 90p for age and gender (11-14). The sedation level achieved was rated with the sedation score determined according to Ramsey Sedation Scoring (RSS) (15).

Patients who could not be sedated with hydroxyzine were scheduled for another day to achieve sedation by CH administration.

Patients who underwent EEG recording were waited to wake up spontaneously. Patients who were crying after waking up or having their eyes open for 30 seconds and continued to be interested in the environment were

considered to be awakened. Patients with sedation longer than two hours were awakened by tactile stimulation. Patients were discharged when the discharge criteria of AAP were met.

2.1.Statistical Analysis

"Statistical Package for the Social Science" (SPSS) 17.0 Windows statistical package program was used for statistical analysis. Numerical values showing normal distribution were given as Mean±Standard Deviation and categorical data as numbers and percentages. The student's t-test was used for comparison of numerical values, and the Chi-square test was used in comparison of the frequencies. P<0.05 was considered as the statistical significance level.

3. Results

A total of 60 patients were included in the study with an age range of 6 months to 12 years. 30 patients received CH, and 30 patients received H. Statistical analysis showed that the groups were comparible in terms of the gender distribution, age, body weight and averages of vital signs measured before medication (body temperature, heart rate, systolic blood pressure, diastolic blood pressure, oxygen saturation and respiratory rate) p>0.05 (Table 2).

Drug applications for sedation in both groups are given in Figure 1. In the first sedation attempt, sufficient sedation was achieved in 96.6% of CH group, and in 76.6% of H group. Seven patients who could not be sedated with H were given CH on another scheduled day (second attempt), and successful sedation was achieved.

An 8-month-old male patient with cerebral palsy+metabolic disease +epilepsy could not be sedated in the CH group (3.3%). Neuromotor developmental retardation was present in 4 of seven patients (23%) who were given H and could not be sedated (Table 1). The comparison of groups in terms of sedation data and side effects in the first sedation attempt is given in (Table 3). Minimal hypoxia was observed in 53% of the CH group and 30% of the H group. The SO_2 (blood oxygen saturation level) of one patient who was given CH decreased to 87%. Although the sedation success rate of CH was significantly higher when compared to H, the frequency of side effects was statistically higher (p = 0.023 and P=0.03, respectively) (Table 3). During sedation, the body temperature of 20% of the CH group (6/30) and 16.6% of the H group (5/30) remained between 35-36ºC for short times. When groups were compared, mean body temperature, heart rate, and respiratory rate were statistically similar in repeated measurements during sedation (p>0.05).

The mean decrease in SO2 was significantly higher in CH group when compared to H group (%3.0±2, %1.6±1.5, respectively) (p=0.046) (Table 3). Systolic and diastolic blood pressure decreased in 33% (10/30) of the patients in the CH group and 23% (7/30) of the patients in the H group. However, Diastolic blood pressure decreased in 30% (9/30) of patients in the CH group and in 16% (5/30) of patients in the H group. Chloral hydrate significantly reduced mean systolic and diastolic blood pressures when compared to H (p=0.007, p=0.003) (Table 3). However, compared to H, CH significantly reduced mean diastolic blood pressure, especially at 60 and 80 minutes (p=0.023). The mean Ramsey Sedation Score (RSS) was significantly higher in the CH group than in the H group (4.8±0.7, 3.5±1.6, respectively) (p<0.001).

Table 1: Patients' diagnosis and final sedation successes of CH and H**.**

Patients' diagnoses	CH	H	P Value
Seizure	17	15	
Successful	17	12	0.09
Failed	0	3	
Seizure (Neuromotor developmental retardation is present)	9	9	
Successful	8	5	0.09
Failed	1	4	
Other causes (acute encephalopathy, ataxia, speech disorder, etc.)	4	6	
Successful	4	6	-
Failed	0	0	

Abbreviations: CH, Chloral hydrate; H, Hydroxyzine, p<0.05 represents a statistically significant difference**.**

4. Discussion

It is generally required to administer sedative agents to children for EEG recording. Some patients do not sleep, and in some muscle movements and anxiety disrupts the recording (2-6). Faytrouny M. et al (8) stated that it is complicated to compare the clinical results with different drugs used in sedation procedures due to the use of different methodologies and criteria.

The sedation success of the CH and H in EEG recording is reported between 70-93% (40-100mg/kg) and 83-89.6% (1-2 mg/kg), respectively, and varies according to dose of the drug, ASA classes of patients, and patient profile (4-8). In present study, the sedation success of CH was 96%, and it was significantly higher than that of H (76.6%) (p=0.023). Although the sedation success rate obtained with H was lower, it was close to the reported rates in the literature. While three of the four patients were sedated with repeated CH administration, no sedation was achieved in any of the seven patients with repeated H administration. Successful sedation was achieved in all patients when CH was administered on another scheduled day to the patients who could not be sedated with H. These data show that CH is more effective in terms of sedation success (Figure 1).

Some studies have reported that the drugs have low sedation success in patients with neuromotor developmental retardation (5-6). The data obtained in present study indicate that although difference was not significant, the sedation success of H is lower in

patients with neuromotor developmental retardation (Table 1).

In the literature, it has been reported that the transition time to sedation in imaging procedures is between 16.2-30 minutes for CH and 23.7-34.6 minutes for H. (3,7,16,17). In our study, the mean transition time to sedation was 19.8±12.4 minutes for CH and was consistent with the literature, and it was 14.6±13.4 minutes for H and was shorter than the literature.

For CH and H, the sedation time has been reported in the range of 20-88 min and 30-85 min, respectively (3, 5, 7, 18). In present literature, it is difficult to comment on the actual sedation time of both drugs, since there is insufficient information about whether patients are immediately awakened by anesthesiologist or waited to wake up spontaneously. In our study, patients whose sedation time was extended to two hours (CH:11/30, H:2/30) were accepted as prolonged sedation and were awakened with tactile stimulation at the end of the second hour. Therefore, the maximum sedation time was two hours. So, the mean sedation time provided by CH was (93±30 minutes) significantly longer than the average sedation time provided by H (45.3±42 minutes) ($p<0.0001$).

The consciousness levels of the patients during the sedation process are determined through scales such as Ramsay Sedation Score (RSS) (15, 19). Fallah R et al. (5) reported the mean RSS as 4.53±1.63 of the patients sedated with CH (40 mg/kg) in EEG recording. To our knowledge, there is no study investigating any sedation score in pediatric patients who received H. In our study, the mean RSS in CH group was 4.8±0.7, and it was significantly higher than the H group (3.5±1.6) ($p<0.001$). The value found for CH was consistent with the literature.

Studies have reported that there may be a transition from planned sedation level to a higher sedation level in the sedation procedures of children (16, 20, 21). In our study, all patients remained at the level of minimal-moderate sedation, and a severe and more advanced sedation was not observed. This result was considered to be associated with the inclusion of patients with ASA class-I-II. The mean total procedure time in CH group was (132±39 min) statistically longer than the mean total procedure time of the H group (74±52 min) ($p<0.0001$). This feature of H may be preferred for sedation in patients who has limited time for outpatient evaluation.

The American Academy of Pediatrics (AAP) recommends recording the heart rate, respiratory rate, blood pressure, and oxygen saturation of pediatric patients who were given drugs for sedation at least every 5 minutes. However, they also reported that the patient's blood pressure could be observed with an interval of 10-15 minutes; if the patient is well balanced, O_2 saturation is good, and peripheral circulation is healthy (9, 22). In our study, while heart rate and oxygen saturation were continuously monitored, other vital signs were recorded every 10 minutes since no significant deterioration was observed. Although the ASA classes of the patients have not been specified in most studies comparing CH and H in EEG recordings (3, 6, 7), it was stated that patients with ASA class-I-II are eligible candidates for minimal and moderate sedation (9, 22). In our study, absence of any significant hemodynamic impairment and side effect support the opinion that ASA Class-I-II patients are suitable candidates for mild-moderate sedation.

Table 2: Demographic data of the groups and the mean of basal vital values measured before medication.

	CH	***H***	***P Value***
Gender			
Female N (%)	15 (25)	15 (25)	p>0.05
Male N (%)	15 (25)	15 (25)	p>0.05
	Mean±SD	**Mean±SD**	
Bodyweight (kg)	14.0±5.2	13.7±5.5	p>0.05
Age (months)	43.3±33	39.7±29	P>0.05
Body temperature (^oC)	36.7±0.29	36.6±0.29	p>0.05
Heart rate (pulse/min)	115.8±13.2	117.3±15.6	p>0.05
Systolic blood pressure (mmHg)	105.6±10.1	102.1±14.1	p>0.05
Diastolic blood pressure (mmHg)	62.5±11.3	54.7±12	p>0.05
O_2 saturation (%)	96.4±1.1	96.3±1.0	p>0.05
Respiratory rate (min)	20.6±2.8	21.8±4.5	p>0.05

Abbreviations: CH, Chloral hydrate; H, Hydroxyzine; SD, Standard deviation, $p<0.05$ represents a statistically significant difference.

Table 3: Comparison of the groups in terms of sedation data and side effects

	CH(N=30)		*H(N=30)*		*P Value*
Sedation Data	**Mean±SD**		**Mean±SD**		
Transition time to sedation (min)	19.8±12.4		14.6±13.4		0.127
Sedation time (min)	93.0±30		45.3±42		0.000
Ramsey Sedation Scoring (RSS)	4.8±0.7		3.5±1.6		0.000
Reduction in body temperature (%)	1.5±1.1		1.2±10		0.214
Reduction in respiratory rate (%)	12±40		10±5.9		0.318
SO_2 reduction (%)	3.0±20		1.6±1.5		0.046
Reduction in heart rate (%)	14±8		13±7		0.706
Reduction in systolic blood pressure (%)	14.0±70		8.9±8.2		0.007
Reduction in diastolic blood pressure (%)	24±23		17±11		0.003
Total time of the procedure (min)	132±39		74±52		0.000
	N	**%**	**N**	**%**	**P Value**
Sedation success (%)	29	96.6	23	76.6	0.023
Side effects					
Prolonged sedation (n)	11	36	6	20	0.03
Vomiting (n)	2	6.6	-	-	
Agitation (n)	1	3.3	-	-	
Those with saturation <90 (n)	1	3.3	-	-	

Abbreviations: CH, Chloral hydrate; H, Hydroxyzine**;** SD, Standard deviation, p<0.05 represents a statistically significant difference.

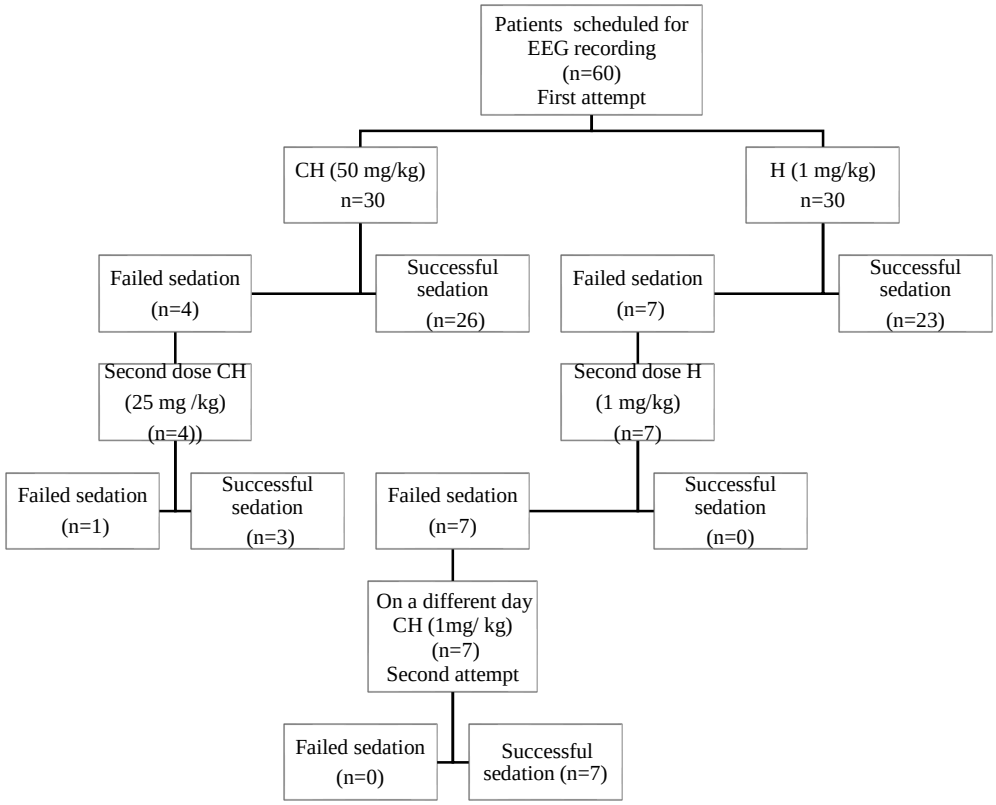

Figure 1: Flow chart of sedation success with CH and H. CH, Chloral hydrate; H, Hydroxyzine.

In ASA class-I-II children, during imaging procedures, Magnetic resonance imaging (MRI), Computerized tomography (CT), Echocardiography (ECO), Electroencephalogram (EEG), etc.), the side effects reported for CH (50-100 mg/kg) are mild hypoxia (4-9%), moderate to severe hypoxia (0.5%), prolonged

sedation (3-3.3%), vomiting (0.4-4%), agitation (0.5-6%), apnea (0.03%), laryngospasm (1.4%), hypercarbia (6.6%), and hypotension (0.4%). They were irritable behavior (4.2%) and nausea-vomiting (2.8%) for H (1-2 mg/kg) (3, 17, 23, 24). A mean reduction of 3±2% was observed in oxygen saturation in the CH group and 1.6±1.5% in the H group (p=0.046). However, in 53% (16/30) of CH group and 30% (9/30) of H group SO_2 ranged between 90-95% (minimal hypoxia) for short episodes. Oxygen saturation of these patients returned to normal spontaneously without any intervention. The SO_2 of one patient who was given CH decreased to 87%. The patient's head was brought to extension, and oxygen was delivered with a mask, and the saturation increased within 4-5 minutes. The minimal hypoxia rates determined in our study were higher than those given in the literature. The SO_2 value was measured as 95% before recording in 5/30 patients in the H group and 7/30 patients in the CH group. This low initiation value may have made it easier for patients to fall below 95%.

An average reduction of 1.5±1.1% in the body temperature was detected in the CH group, and 1.2±1% in the H group (p>0.05). During sedation, there were times when the body temperature of 20% of the patients in the CH group (6/30) and 16.6% of the patients in the H group (5/30) remained between 35-36 ºC. This decrease in body temperature may be related to the temperature of the environment or the decrease in the body temperature of the sedated patients. There was no information in the literature that both drugs cause hypothermia.

In a study performed by Heistein LC. et al. (17) 1092 pediatric patients were given CH for sedation before echocardiography, and it was found that the heart rates of the sedated patients decreased at an average of 14±10%, and their blood pressures decreased at an average of 23±13%. It was also pointed out that the changes caused by sedative drugs in heart rate and blood pressure were similar to changes occurring during sleep.

In this study, an average of 14±7% decrease in systolic blood pressure and an average of 24±23% decrease in diastolic blood pressure were detected in the CH group, while an average of 8.9±8.2% decrease in systolic blood pressure and an average of 17±11% decrease in diastolic blood pressure were detected in the H group. The CH significantly reduced the mean systolic and diastolic blood pressure compared to H (P=0.007, p=0.003).

Systolic and diastolic blood pressures decreased of 33% (10/30) of patients in the CH group and 23% (7/30) of patients in the H group. However, diastolic blood pressures decreased of 30% (9/30) of the patients in the CH group and 16% (5/30) of the patients in the H group. These decreases, which did not cause clinical findings and were normalized during follow-ups, were not intervened. Also, compared to H, CH significantly reduced diastolic blood pressure, especially at 60th and 80^{th} minutes (p=0.023).

In a study performed on healthy pediatric patients, Soergel et al. (25) found a 13.6±6% reduction in systolic blood pressure and a 23±9% reduction in diastolic blood pressure during sleep at night, and suggested using pediatric reference values separately to assess blood pressure while awake and asleep. In our study, a mean reduction of 14±8% in the CH group and 13±7% in the H group were found in heart rate. None of the patients developed bradycardia. The heart rate and blood pressure reduction percentages of the patients who were administered CH and H during the sedation were compatible with the literature. These reductions may either be the result of the drugs or sedation itself.

In addition, nausea, vomiting, and irritable behaviors were observed in a small patient group, and no severe reactions were encountered (3, 7, 23, 26). In our study, no severe side effects were seen, and no intervention was required for both drugs.

It was reported that both drugs were safe with similar side effects when the following conditions suggested by the AAP (9, 22) were established in the sedation applications of pediatric patients; the patients with hypoxia risk should be excluded before the procedure, the sedation process should be managed by appropriately trained personnel, and appropriate doses of CH and H should be used.

Fong CY et al. analyzed 13 studies comparing CH administration with other sedative drugs and music therapy in children who were undergoing CNS imaging and EEG recording. They stated that in the analyzed studies, only a few of them had given information about operation success, additional sedative agent requirements, and the level of sedation determined through reliable and valid scales. They also highlight the need for further studies on the adverse effects of CH (27). In our study, the effectiveness of CH and H and their effects on vital signs were investigated more comprehensively. In addition, our study contributes to the literature in order to overcome the shortcomings mentioned above.

5. Conclusions

Our study results indicate that CH, which has high sedation success in EEG recording in pediatric patients, can be preferred primarily. However, in patients with limited time for EEG recording, H may be preferred because of the significantly shorter total procedure time. The mean Ramsey Sedation Score (RSS) was significantly higher in the CH group than in the H group. During EEG recordings of patients with neuromotor developmental retardation, CH may be preferred for sedation. More comprehensive studies are required about the effects of CH blood pressure-lowering effect on human body systems.

Main points of the article

*It is generally required to administer sedative agents to children who cannot be put to sleep or are unable to communicate in order to reduce anxiety and muscle movements for EEG. Chloral hydrate (CH) and
*Hydroxyzine (H) have been used as sedative agents in many EEG centers for a long time.
*The effect of CH and H on vital signs has not been studied in detail previously.
*Our study results indicate that CH, which has high sedation success in EEG recording in pediatric patients, can be preferred primarily.
*The CH significantly reduced the mean diastolic blood pressure compared to H
*The mean Ramsey Sedation Score (RSS) was significantly higher in the CH group than in the H group. Therefore during EEG recordings of patients *with neuromotor developmental retardation, CH may be preferred for sedation.

Restrictions of the study

The relatively low number of patients, medications not to be given in different doses, absence of control group is the restriction of our study.

Ethics Committee Approval

The Ethics Committee's approval of the Atatürk University Faculty of Medicine is obtained for the study. (Decision number 25 of 07.12.2007 dated meeting no. 6).

Informed Consent

Informed Consent from Family

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Conflict of Interests

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Author Contributions

Concept-M.A.G., HT.; Design-M.A.G., M.G., H.T.; Supervision-H.T.; Data Collection and/or Processing-M.A.G; Analysis and/or Interpretation - M.A.G., M.G., H.T; Literature Search-M.A.G., M.G; Writing Manuscript-M.A.G., M.G., H.T.; Critical Review-M.A.G., M.G., H.T.

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Clinicopathological Characteristics of Endometrial Carcinosarcomas: A Single-Center Experience

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Abstract: Endometrial carcinosarcoma (ECS) is epidemiologically and clinically similar to endometrial adenocarcinoma (EA) and is considered an aggressive variant of EA. Considered as carcinomas with sarcomatous and carcinomatous components, these tumors are rare, with a very poor prognosis. The definitive diagnosis of ECS is based on histopathological examination. We aimed to share the clinicopathological characteristics of histopathologically diagnosed ECS cases. We analyzed 26 materials diagnosed as ECS after histopathological examinations that were sent to our department as total abdominal hysterectomy in the last 7 years. The histological type of the carcinoma component was serous carcinoma in 14 cases, and endometrioid adenocarcinoma in 12 cases. The histological type of sarcoma component was endometrial stromal sarcoma in 16 cases, fibrosarcoma in 2, leiomyosarcoma in 2, and chondrosarcoma in 6. According to FIGO staging, 10 of the cases were stage IA, 2 was stage IB, 2 was stage IIIA, 10 were stage IIIC2, and 2 was stage IVB. According to pTNM pathological staging, 10 of the cases were pT1a, 6 were pT1b, 4 were pT3a, 4 were pT3b, and 2 was pT4. Since the sarcomatous component of ECS is unlikely to metastasize, the prognosis is believed to be shaped by the characteristics of the epithelial component. Consistent with the literature, we found all lymph node and distant organ metastases to be consisted of carcinomatous components. ECS is a rare, and extremely aggressive malignancy with poor prognosis. There is still no typical laboratory finding or specific imaging for definitive diagnosis. Thus, the diagnosis of ECS can only be made following histopathological examination. Similarly, FIGO or pTNM staging can only be made after histopathological examination using the appropriate procedure.

Keywords: Endometrial Carcinosarcoma, Malignant Mixed Müllerian Tumor, Histopathology.

1. Introduction

Also called malignant mixed müllerian tumors, endometrial carcinosarcomas (ECS) are extremely rare (1).

Formerly in the uterine sarcoma group, these tumors are now considered as carcinomas with sarcomatous and carcinomatous components and monoclonal

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development (2, 3). Epidemiologically and clinically similar to endometrial adenocarcinomas, ECS is considered an aggressive variant of endometrial adenocarcinoma (4). With a very poor prognosis, ECS accounts for less than 5% of all uterine cancers but is responsible for over 15% of deaths. Similar to endometrial carcinomas, it is associated with obesity, nulliparity, exogenous estrogen, and tamoxifen use. Also, a history of exposure to pelvic radiation is associated with increased ECS risk (5). They often occur in the postmenopausal period. Anemia can be observed in 10% of patients due to vaginal bleeding (6). For over 10% of cases, signs of metastasis can be the first finding (1, 6). Pelvic ultrasonography (USG) is most often the first-line imaging method, although it cannot distinguish ECS from endometrial adenocarcinoma. Using Computed Tomography (CT) or Magnetic Resonance Imaging (MRI), ECS is often detected as a heterogeneous and polypoid mass that extends into the endocervical canal, with an intense increase for a long period of time. These two methods can help detect myometrial invasion depth, lymph node involvement, and metastasis (7). No typical laboratory finding has been associated with the diagnosis of ECS. However, an excessive increase of CA-125 may indicate more advanced disease (8). Still, the definitive diagnosis of ECS is only made after histopathological examination. Here, we aimed to share the clinicopathological characteristics of histopathologically diagnosed ECS cases, a rare malignancy.

2. Material and Methods

We included 26 materials diagnosed with ECS after necessary histopathological examinations that were sent to the Department of Medical Pathology of the Faculty of Medicine at Atatürk University between January 2013 – June 2020 as total abdominal hysterectomy materials. Beside total abdominal hysterectomy, bilateral salpingo oophorectomy and pelvic-paraaortic lymph node dissection were performed in all patients. Hematoxylin-Eosin (H&E) and immunohistochemical preparations and pathology diagnosis reports belonging to each case were obtained from the archives of our department; all cases were re-evaluated by two pathologists. pTNM staging was performed again according to the 8th Edition, Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) and clinical staging was performed again according to the International Federation of Gynecology and Obstetrics (FIGO) 2018. The histological type of carcinomatous and sarcomatous components, macroscopic tumor diameters, myometrial invasion depths, the presence or absence of regional lymph node metastasis, survival rates, and the presence or absence of homologous or heterologous components were investigated. Cases with unavailable clinical data or pathology preparation were excluded. Clinical characteristics like age and the year of the case were obtained from the database of our hospital. Approval was obtained from the ethics committee of the Faculty of Medicine at Atatürk University (08-01/10/2020).

3. Results

The patients had a mean age of 59 years, ranging from 51 to 67 years. Mean macroscopic tumoral diameter was 7.3 cm, ranging from 3 to 13 cm. The tumors were invasive to the 1/2 outer part of myometrium in 16 cases and to the 1/2 inner part of myometrium in 10 cases. According to the FIGO staging, 10 cases were stage IA, 2 was stage IB, 2 was stage IIIA, 10 were stage IIIC2, and 2 was stage IVB. According to pTNM pathological staging, 10 cases were pT1a, 6 were pT1b, 4 were pT3a, 4 were pT3b, and 2 was pT4. In 12 patient there was regional lymph node metastasis and in all case it consisted of carcinomatous component metastasis (12 were pN2a and 14 were pN0). Considering distant organ metastasis, 2 was M1 and 24 were M0. Lung metastasis was observed in 2 (8%) patient, and it consisted of carcinomatous component metastasis. Lymphovascular invasion was observed in 24 cases. 10 cases developed from the endometrial polyp floor. Cervical glandular and stromal involvement was observed in 10 cases.

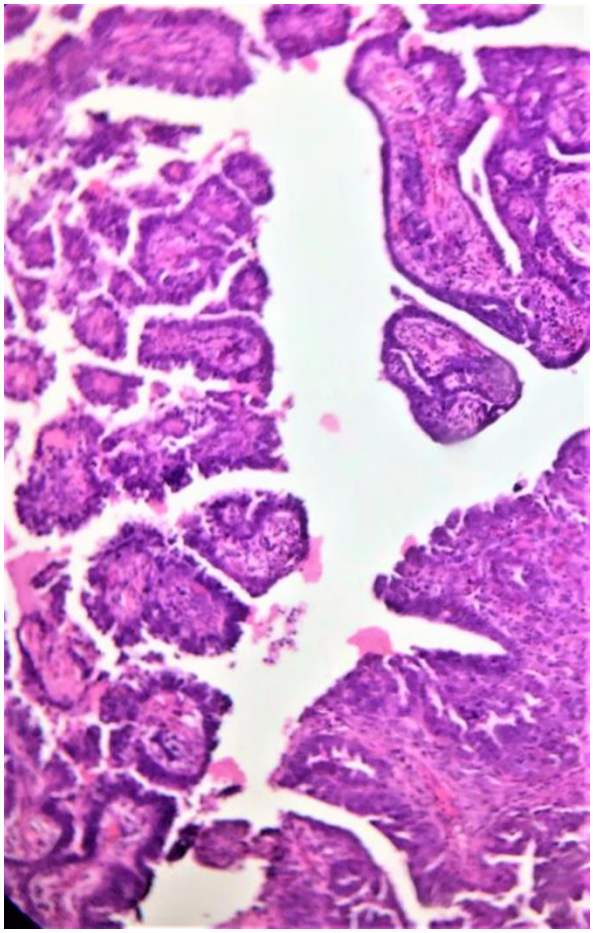

Figure 1: Serous carcinoma areas that form the epithelial component of carcinosarcoma.

The histological carcinoma component was serous carcinoma in 14 cases and endometrioid adenocarcinoma in 12. 6 of our cases were has heterologous sarcomatous components and the sarcomatous component was chondrosarcoma in all 6. 20 of our cases were has homolog sarcomatous components and the sarcomatous component was endometrial stromal sarcoma in 16 cases, fibrosarcoma in 2, leiomyosarcoma in 2. (Table 1 and Figures 1, 2, 3).

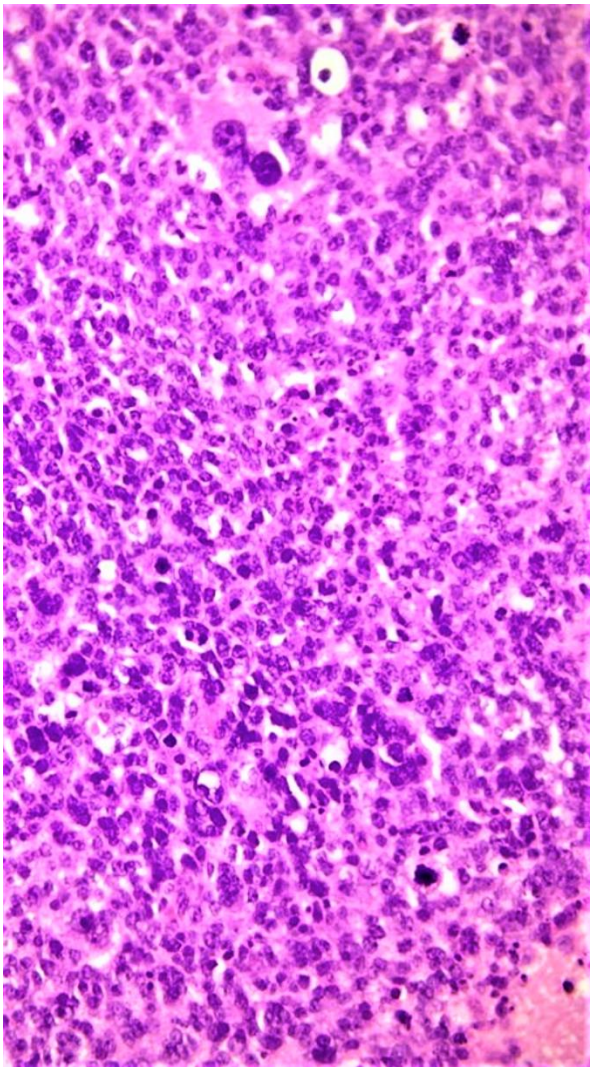

Figure 2: Areas of sarcomatous component of carcinosarcoma.

4. Discussion

ECS is a rare malignancy that often presents with an aggressive clinical picture and poor prognosis. Mean age of incidence has been reported as the seventh decade, with a median age of 62 to 67 years at diagnosis. (9). Bosquet et al. reported 95% of their patients to be in the postmenopausal period, with a mean age of 64.6 years (10). Similar to their findings, all our cases were in the postmenopausal period, with a mean age of 59 years.

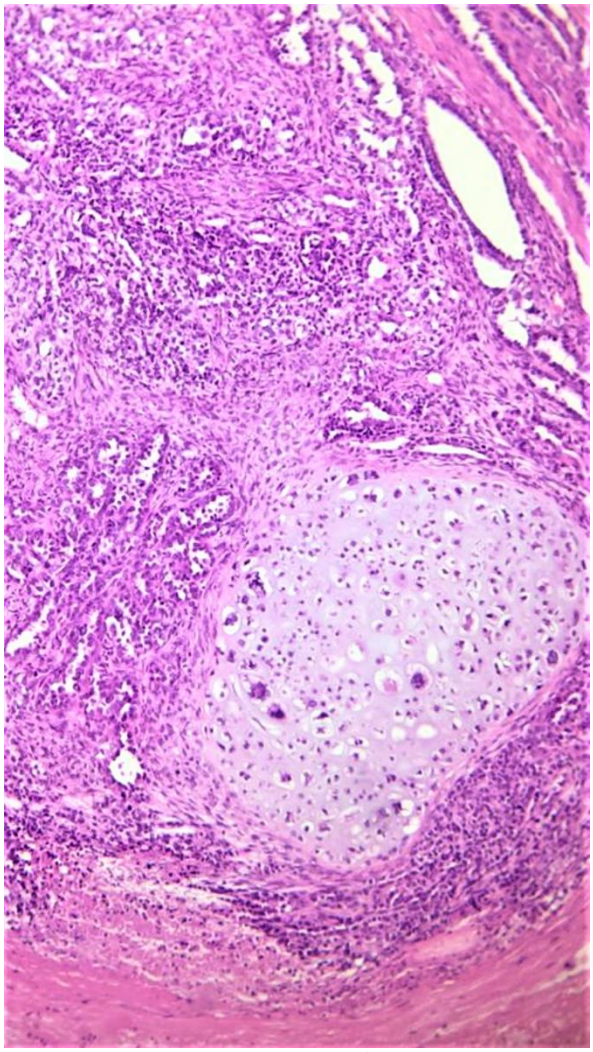

Figure 3: The carcinoma component of carcinosarcoma and the chondrosarcoma area that forms the heterologous element of the sarcomatous component.

ESC has a different biological behavior from other uterine sarcomas. The FIGO 2009 classification has included it among endometrial carcinomas due to similar risk factors and clinical behaviors (11). Recently, however, ECS is believed to develop from the metaplastic transformation of a single neoplastic cell type, arising from the epithelial-mesenchymal transition (12). It is divided into two groups as homologous and heterologous depending on the mesenchymal components (13). Homologous ECS contains mesenchymal components made up of tissues that are normally found in the uterus. On the contrary, heterologous ECS includes sarcomatous components that are not found in the uterus. Most ECS cases have a single sarcomatous component, most commonly high-grade endometrial stromal sarcoma (ESS) in homologous ECS. For heterologous ECS, the most common sarcomatous component is rhabdomyosarcoma (14). In the present study, the sarcomatous component was ESS in 62% of our cases, consistent with the literature. 6 of our cases were

heterologous and the sarcomatous component was chondrosarcoma in all 6, unlike the literature. Recent research reports that most ECS cases only have one carcinomatous component, most commonly high-grade serous carcinoma. In rarer cases, it may be in the form of endometrioid adenocarcinoma or clear cell carcinoma (14). According to our findings, 54% of the carcinoma components were high-grade serous carcinomas, similar to the latest knowledge in the literature.

Table 1: Demographic and Histopathological Features of Cases.

Age	Tumor Diameter (cm)	pT	pN	pM	FİGO Stage	Carcinoma Component	Sarcoma Component
52	8	pT1a	pN0	pM0	IA	Serous Carcinoma	Endometrial Stromal Sarcoma
67	12	pT3b	pN2a	pM0	IIIC2	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
51	3.5	pT1a	pN0	pM0	IA	Endometrioid Carcinoma	Fibrosarcoma
58	5	pT1b	pN2a	pM0	IIIC2	Serous Carcinoma	Endometrial Stromal Sarcoma
65	7.5	pT1b	pN0	pM0	IB	Serous Carcinoma	Endometrial Stromal Sarcoma
56	6	pT3a	pN0	pM0	IIIA	Serous Carcinoma	Leiomyosarcoma
67	8.4	pT4	pN2a	pM1	IVB	Serous Carcinoma	Endometrial Stromal Sarcoma
54	3	pT1a	pN0	pM0	IA	Endometrioid Carcinoma	Chondrosarcoma
55	13	pT1a	pN0	pM0	IA	Serous Carcinoma	Chondrosarcoma
60	3,5	pT1b	pN2a	pM0	IIIC2	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
61	9	pT3a	pN2a	pM0	IIIC2	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
66	12	pT3b	pN2a	pM0	IIIC2	Serous Carcinoma	Chondrosarcoma
52	3.5	pT1a	pN0	pM0	IA	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
66	11	pT3b	pN2a	pM0	IIIC2	Serous Carcinoma	Endometrial Stromal Sarcoma
66	6.5	pT1b	pN0	pM0	IB	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
53	2.4	pT1a	pN0	pM0	IA	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
54	12	pT1a	pN0	pM0	IA	Serous Carcinoma	Chondrosarcoma
54	4.5	pT1a	pN0	pM0	IA	Serous Carcinoma	Fibrosarcoma
56	6	pT1b	pN2a	pM0	IIIC2	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
52	4	pT1a	pN0	pM0	IA	Endometrioid Carcinoma	Fibrosarcoma
62	10	pT3a	pN2a	pM0	IIIC2	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
54	3	pT1a	pN0	pM0	IA	Endometrioid Carcinoma	Chondrosarcoma
59	4.5	pT1b	pN2a	pM0	IIIC2	Endometrioid Carcinoma	Endometrial Stromal Sarcoma
61	8.5	pT3a	pN2a	pM0	IIIC2	Serous Carcinoma	Endometrial Stromal Sarcoma
65	11	pT3b	pN2a	pM0	IIIC2	Endometrioid Carcinoma	Chondrosarcoma
67	9.5	pT4	pN2a	pM1	IVB	Serous Carcinoma	Endometrial Stromal Sarcoma

Like in endometrial carcinomas, metastasis can be observed in regional lymph nodes such as paraaortic and pelvic lymph nodes, peritoneal surfaces, distant organs, and particularly the lungs. Due to the aggressive behavior of ECSs, distant organ metastasis occurs in over 10% of patients at initial diagnosis (1). Similar to the literature, in our study, lung metastasis was observed in 2 (8%) patient, and it consisted of carcinomatous component metastasis. Since the sarcomatous component of ECS is unlikely to

metastasize, the prognosis is believed to be shaped by the characteristics of the epithelial component (1). Here, we observed lymph node metastasis in 12 (46%) cases, all consisting of carcinomatous components, again similar to the literature. In ECS, most of the patients are advanced staged at the time of diagnosis. There were 71 cases of ECS in the studies of Akahira et al. 36 of them (51%) were advanced stage cases (28 at stage I, 7 at stage II, 24 at stage III and 12 at stage IV) (4). Similar to Akahira et al's study in our cases, 14 (54%) were at the advanced stage (2 at stage IIIA, 10 at stage IIIC2, and 2 at stage IVB) and 12 (46%) were at stage I at diagnosis.

Staging should be done with taking into consideration the pathological TNM staging by UICC/AJCC and the clinical staging by FIGO. Although mostly parallel, the pTNM staging and FIGO clinical staging have certain key differences. In clinical staging, the presence of regional lymph node metastasis (presence of pelvic lymph node metastasis–stage IIIC1; presence of paraaortic lymph node metastasis–stage IIIC2) FIGO classifies as stage IIIC (15, 16). Temkin et al. identified the presence of lymph node metastasis in ECS as a poor prognostic factor, highlighting the significance of extensive lymph node dissection (17). Of our cases, 4 with pT1b, 2 with pT3a, and 4 with pT3b based on pathological staging were classified as stage IIIC2 according to FIGO clinical staging due to paraaortic lymph node metastasis. Considering our findings and the findings in the literature, we believe that cases with insufficient lymph node sampling are ineligible for proper FIGO staging and patients cannot benefit from optimal treatment protocols.

It can be difficult to distinguish ECS from uterine sarcoma using a prominent mesenchymal component (sarcomatous overgrowth), which emphasizes the significance of proper pathological-anatomical examination (13). For a correct diagnosis, both components should be observed in the histopathological examination of the uterus (18). Symptoms, imaging methods, and/or laboratory findings cannot distinguish ECS from endometrial carcinoma or uterine sarcoma. Endometrial sampling by endometrial biopsy or curettage is most often performed before surgery. Since ECS has endometrial origins, endometrial sampling is more appropriate for diagnosis. Alas, small biopsy materials that do not reflect the entire lesion are apparently not an accurate test for diagnosing ECS (19). Since the diagnosis of ECS is based on histomorphologically demonstrating carcinomatous and sarcomatous components along with stroma invasion, definitive diagnosis depends on the pathological evaluation of the hysterectomy material.

5. Conclusions

ECS is a rare, and extremely aggressive malignancy with poor prognosis. According to the latest staging guidelines, it is classified among high-grade endometrial carcinomas that include sarcomatous metaplasia. There is still no typical laboratory finding or specific imaging for definitive diagnosis. Thus, the diagnosis of ECS can only be made following histopathological examination. Similarly, FIGO or pTNM staging can only be made after histopathological examination using the appropriate procedure.

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Conflict of Interests
All authors declared that there is no conflict of interest.

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Author Contributions
Ceylan O and Özmen S originally conceived the idea and hypothesis. Özmen S designed the study. Ceylan O made the research organization. Özmen S collected the data. Ceylan O interpreted the results. Ceylan O and Özmen S drafted the manuscript. All authors reviewed and approved the manuscript.

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Evaluation of Main Inflammatory Markers on Peripheral Vertigo Attack

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Abstract: The aim of this study is to evaluate the main inflammatory markers; neutrophile lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR), mean platelet volume (MPV), C-reactive protein (CRP), lactate dehydrogenase (LDH) and creatine kinase (CK) in patients with acute peripheral vertigo attack. The records of patients files and laboratory results were analyzed retrospectively. The measures of complete blood count and basic biochemical test were compared in acute peripheral vertigo attack between patient and control group. The sample consists of 119 patients and 98 healthy controls are included in the control group. There is not any statistically significant change of main inflammatory measures between two groups. Peripheral vertigo can be caused by many diseases with various pathologies. There may be various mechanisms besides inflammation and atherosclerosis of microvascular structures. The results of this study states that etiology of peripheral vertigo attack may not be related with ischemia and inflammation; it may be related with viral etiology.
Keywords: Vertigo, Inflammation Markers, Neutrophile Lymphocyte Ratio, Platelet Lymphocyte Ratio.

1. Introduction

Vertigo is estimated to affect 20% to 30% of people at some point in their lifetime. Vertigo attack is one of the common reasons for admission to the emergency department of the hospital (1). Vertigo is the perception of movement either of the self or of the surrounding objects, and is usually refers to a feeling of rotation, swaying, or tilting of the body or surrounding environment (2). The cause of vertigo may be central due to central nervous system pathologies or peripheral due to diseases effecting the inner ear and vestibulocochlear nerve. The most common peripheral vestibular disorders in patients with vertigo include Meniere's disease, benign paroxysmal positional vertigo, vestibular neuritis, labyrinthitis, perilymphatic fistula and acoustic neuroma (3).

Complete blood cell count and routine biochemical parameters of blood provide useful information about patient's general health condition. Therefore, these laboratory tests are applied first nearly all patients who admitted to the emergency department with vertigo attack. The neutrophile to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and mean platelet volume (MPV) have been suggested as an indicativemarkers of systemic inflammation and thrombotic events (4). Higher levels of NLR and PLR were detected in patients with cardiovascular,

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oncological and neurological diseases as well as some otological diseases like facial paralysis and idiopathic sensorineural hearing loss (5). Atherosclerosis and inflammation in microvascular structures of inner ear are detected in patients with higher level of NLR, PLR and MPV (6). C-reactive protein (CRP), lactate dehydrogenase (LDH) and creatine kinase (CK) are novel potential markers that can be easily calculated in routine biochemical blood test. Clinical inflammatory status may be predicted by evaluating these laboratory tests (7). These markers are useful for scanning and detecting the prognosis of diseases with inflammatory origin.

In the otolaryngology field, several authors have described potential relationships with peripheral vertigo and inflammatory parameters. Possible relationship of these parameters with vertigo provides more convenience in early diagnosis and treatment in emergency department especially for practitioners. More literatures are required for this issue to enlighten this possible inflammatory etiology and availability of these laboratory parameters in emergency department for acute peripheral vertigo attack management. Therefore; the evaluation of the changes of these laboratory results is more important for determining the significance of usage of these parameters in the management of acute peripheral vertigo attack. The aim of this study is to assess the significance of NLR, PLR, MPV, CRP, LDH and CK in patients with acute peripheral vertigo attack and to compare the levels of these parameters in patients with peripheral vertigo attack and healthy controls by scanning the patient files retrospectively.

2. Material and Methods

This retrospective study included 119 patients who had acute peripheral vertigo attack and 98 healthy controls from January 2019 to July 2020 in the second degree hospital. The control group was composed of subjects who underwent complete blood cell count and basic biochemical test as part of routine check up. The patient group was composed of subjects who admitted to emergency department of the hospital with acute peripheral vertigo complaint.

All patients underwent detailed neurological, otological and cardiovascular examinations by practitioner duty on emergency department. Complete blood cell count, basic biochemical test, electrocardiography (ECG) and cranial imaging were performed for all patients. Main inflammatory markers were evaluated to determine the general health status at the admission of the emergency department. Exclusion criteria were: abnormal neurological examination, subsequent diagnosis with central nervous system disease, abnormal cranial imaging, sudden hearing loss and abnormal ECG findings. Patients who had acute vertigo, nausea, vomiting, postural instability and absence of sudden hearing loss, otitis media and neurological signs were included in this study. Patients who developed vertigo symptoms within 3 days and patients with new onset of symptoms were included in this study to discriminate patients with chronic peripheral vertigo. These patients were considered as presented with acute peripheral vertigo attack.

The demographic findings and laboratory data were screened from the database of our hospital and recorded. Date were analyzed and compared between patient and control groups. The NLR and PLR were calculated as a simple ratio between absolute neutrophile to lymphocyte counts and platelets to lymphocyte counts. MPV, CRP, LDH and CK levels were recorded and analyzed between groups.

2.1. Statistical Analyses

The collected data were exported to SPSS version 19.0 for Windows (IBM, Armonk, NY) for statistical analyses. Descriptive data were expressed as mean ± standard deviation. The independent samples t test was used for comparison of two groups. Statistical significance was defined as $p<0,05$.

Sample size of the study was planned due to the time interval (Jan 2019-July 2020) independent from power analysis. The power analysis of the study was calculated after data collection. G Power version 3.1.9.7 for Windows (HH Düsseldorf University, Germany) were used for power analysis. Actual power of the study was calculated as 0,9506.

3. Results

The sample group consists of 119 patients; the mean of age was 48,1±16,68 years and 64,72% was female and 36,28% was male. The control group consists of 98 patients; the mean of age was 38,03±13,85 years and 54,08% was female and 45,92% was male.

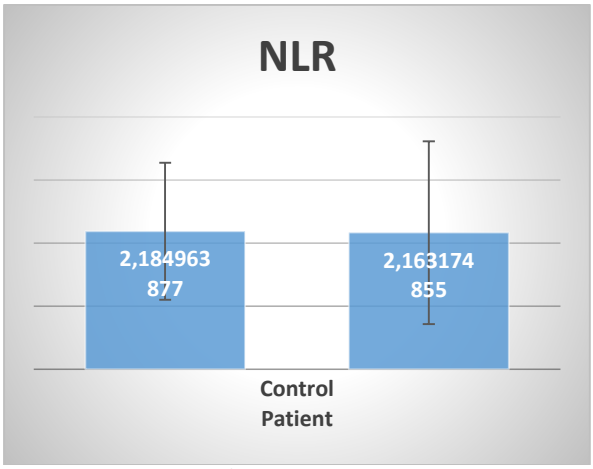

Figure 1: Comparison of neutrophile to lymphocyte ratio (NLR) between the patient group and the control group.

The mean of NLR value of patient group was 2.16±1.44 and the mean of NLR value of control group was 2.18±1.08 (Table 1). There is not statistically significant change between two groups in this ratio (Figure 1).

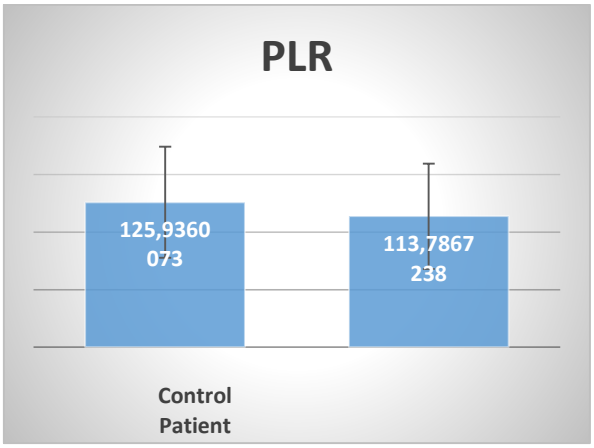

Figure 2: Comparison of platelet to lymphocyte ratio (PLR) between the patient group and the control group.

The mean of PLR value of patient group was 113.78±45.73 and the mean of PLR value of control group was 125.93±48.22 (Table 1). There is not statistically significant change in PLR between two groups (Figure 2).

The mean of MPV value of patient group was 9.99±0.98 and the mean of MPV value of control group was 10.19±0.97. There is not any significant change observed between two groups. The mean of CRP, LDH and CK were 5.86±5.58, 190.86±49.47 and 80.63±63.49 in the patient group. The mean of CRP, LDH and CK were 4.16±3.21, 180.88±40.74 and 97.25±49.45 in the control group (Table 1). There is not any statistically significant relation observed also (Figure 3).

4. Discussion

Symptoms such as nausea, vomiting, sweating, nystagmus and bradycardia are commonly seen in patients with peripheral vertigo. Attacks of vertigo can seem frightening to patients; anxiety and worsening of emotional status of patient are commonly observed. This instable condition may cause serious events like increasing the levels of stress related hormones and cytokines (8). These events cause stress and inflammation therefore changes of inflammatory markers may be observed. The inflammatory process induces some changes in peripheral blood cells and changes of the biochemical parameters in the blood serum (9).

Compete blood cell counts are widely used to evaluate patients' general condition so it is the first and basic test in peripheral vertigo attack with basic biochemical test in the emergency department. NLR and PLR have been proposed as inflammatory markers. These ratios have been used in otolaryngology, cardiovascular medicine and oncology to evaluate the inflammatory status and to assess the prognosis of the disease. MPV, CRP, LDH and CK indicates the inflammatory status as well, can easily calculated from basic blood test (10). These markers are highly repeatable, inexpensive and available predictors for detecting systemic inflammation moreover they are frequently used and evaluated quickly in emergency department.

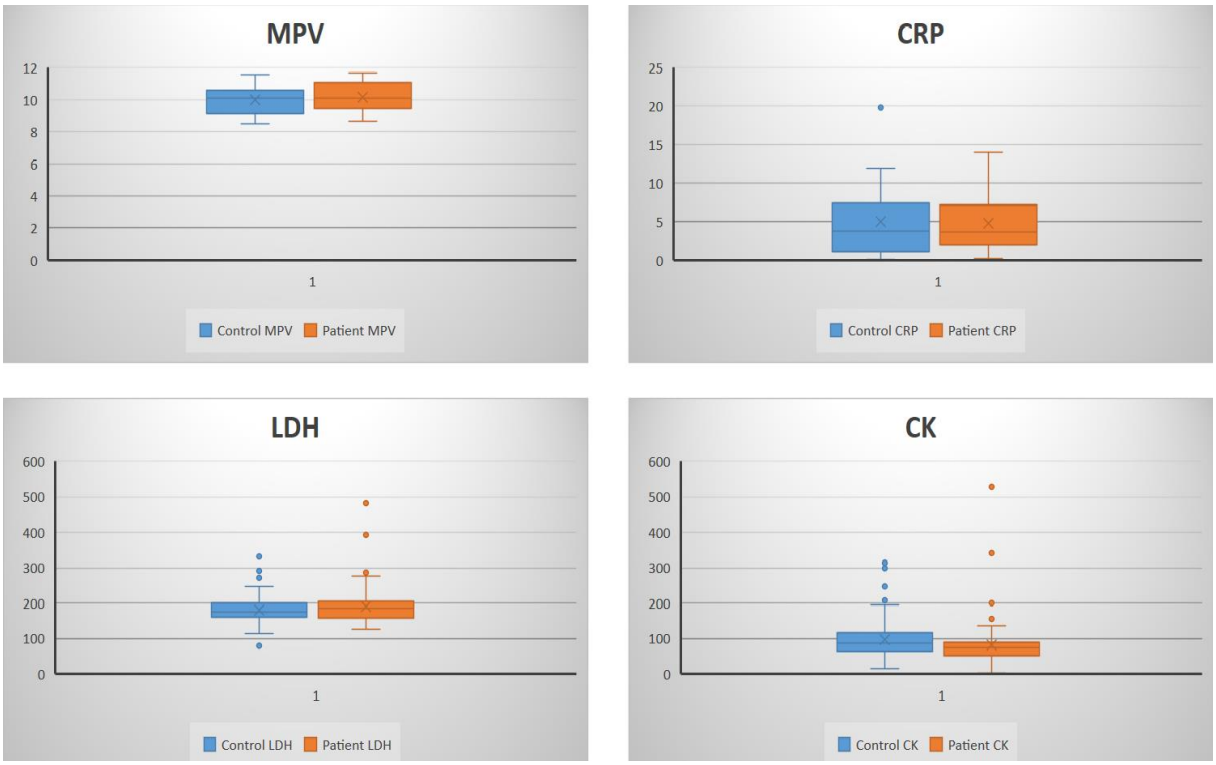

Figure 3: Comparisons of mean platelet volume (MPV), C reactive protein (CRP), lactate dehydrogenase (LDH), creatine kinase (CK) between the patient group and the control group.

Table 1: Results of Age, Sex, NLR, PLR, MPV, CRP, LDH and CK between two groups.

	Control Group n=98	**Patient Group n=119**	**Statistical Significance**
Age	38.03±13.85	48,1±16,68	
Sex (Female)	54.08%	64,72%	
NLR	2.18±1,08	2.16±1.44	t value: 10.689 p value : 0.136
PLR	125.93±48.22	113.78±45.73	t value: 6.504 p value : 0.122
MPV	10.19±0.97	9.99±0.98	t value: - 0.211 p value : 0.832
CRP	4.16±3.21	5.86±5.58	t value: - 0.196 p value : 0.844
LDH	180.88±40.74	190.86±49	t value: - 1.599 p value : 0.111
CK	97.25±49.45	80.63±63.49	t value: 2.454 p value : 0.114

NLR is a potential marker of inflammatory state and a predictor of prognosis in many diseases like coronary artery disease, renal, thyroid disease and solid tumors. Ozler et al. investigated the relationship between NLR and facial paralysis and there was a correlation between NLR values and prognosis of facial palsy (11). PLR and MPV have been proposed as a marker indicating platelet activation and inflammation associated with cardiovascular and cerebrovascular diseases. Gary et al. have concluded that a higher levels MPV and increase in platelet counts may lead to inflammation and ischemic events (12). Sagit et al. and Ulu et al. have found increased level of MPV in idiopathic sensorineural hearing loss (13, 14). Sea et al. demonstrated that NLR and PLR value were significantly higher in the patients with idiopathic sensorineural hearing loss (6). While the etiologies are uncertain, the proposed mechanisms are similar for sudden hearing loss and vertigo. The results of these studies may support the role of inflammation and thrombosis in peripheral vertigo attack. Recently, Ozbay et al. investigated NLR in peripheral vertigo patients. In that study, higher NLR in patients group was interpreted and they concluded that the potential inflammation and thrombotic mechanisms in the formation of vertigo (15). According to these literatures; the inflammatory response may contribute to thrombosis, then results to microvascular occlusion and labyrinthine ischemia and vertigo attack may be formed due to this process. However, it is difficult to suggest that this mechanism is accurate.

Peripheral vertigo can be caused by many diseases with various pathologies. Therefore, there may be various mechanisms besides inflammation and atherosclerosis. However, there was not any correlation found in these markers between two groups in this study. Temirbekov et al. also investigated NLR, PLR and MPV in peripheral vertigo and found no difference in the patient's group like our study (1). The authors who found that some relationships with these markers with vertigo, think that ischemic and inflammatory etiology could be a basis of vertigo. The results of this study states that etiology of peripheral vertigo attack may not be related with ischemia and inflammation it may be related with viral etiology. In this study, patient's group consists of acute peripheral vertigo attack, this group is very heterogenous with many different etiologies. Insignificant result of this study may be related to this heterogeneity.

The main limitations of this study are small sample size and type of method. Retrospective patient file evaluation is the important disadvantage of this study. Power analysis were calculated after data collection so sample size could not be planned at the beginning of the study. Detailed neuro-otological examination and vestibular tests could not applied for differential diagnosis of peripheral vertigo therefore various types of peripheral disorders were evaluated at the same time in this study. The insignificant result can be related with evaluating the various types of peripheral disease.

5. Conclusions

NLR, PLR, MPV, CRP, LDH and CK can reflect the inflammatory status of body. When evaluating the inflammatory diseases or diseases with inflammatory etiology, these markers should be taken into consideration. Acute peripheral vertigo attack can be caused by many diseases with various pathologies. Diagnosis or predicting the prognosis of peripheral vertigo may not be managed with evaluation of these markers. In this study, there is not any statistically significant relation with these markers in peripheral vertigo. Detailed neuro-otological examination and vestibular tests are still good and useful methods for

peripheral vertigo. More comprehensive, detailed prospective studies with large groups can be useful for detection of the importance of these markers in peripheral vertigo.

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Conflict of Interests
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Author Contributions
Dilci A and Cevizci R contributed to the conception and design of the study. Dilci A contributed to the collection of the data and statistical analysis and evaluation of the results. Dilci A contributed to the creating and writing of manuscript. Cevizci R contributed to revising the work and final approval of the version.

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Evaluation of Carotid Artery Plaques with B-Flow Sonography and Comparing the Results with Color, Power Doppler US and DSA

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Abstract: To evaluate carotid artery lesions with B-flow sonography and compare the findings with color, power Doppler sonography and digital subtraction angiography (DSA). Forty-Four patients (30 males, 14 females; mean age 64±9; age range: 42-81) with 61 carotid artery lesions were involved in the prospective study. The lesions were evaluated with color, power Doppler and B-flow sonography. All images were reviewed and graded independently by two radiologists for surface delineation, plaque morphology and overall image quality. The grades for each technique were compared with Friedman and Wilcoxon tests. Simple regression analysis was used to compare the percentage of stenosis calculated with different sonographic techniques and DSA for lesions causing more than 60% stenosis. Statistical analysis with Friedman test revealed that B-flow is superior to other sonographic techniques for all three parameters (plaque surface delineation, plaque morphology and overall image quality). Wilcoxon test also showed B-flow is superior to other methods for the evaluation of plaque surface delineation and plaque morphology (p=0.000); while no significant difference was found between B-flow and power Doppler imaging for the evaluation of overall image quality (p=0.09). Kappa scores reflected moderate to good interobserver correlation (0.297-0.659). The percentage of stenosis calculated with both B-flow and power Doppler sonography correlated with DSA significantly (p=0.000). B-flow sonography is a technique that provides visualization of the blood flow and the morphology of the surrounding vessel wall simultaneously. This technique maintains more efficient evaluation of carotid artery lesions by eliminating artifacts such as aliasing and overwriting.

Keywords: Ultrsonography, B-Flow, Doppler, Atherosclerosis, Carotid Artery, Stenosis.

1. Introduction

In westernized societies, atherosclerosis is one of the leading causes of ischemic stroke (1). Especially atherosclerosis of internal carotid artery (ICA) is one of the major causes of stroke. The stroke risk can be reduced with early diagnosis and appropriate treatment (2, 3). Large multicenter trials such as North American

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Symptomatic Carotid Endarterectomy Trial (NASCET) and European Surgery Trial (ECST) have demonstrated that the stenosis grade of ICA is particularly important in therapy planning. Recent studies showed that surface characteristics and morphology of atheroma plaques are also among predictors of stroke risk (4-6).

Digital subtraction angiography (DSA) has been accepted as the "gold standard" in the evaluation of ICA-stenoses with a sensitivity, specificity, and accuracy of 95%, 99%, and 97%, respectively (7). On the other hand, DSA has shortcomings such as high cost, invasiveness, ionizing radiation, risk of nephrotoxicity, and neurologic complications which initiates the search for alternative diagnostic methods for evaluation of ICA-stenosis.

Computerized tomography (CT) is one of the modalities frequently used in the evaluation of ICA-stenosis. Main disadvantages are the use of ionizing radiation, risk of nephrotoxicity, suboptimal stenosis grading especially in calcified plaques (8).

Magnetic resonance imaging (MRI) without the need for ionizing radiation and good sensitivity particularly for the evaluation high-grade stenosis is an alternative method for carotid stenosis detection. However relatively high costs, long examination duration, unable to perform in patients who have a pacemaker or claustrophobia limits its usage (8, 9).

Color Doppler sonography (with many advantages e.g., wide accessibility, noninvasive nature, application without ionizing radiation exposure) is one of the most used methods in evaluating carotid arteries. On the other side color doppler sonography has its own limitations; B-flow sonography is a relatively new method that may overcome the shortcomings of color Doppler ultrasonography. With B-flow ultrasound, it is possible to image blood flow and vessel wall simultaneously without the limitations of Doppler imaging such as loss of signal at some detection angles, limitations of wall filter and aliasing. In this study, we aimed to evaluate carotid artery lesions with B-flow sonography and compare the findings with color, power Doppler sonography and digital subtraction angiography (DSA).

2. Material and Methods

2.1. Data collection and Patients

This is a single-center study performed at Gazi University Hospital. The study was approved by the Ethics Committee of Gazi University, and all the patients signed informed consent forms.

From March to December 2004, 44 consecutive patients (14 females, 30 males) who had atherosclerosis in ICA were prospectively included. The mean patient age was 64±9 (range, 42-81 years). Patients who have ICA stenosis>60% were evaluated with DSA.

2.2. Image assessment

All color doppler, power doppler, and B-flow examinations were performed with LOGIQ sonography system (GE Medical Systems, Milwaukee, WI, USA) with the aid of a 4-10 MHz linear transducer. During the color and power doppler examinations related parameters (color gain, wall filter, and velocity scale) were optimized to minimize artifacts. During B-flow imaging "sensitivity" was set to 8, "background: on", and focus was adjusted posterior to vessel. In all three methods, gain was set to obtain the best possible image quality. Images and movies were recorded via "Logiqworks" archiving system (GE Medical Systems, Milwaukee, WI, USA).

Plaque surface delineation, morphology of plaques and overall image quality were independently assessed by two experienced radiologists using a three-point scale (1=the plaque was vaguely characterized/image quality: poor, 2=the plaque was moderately characterized/ image quality: average, and 3=the plaque was clearly characterized/image quality: excellent).

20 patients who had ICA-stenosis>60% were also evaluated with DSA. DSA was performed via a 5F catheter. Left and right carotid arteries were visualized at least in two projections. For each projection 6 ml, 320 mg/ml non-ionic iodinated contrast media was used.

The ICA-stenosis grades from the images of ultrasonography and DSA were independently measured by two radiologists.

2.3. Statistical Analysis

Statistical analysis was performed with the aid of SPSS for Windows (SPSS, IBM, USA). Values are presented as means ± standard deviations and ranges. The comparisons of quantitative data were evaluated using Friedman and Wilcoxon tests. Correlation between stenosis grades measured with Color Doppler, power Doppler, B-flow and DSA were analyzed with simple regression analysis. Interobserver agreement was analyzed via Kappa score. p-value<0.05 was considered to indicate a statistically significant difference.

3. Results

Of the total 61 atheromatous plaques; 32 (54.2%) were at right ICA, 21 (35.6%) plaques were at left ICA, 3 (5.1%) were at left external carotid artery (ECA) and 1 (1.7%) was at right ECA.

There was a statistically significant difference among the three parameters between B-flow, color, and power Doppler sonography (p=0.000) (Table 1).

Table 1: Friedman test results.

	Surface delineation	**Plaque morphology**	**Overall image quality**
Color Doppler	1.43	1.67	1.48
Power Doppler	1.81	1.80	2.15
B-Flow	2.75	2.52	2.37

Lowest value 1 represents poor image quality, where highest value 3 represents excellent image quality.

Post hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a significance level set at $p<0.017$. In comparison to power and color doppler sonography B-flow sonography found to be superior in the assessment of plaque surface and morphology ($p=0.000$). In terms of overall image quality B-flow and power Doppler sonography were found to be superior to color Doppler sonography ($p=0.000$). However, in terms of overall image quality, there was no significant difference between B-flow sonography and power Doppler sonography ($p=0.09$) (Table 2).

Table 2: Wilcoxon test results.

Surface delineation	Plaque morphology	Overall image quality
PD>CD ($p=0.000$)	PD>CD ($p=0.000$)	PD>CD ($p=0.000$)
BF>CD ($p=0.000$)	BF>CD ($p=0.000$)	BF>CD ($p=0.000$)
BF>PD ($p=0.000$)	BF>PD ($p=0.000$)	BF=PD ($p=0.009$)

CD: Color Doppler; PD: Power Doppler; BF: B-flow.

Kappa score showed moderate to good interobserver agreement (0.297-0.659) (Table 3).

Table 3: Interobserver agreement (Kappa score).

	Surface delineation	Plaque morphology	Overall image quality
Color Doppler	0.576	0.319	0.659
Power Doppler	0.350	0.297	0.316
B-flow	0.400	0.435	0.374

In patients who had stenosis greater than 60% were also compared in terms of stenosis grade with B-flow, power/color Doppler sonography and DSA. Figure 1 summarizes the stenosis grade of 20 patients who were evaluated with DSA.

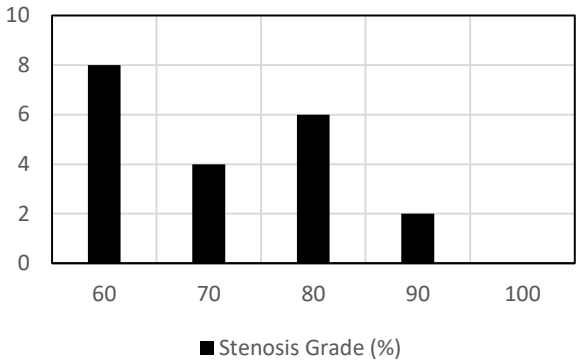

Figure 1: Distribution of stenosis grade of patients who were evaluated with DSA.

The serum MPO levels of MS patients (median=9192.30) were lower than the MPO levels of the healthy control group (median=1076.91) and this decrease was statistically significant (p=0.034) (Table 1). No significant correlation was found between MS patients' serum MPO levels and age, EDDS score, disease duration, ARR (p>0.05) (Table 2). There was no significant difference between the serum MPO levels of MS patients, and the DMT groups used (p=0.558) (Table 2).

When MS patients were classified by disease types, the disease duration and EDSS score of SPMS patients were significantly higher compared to RRMS patients, but there was no significant difference between these two groups in terms of age, ARR and MPO levels (Table 3).

All three sonography methods showed statistically significant correlation with DSA in terms of stenosis measurement ($p<0.0001$). Correlation coefficient between B-flow sonography and catheter angiography ($p_{BF}=0.969$) was higher than power and color doppler sonography ($r_{PD}=0.894$, $r_{CD}=0.886$, respectively). Figure 2 shows linear regression analysis results of color Doppler and B-flow sonography.

In stenosis grading, the difference between B-flow sonography and DSA was -6% to +5%, whereas the difference between power Doppler and DSA was -16% to +8%. DSA and B-flow sonography showed among 15 (75%) patients the highest correlation (Figure 3). On the other hand, DSA and power Doppler sonography showed among 3 (15%) patients, DSA and Doppler sonography showed among 2 (10%) patients the highest correlation.

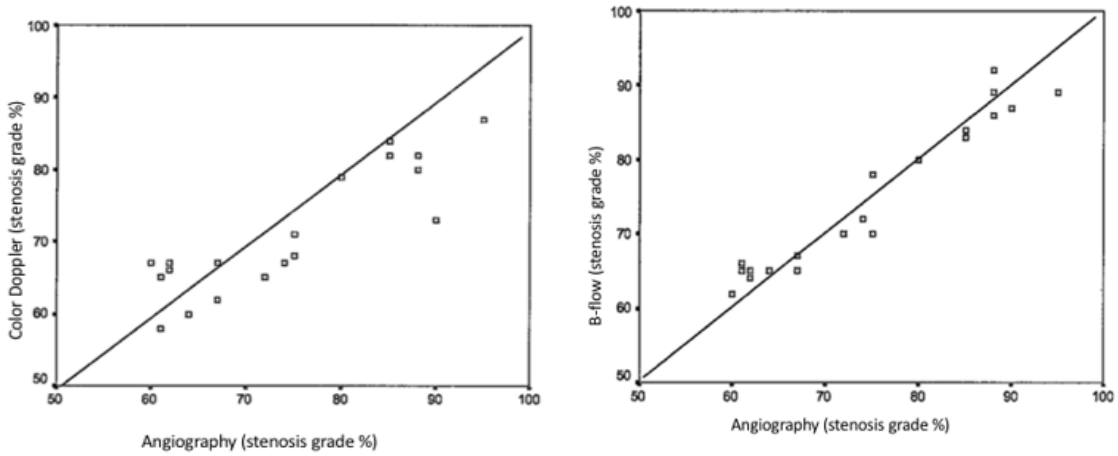

Figure 2: Linear regression analysis results.

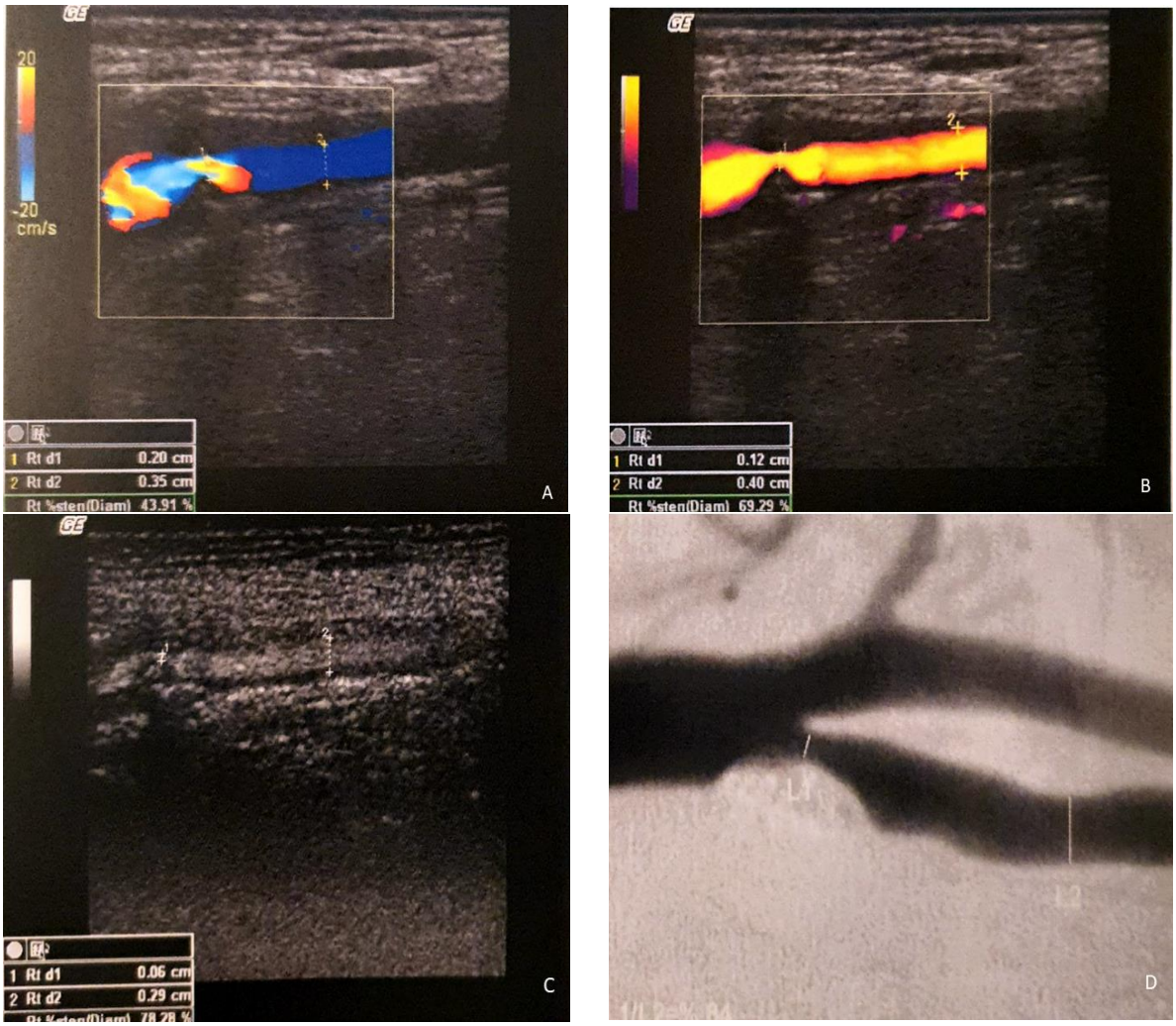

Figure 3: 70-year-old male patient with stenosis in left internal carotid artery graded as 44 % in color Doppler **(A)**, 69% in power Doppler **(B)**, and 78% in B-flow **(C)**. Here B-flow imaging correlated well with DSA **(D)** which graded the stenosis as 84%.

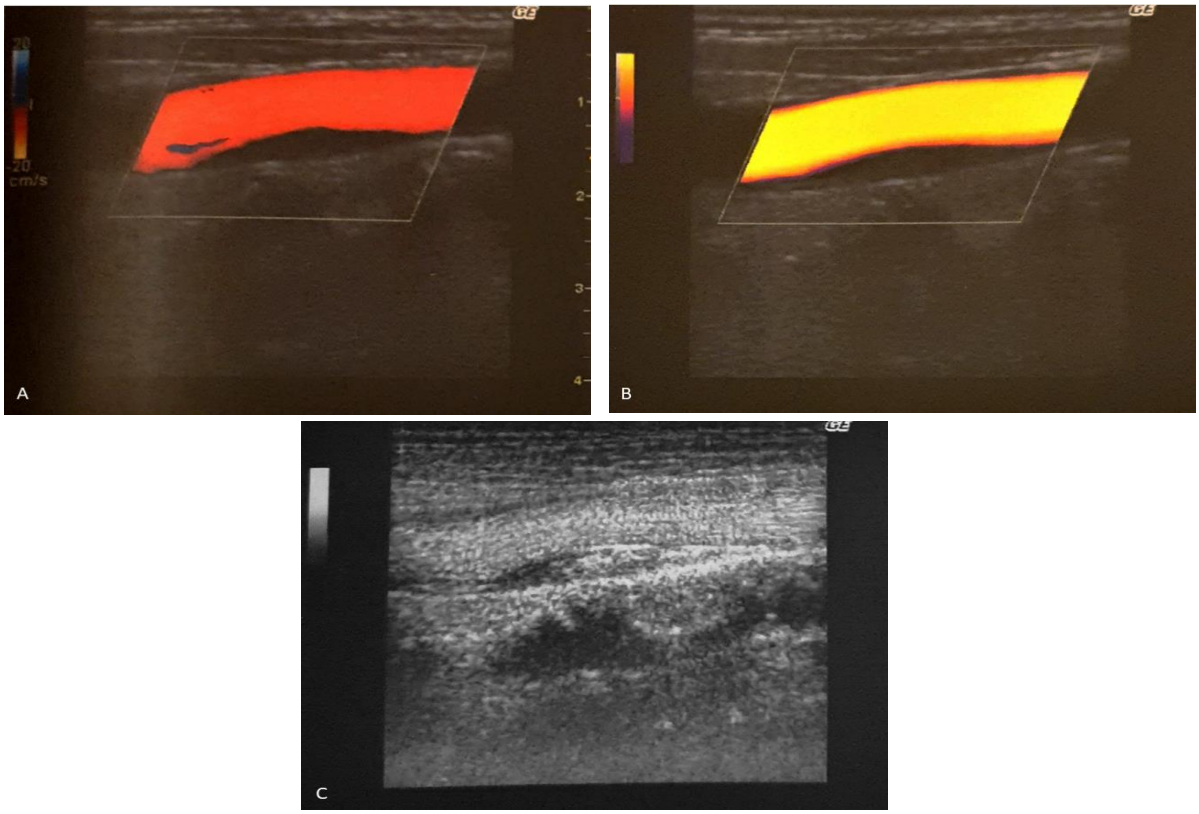

Figure 4: 59-year-old male patient with a fibrofatty plaque in the right common carotid artery. Plaque surface delineation and morphology cannot be optimally visualized with color **(A)** and power Doppler **(B)** imaging. With B-flow imaging **(C)** the heterogeneous structure of the plaque and the surface delineation can be visualized.

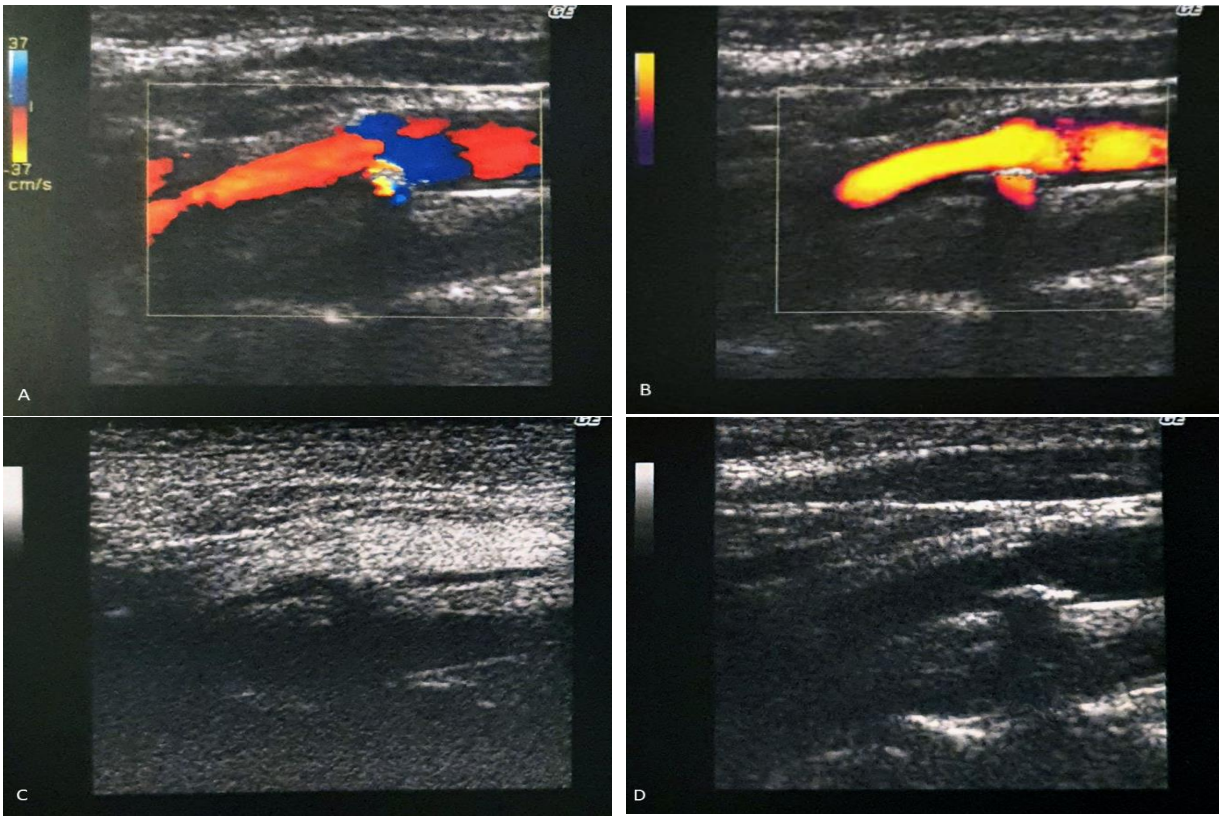

Figure 5: 74-year-old male patient color with a calcified plaque in the carotid artery. Color **(A)** and power Doppler **(B)** suffer from "twinkling artifact" seen posterior to the calcified atheroma plaque. B-flow **(C)** demonstrates the plaque and flow without artifacts. Characteristics of plaque in B-mod **(D)**.

4. Discussion

Atherosclerosis of carotid arteries is a common disease which causes high mortality and morbidity. Defining the nature of atheroma plaque, as well grading the stenosis plays an important role in determining the appropriate treatment. Although DSA is the traditional method in carotid artery stenosis measurement, because of high costs, ionizing radiation and complication potential there is a search for alternative noninvasive methods (10-12).

Large population trials like NASCET and ECST showed that in high-grade carotid artery stenosis, patients get more benefit from carotid endarterectomy in comparison to medical treatment. It is shown that symptomatic patients have a larger intraplaque hemorrhage in comparison to asymptomatic patients (13, 14). Another study confirmed that patients who have type 1 and 2 plaques encounter more transient ischemic attacks (15, 16). These studies showed that the assessment of plaque morphology may be as important as grading stenosis.

Color and power Doppler sonography have some technical limitations in plaque morphology imaging. In color and power Doppler sonography, grayscale and flow images were generated separately with different techniques. Color codes generated with Doppler sonography technique are overwritten over gray-scale images. The gray-scale information of the vessel wall and plaques were partially covered by color codes of Doppler imaging. Aliasing artifact which is seen in high grade stenosis also limits the evaluation of plaque morphology. Separately generating grayscale and Doppler images demands high computational power which causes a drop in frame rate and spatial resolution. Angle dependent nature of Doppler sonography may also limit assessment of carotid artery stenosis. Increased flow velocity is a good predictor of high-grade stenosis. However, in patients with arrhythmias or aortic insufficiency estimation of stenosis grade with of carotid arteries, arteriovenous malformations, carotid body tumors or high-grade stenosis/occlusion in the contralateral carotid artery may cause a false increase in flow velocities (17). B-flow sonography uses digitally coded ultrasound waves which enhance the weak signal derived from streaming blood, simultaneously suppresses the signals from the surrounding stationary tissues. This allows displaying the flow and surrounding tissues in the same spatial plane with the same, high frame rate. With B-flow imaging some limitations of color and power Doppler are overcome, so that blood flow, vessel wall and neighboring tissues can be real-time visualized.

Frame rate, spatial and contrast resolution are higher in B-flow imaging than Doppler methods. High frame rate and spatial resolution of B-flow gives the opportunity for a real-time demonstration of complicated hemodynamic flow patterns. Simultaneously evaluation of surface characteristics of plaque and altered flow dynamics may help to better grade cerebrovascular event risk. Additionally, high contrast and spatial resolution help to image the vessel wall and soft tissue planes simultaneously with blood flow. B-flow imaging is user friendly with fewer settings (sensitivity, background (on/off), focus position).

There are many studies about B-flow imaging of carotid arteries. However, most of the studies were focused on stenosis grading, there is a limited number of studies about plaque structure assessment (18-22). Most of these studies were focused on comparing B-flow imaging with power Doppler and DSA. These studies revealed that B-flow imaging showed a higher correlation with DSA than power Doppler imaging. Mikami T et all. studied pathological flow patterns in carotid stenosis with B-flow imaging. In some patients, a prestenosis reverse flow was observed which may cause embolus (23). In another study in post stenotic flow changes of gray-scale intensity was analyzed, which showed no correlation with systolic flow velocity (19).

Distinctly in our study, we evaluated the surface and structural characteristics of plaques with B-flow imaging and compared it with color and power Doppler sonography. We found that in imaging plaque surface and structural characteristics B-flow imaging was superior to color and power Doppler imaging. Lower contrast and spatial resolution in color and power Doppler imaging makes imaging the plaque characteristics difficult (Figure 4).

Additionally, “overwriting” and “aliasing” artifacts covers the plaque surface making it impossible to evaluate. B-flow imaging with high contrast and spatial resolution and without the above-mentioned artifacts has great advantages.

The twinkling artifact is caused by structures causing high reflection such as calcified plaques. This artifact generates blue and red color codes posterior to calcified plaques which causes difficulties in plaque and flow analysis. In our study we did not observe any twinkling artifacts in B-flow imaging, which helped in analyzing calcified plaques (Figure 5).

In our study power Doppler and B-flow imaging showed high correlation with DSA in grading ICA-stenosis. The correlation coefficient between B-flow imaging and DSA was higher than the doppler methods. B-flow imaging without the “overwriting” artifact enables a more accurate measurement of stenosis. In some cases, with tortuous vessels -because of angle dependent nature of color Doppler imaging- true plane stenosis analysis is exceedingly difficult. In moderate stenosis even with optimal PRF and Doppler gain settings vessel lumen is not filled completely with Doppler color codes, which causes undegrading the stenosis. Our findings showed that B-flow imaging correlates well with the gold standard DSA in carotid artery stenosis grading. Another important result is that in B-flow imaging the plaque morphology can be evaluated simultaneously with flow information.

NASCET and ECST recommend endarterectomy in high-grade ICA-stenosis. When stenosis over 70% is taken as cut off point, in color Doppler imaging 3

patients were undegraded, whereas with B-flow imaging none of the patients were undegraded.

Compared to our study Umemura et all. showed a higher correlation between B-flow imaging and DSA (r=0.977 vs r=0.969) (21). On the other hand, in a similar study, Yurdakul et all showed a lesser correlation between B-flow imaging and DSA compared to our study (22). However, in all studies correlation coefficients are extremely close. These results encourage the use of B-flow imaging in carotid artery stenosis grading.

Posterior acoustic shadowing caused by calcified plaques and insufficient signal obtained from deeply coursing vessels are the major limitations of B-flow imaging. Most patients have superficial carotid arteries, as well when necessary deeply coursing vessels can be better evaluated with low-frequency probes.

5. Conclusions

As a result, B-flow imaging with real-time flow information on gray-scale images, with less susceptibility to artifacts is a complementary method to Doppler imaging in carotid stenosis grading and plaque structure analysis.

Conflict of Interests

The authors declare that there is no conflict of interest.

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Author Contributions

Gümüş T, Yücel C, Oktar S and Ilgıt ET had the idea for and designed the study and drafted the original manuscript. Gümüş T, Oktar S, Özdemir H and Önal B collected the data. Özdemir H, Önal B and Ilgıt ET contributed to literature search and checked the data. Gümüş T, Yücel C, Önal B and Oktar S contributed to the statistical analysis. Yücel C, Özdemir H and Ilgıt ET contributed to critical revision of the manuscript. All authors read and approved the final manuscript.

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Hospital Infection Rates and Resistance Profiles in the Neonatal Intensive Care Unit

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Abstract: Nosocomial infections in newborns have characteristics not seen in any other group of patients. Newborns treated in intensive care are a group of patients with very weak defense system prone to infections. The incidence rate of nosocomial infections in newborns is one of the highest and there are differences in this rate between developed and developing countries. In this study, it was aimed to retrospectively assess the frequency and antibiotic resistance of microorganisms isolated from patients in neonatal intensive care units. The study was conducted in neonatal intensive care unit and sent to a microbiology laboratory between 1.1.2020 and 12.12.2020. To determine the foci of infection, blood, urine sample were taken and tracheal aspirate were taken from ventilated patients. Pediatric BACTEC FX (Becton Dickinson MD, ABD) bottles were used for blood samples. 5% sheep blood agar and eosin methylene blue (EMB) medium were used for tracheal aspirate cultures. Quantitative measurements were made on the tracheal aspirate cultures. Growths with colony number>10^5 cfu/ml were considered positive growth. CDC's diagnostic criteria were used for the diagnosis of hospital-acquired pneumonia in order to exclude colonization in patients with significant growth. Infections were detected in 29 (4.7%) of the 612 newborn patients. All of them were 8 different pathogens. It consists of 15 (51.7%) male and 14 (48.3) female infants by gender. Among them, growth were detected in 6 (20.6%) blood cultures,1 (3.4%) tracheal aspirate and 22 (75.8%) urine samples. Of the microorganisms isolated, 24 (82.8%) were Gram-negative bacteria, and 5 (17.2%) were Gram-positive bacteria; The most commonly isolated pathogens among gram negative were Escherichia coli (75.9%) and 6 (27.3) of them are ESBL(Extended Spectrum Beta-Lactamases) posıtıve. Followed by *Klebsiella pneumonia* n:1 (3.4%), and n:1 (3.4%). Klebsiella oxytoca. And gram positive were Staphylococcus haemolyticus 1 (3.4%), Streptococcus acidominimus 1 (3.4%), Streptococcus mitis1 (3.4%), Streptococcus oralis 1 (3.4%) and Streptococcus vestibularis 1 (3.4%), Respectively. **Keywords:** Antibiotic, Microorganism, Neonatal Intensive Care.

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1. Introduction

Neonatal intensive care units are at high risk for developing nosocomial infections (NIs) (1). Risk factors are exposure to invasive medical devices such as mechanical ventilators and central venous catheters (CVCs) and resistant microorganisms Surveillance of NIs is a very important. Due to few reports of surveillance in neonatal units in developing countries, we planned this study. In addition, we aimed to determine the flora distribution and antibiotic resistance profile in our hospital (1, 2).

2. Material and Methods

Traceal aspırate and urıne samples are detected by conventıonal methods. Blood samples taken from the patients were placed in the BACTEC FX (Becton Dickinson MD, ABD) automated system device. The susceptibility of the samples with a growth signal was determined on the BD Phoenix TM 100 (Becton Dickinson Co Sparks MD, ABD) antibiogram device. In order to test each isolate for in vitro antimicrobial susceptibility, in accordance with the Clinical and Laboratory Standards Institute criteria (3), The standard inoculum, adjusted to 0.5 McFarland standard turbidity, was evenly distributed over the surface of Mueller Hinton agar (Oxoid, Ltd. UK). Antimicrobial discs (Oxoid, Ltd. UK) amicasin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), penicillin (10 µg), tetracycline (30 µg), cefoxitin (10 µg), etc were applied to Mueller Hinton agar plates using an automatic disk dispenser. Following an overnight incubation at 37 °C, the zone of inhibition was measured and interpreted a s susceptible, intermediate or resistant according to European Committe on Antimicrobial Susuceptibility Testig Standard Criteria (3). Our study, which was reviewed by the Institutional Health Research Ethics Review Committee of the City and Pandemi Hospital, on 21.12.2020, was ethically approved with the decision number 23-229.

2.1. Statistical Analyses

Descriptive statistics were used The Chi square. p value <0.05 was considered statistically significant.

3. Results

The most commonly isolated pathogenes were *Escherichia coli* (75.9%) and 6 (27.3) of them were ESBL (Extended Spectrum Beta-Lactamases) posıtıve in the recent year. All of ESBL positive *E.coli* are urine samples and 4 women, 2 men babies. ESBL negative samples are 16. One of the these samples is a male patient and is a tracheal aspirate sample. A blood culture sample consisting of 2 men and 1 female. Urine culture samples from 7 men and 5 women are ESBL negatıve E.coli samples. E.coli and antibiotic susceptibilty are shown in Table 1.

In antibiotic Susceptibilty profiİe of Klebsiella oxytoca 1 (%3.4) isolated from a women's baby; resistance is detected only to ampicillin and is urine sample.

In another urine sample and in the female patient K. pneumonia n:1 (3.4%) has been detected. The antibiotic resistance profile is the same as for K.oxytoca. Staphylococcus. haemolyticus n:1(3.4%) from Gram positive bacteria was isolated from the blood sample. MSKNS showed (Methicillin Sensitive Coagulase Negative Staphylococcus) profile. Another sample was blood culture n:1 (3.4%) and the isolated microorganism *Staphylococcus acidominimus*, and antibiotic sensitivity is shown in Table 2.

4. Discussion

Intensive care units take up 30% of the prevalence of infection worldwide (4). This rate emerges as a significant cause of morbidity and mortality (4). The most common types of NIs are: surgical site infections, blood stream infections, urinary tract infections (5) respiratory infections, gastroenteritis, pneumonia and meningitis and other soft tissue infections (6). Long-term hospitalization of patients, invasive interventions, low birth weight, total parenteral nutrition congenital anomalies increase the risk of infection (7) and the fact that the immune system is undeveloped in newborns also facilitates the development of health care-related infections (8). Gram-positive bacteria have been reported in neonatal nasocomial infections indeveloped countries, while Gram-negative bacteria have been reported in developing countries. (9-11). In our study, Infections were detected in 29 of the 612 newborn patients and showed that 4.7% of the patients had bacterial infections. Our study found low rates compared to some studies conducted in the world (12-17). These differences may be due to patient-related factors, equipment quality, financial resources, surveillance studies, raising awareness, and the competence of the surgical team (17). In the present study, high rate of Gram negative 24 (82.8%) bacteria were the causative agents of nosocomial infections than Gram-positives 5 (17.2%).The most frequently isolated GNB (Gram negative bacteria) E.coli 22 (75.9%) and 6 (27.3) of them are ESBL (Extended Spectrum Beta-Lactamases) posıtıve. All other pathogens were detected equally.In Our study, these isolates which are E.coli (ESBLnegative) were %66.7 rate resistant to universally recommended antibiotics (ampicillin, gentamicin) for empirical treatment. As in other studies, there was resistance to these 2 antibiotics in our results (18-21). Among Gram-negatives, *E. coli* was resistant to ceftazidime 2/16 (12.5%), amoxicillin-clavulanate 7/16 (43.8%) In the our study, high rate of Gram negative (82.8%) bacteria were the causative agents of nosocomial infections.

And this present study the most causative pathogenes is *E.coli*. As in other studies, there was resistance to these 2 antibiotics in our results. An another study, pathogenic microorganisms isolated as a hospital acquired infections had the most GNB Gram Negative Bacteria (79.8%), and the most isolated GNB was K.pneumoniae (N: 22% 29.3) (9). Mutlu et al. (22) in their study of six-year gram-negative sepsis at neonatal nasocomial infection Gram-positive microorganisms 68%, Gram-negative 32% Serratia marcescens (16.4%), Klebsiella spp as a factor of septicemia, (14.7%), Pseudomonas spp. (12%) reported, respectively. Mireya et al.(23) KNS rate 66.6%, Enterococcus 3.3% staphococcus aureus 1.1%, E. coli 13.3% Enterobacter 8.8%, Pseudomonas 4.4%, Klebsiella 2.2% were identified. Olukman et al. (24) determined hospital infections as Gram-negative infections as 44%, Gram-positive infections as 36%, and fungal infections as 20%. The most common microorganisms found as KNS, S. Aureus and Candida in a study (25) Also, in another study, the most hospital acquered infection pathogens were GNB (54.4%) and the most common pathogens were *K. pneumoniae* (19.6%) (26).

Table 1: E.coli and antibiotic susceptibilty.

	E.coli																	
	Tracheal aspirat						Blood samples						Urine					
	Male			Female			Male			Female			Male			Female		
Antibiotic	R	S	IED	R	S	IED	R	S	IED	R	S	IED	R	S	IED	R	S	IED
AMC	1						2			1			2	5		1	4	
AM	1						2				1		3	4		2	3	
SAM	1						2				1			7			5	
PTZ		1						2		1				7			5	
CAZ	1						1	1			1		2	5			5	
CRO	1						1	1			1			7			5	
FEP	1						1	1				1	2	5			5	
N			1				1	1		1			1	6			5	
AK	1						1	1			1			7			5	
LEV		1						2		1			1	6			5	
IPM-MEM- ETP		1						2			1			7			5	
CT		1			N/A			2			1			7			5	

S: Sensitive, R: Resistant, and, IED: Increased exposure to the drug CN: Gentamycin, AMC: Amoksisilin clavulanik asit, AM: Ampicillin, SAM: Ampicillin Sulbactam, Piperasilin tazobaktam: PTZ, Ceftazidime: CAZ, Ceftriakson: CRO, Cefepime: FEP, Gentamicin: CN, Amikacin: AK, Levofloxacin: LEV, Imipenem: IPM, Meropenem: MEM, Ertapenem: ETP, Colistin: CT.

Table 2: *S.acidominimus* and antibiotic susceptiblty.

	S.acidominimus					
	Blood samples					
	Male			Female		
Antibiotic	R	S	IED	R	S	IED
AMC	2			1		
AM	2				1	
SAM	2				1	
PTZ		2		1		
CAZ	1	1			1	
CRO	1	1			1	
FEP	1	1				1
CN	1	1		1		
AK	1	1			1	
LEV		2		1		
IPM-MEM- ETP		2			1	
CT		2			1	

AMC: Amoksisilin clavulanik asit, AM: Ampicillin, Penicillin: P, Oxacillin: OX, Cefoxitin: FOX, Levofloxacin: LEV, Erythromycin: E, Clindamycin: DA, Vancomycin:VA, Teicoplanin: TEC, Linezolid: LNZ. S: Sensitive, R: Resistant and IED: Increased exposure to the drug. *Streptococcus mitis* was isolated from the urine sample of a male patient. It was detected resistant to AMC and AM while was detected sensitive to DA and VA. *Streptococcus oralis* is isolated n:1(3.4%) male patients and ıt was evaluated as *contamination. Finally, Streptococcus vestibularis was isolated from the blood culture of a female patient n:1(3.4%). Antibiotic susceptibilities were determıned for AM, P, DA and TEC sensıtıves, respectively*.

Maoulain et al.(27), they reported that 79.6% of NCI was ESBL-producing GNB, and the pathogen that caused the most NCI was *K pneumoniae* (39.7%). Our results are not compatible with these literatures. But, Studies conducted in other countries are similar to our data (31, 32). Differences and similarities in our results may be due to environmental factors, host-patient and microbial factors (31, 32). Among Gram-positive bacteria, *Staphylococcus. haemolyticus n:*1 (%3.4*)* from Gram positive bacteria was isolated from the blood sample. MSKNS showed (Methicillin Sensitive Coagulase Negative Staphylococcus) profile. Another sample was blood culture n:1 (3.4%) and the isolated microorganism *Staphylococcus acidominimus*, and antibiotic sensitivity *is* Penicillin**,** Oxacillin, Cefoxitin resistance. Another sample is streptococcus mitis was isolated from urine sample and was detected resistant to Amoksisilin clavulanik asit, and Ampicillin Streptococcus vestibularis which is sensitive all of antibiotics was isolated from blood culture. The most common infections in NICUs; blood circulation infections (BSI), pneumonia and urinary tract infections (33). In our study, the highest rate of urinary system infections was found. This result may be an increased incidence of urinary tract infections in children. In addition, the fact that respiratory samples such as bronchoscopic sampling or deep tracheal aspirate are taken less than blood cultures may also be effective in these results. From another angle, the higher incidence of Gram-negative infections in our study may be due to health workers not washing their hands adequately and/or contaminated medical equipment.

5. Conclusions

Host and therapeutic risk factors for nosocomial infections should be identified with a surveillance study in the neonatal intensive care unit (NICU). The impact of the staff and the environment on the nosocomial infection rate should be evaluated and the flora of each unit should be determined. The training of the staff should be developed, the awareness of sterilization, surveillance programs should be determined regularly by the experts in order to prevent the increase and spread of resistant strains. In the detection of hospital-borne infections, epidemiological analyses should be conducted with a strong-quality microbiologist and neonatalogist. It is necessary and continuous to take rigid measures in determining the factor of infection in the hospital and taking precautions and in the formulation of antibiotics.

Limitation of the Study

Conducting this study in a pandemic hospital may be related to the limitation of the study, having only treatment procedures for the factor in the covid process.

Conflict of Interests
None
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None

Author Contributions

Celebi O; Concept, design, supervision and resources (Celebi O contribution is %60), Celebi D; Literature search, writing manuscript, analysis and interpretation (Celebi D contribution is %40).

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Low Serum Myeloperoxidase Levels in Multiple Sclerosis Patients

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Abstract: Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system characterized by inflammation, demyelination, and neurodegeneration. It may lead to physical disability, acute neurological, and cognitive problems. The specific etiology of MS has not been clearly defined to date. One of the key factors that play a role in the pathogenesis of MS is oxidative stress, which increases inflammation and neurodegeneration. Myeloperoxidase (MPO) is one of the enzymes secreted by activated inflammatory cells and is produced by monocytes, macrophages, microglia, and neutrophils. At the same time, myeloperoxidase is one of the components of oxidative stress. MPO has been investigated many times in MS patients, but peripheral blood levels of MPO have been studied very few times. This study investigated serum MPO levels in MS, and the relationship of these levels with patients' age, disease duration, prognosis, annualized relapse rate, expanded disability status scale (EDSS) scores, and disease-modifying drug therapies (DMT) used. The study included 50 MS patients and 50 healthy controls, and their demographic and clinical characteristics were determined. Serum MPO levels were significantly lower in MS patients than in the healthy control group ($p=0.034$). No significant correlation was found between MPO levels and patients' age, EDSS scores, disease duration, DMTs used, and disease progression ($p>0.05$). These results show that low MPO levels in MS patients have an important role in the pathogenesis of MS. There is a need for further studies on this subject.

Keywords: Multiple Sclerosis; Myeloperoxidase; Oxidative Stress.

1. Introduction

Multiple sclerosis (MS) is an autoimmune, neurodegenerative disease of the central nervous system (CNS) caused by immune dysfunction. It is characterized by demyelination, chronic inflammation, neuronal, and oligodendrocyte loss (1-3). The onset of MS is typically between the ages of 20 and 40 years, it is more common in women and is one of the most important causes of nontraumatic neurological disability in young adults (4). In 1996, the National Multiple Sclerosis Society (NMSS) MS Clinical Research Advisory Committee defined four clinical phenotypes of MS: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP), and progressive-relapsing (PR) (5). The most common type in more than 80% of MS patients is RRMS, which is characterized by variable neurological symptoms and complete or incomplete recovery during remission. More than half of individuals in the RR stage of MS

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develop the SP form of MS following the accumulation of neurological deficits (6). Although the pathogenesis of MS is still not completely known, it is defined as an inflammatory demyelinating disease, and axonal damage of CNS directly correlates with the intensification of inflammatory processes and oxidative stress (7).

Myeloperoxidase (MPO) is one of the enzymes secreted by activated inflammatory cells such as neutrophils, monocytes, macrophages, and microglia (8, 9). In human neutrophils, it is part of the host defense system against microorganisms. MPO catalyzes the formation of hypochlorous acid (HOCl) and, other cytotoxic oxidants which has powerful activity against a variety of bacteria, viruses, and fungi (9). It produces highly reactive molecules such as hypochlorite, tyrosyl radicals, and aldehydes that can covalently modify lipids, which in turn causes more local tissue damage and increases the inflammatory cascade (10, 11). MPO is found at high levels in active MS plaques in humans (12, 13). However, it has been previously observed that mice devoid of MPO develop experimental autoimmune encephalomyelitis (EAE) and have higher morbidity and mortality compared to their wild-type counterparts (14). Therefore, the role of MPO in inflammatory demyelination remains unclear.

MPO has been studied many times in MS patients, but peripheral blood levels of myeloperoxidase have been studied very few times. This study investigated myeloperoxidase levels in MS patients and healthy control group, and the relationship of these levels with MS patients' age, disease duration, prognosis, annualized relapse rate (ARR), and expanded disability status scale (EDSS) scores.

2. Material and Methods

The study included 50 MS patients aged 18 years and over who were admitted to the Neurology MS outpatient clinic of Ataturk University, Faculty of Medicine between November 6, 2020 and December 21, 2020 and who were diagnosed with MS according to the 2010 Mc Donald diagnostic criteria, and 50 age- and sex-matched healthy controls after obtaining informed consent. Those who received oral or parenteral steroid therapy in the last three months, those with other systemic diseases, MS patients during the attack period, and those who had an attack in the last three months were excluded from the study. The demographic characteristics of the individuals in both groups, MS patients' disease-modifying therapies (DMT), disease duration, the ARR, and disease type were determined. The neurological examinations of MS patients were performed by the same neurologist, and their clinical characteristics and EDSS scores were determined. Venous blood samples were collected from the individuals. Thirty minutes after the collection of blood samples, the samples were centrifuged at 4000 rpm for 10 minutes and serums were taken. The samples were kept at -80 ^{o}C until analysis. Serum MPO levels were measured by the manual method. The test principle of the MPO determination is based on the kinetic measurement of the absorbance at 460 nm wavelength of the yellowish-orange complex formed as a result of oxidation of MPO and o-dianisidine in the presence of H_2O_2. MPO analysis results were calculated as U/mg protein (15). Local ethics committee approval was obtained for our study (09/19/05.11.2020).

2.1. Statistical Analysis

Statistical analysis was carried out using SPSS 22.0 Software package. The normality of data was evaluated by the Kolmogorov-Smirnov test. In comparison of numerical data, the Mann-Whitney U test was used when the number of groups was two for those who did not comply with the normal distribution, and the Kruskal Wallis test was used when the number of groups was more than two. The chi-squared test was used for comparison of categorical variables. Spearman's Rho test was used for correlation analysis of non-normally distributed data. The level of statistical significance was set at $p<0.05$ in all tests.

3. Results

Serum MPO levels were measured in multiple sclerosis patients (n=50) and healthy controls (n=50), and the data were compared. Of MS patients, 70% were female and 30% were male, with a mean age of 36.32 years. Of MS patients, 84% had RRMS and 16% had SPMS. The mean EDSS score of the patients was 2.30±1.34, the mean disease duration was 5.92±4.94 years and the ARR was 0.97±0.49. Of the patients, 18 (36.0%) were using interferon beta-1a, 7 (14.0%) were using interferon beta-1b, 7 (14.0%) were using fingolimod, 6 (12%) were using glatiramer acetate, 5 (10%) were using dimethyl fumarate, 3 (6.0%) were using teriflunomide, and 4 (8.0%) were using natalizumab (Table 1).

The serum MPO levels of MS patients (median=9192.30) were lower than the MPO levels of the healthy control group (median=1076.91) and this decrease was statistically significant (p=0.034) (Table 1). No significant correlation was found between MS patients' serum MPO levels and age, EDDS score, disease duration, ARR (p>0.05) (Table 2). There was no significant difference between the serum MPO levels of MS patients, and the DMT groups used (p=0.558) (Table 2).

When MS patients were classified by disease types, the disease duration and EDSS score of SPMS patients were significantly higher compared to RRMS patients, but there was no significant difference between these two groups in terms of age, ARR and MPO levels (Table 3).

Table 1: Demographic and clinical characteristics and serum myeloperoxidase levels of MS patients and healthy controls.

	MS (n=50)	**Healthy control (n=50)**	**p**
Gender n (%)			
Female	35 (70%)	35 (70%)	1*
Male	15 (30%)	15 (30%)	
Age, years			0.849**
Mean±SD;	36.2±9.4;	36.3±9.8;	
Median(Min-Max)	35 (19-59)	35 (19-57)	
Disease Type n (%)		-	
RRMS	42 (84%)		
SPMS	8 (16%)		
Disease duration, years		-	
Mean±SD;	5.92±4.94;		
Median(Min-Max)	4 (1-22)		
EDSS		-	
Mean±SD;	2.30±1.34;		
Median(Min-Max)	2 (0-6)		
ARR		-	
Mean±SD;	0.97±0.49;		
Median(Min-Max)	0.41 (0.2-1.59)		
DMT n (%)		-	
Interferon beta-1a	18 (36%)		
Interferon beta-1b	7 (14%)		
Glatiramer acetate	6 (12%)		
Fingolimod	7 (14%)		
Dimethyl fumarate	5 (10%)		
Teriflunamide	3 (6%)		
Natalizumab	4 (8%)		
MPO *(U/mg protein)*			
Mean±SD;	8611.22±6688.80;	10303.50±3775.38;	**0.034****
Median(Min-Max)	9192.30 (1038.46-40200)	1076.91 (2076.92-16269.23)	

*****Chi square Test, ******Mann-Whitney U Test, **MS:** Multiple Sclerosis, **RRMS:** Relapsing-remitting MS, **SPMS:** Secondary Progressive MS, **EDSS:** Expanded Disability Status Scale, **ARR:** Annualized relapse rate, **MPO:** Myeloperoxidase, **DMT:** Disease-modifying therapy.

Table 2: Correlation of serum MPO levels with age, EDSS, disease duration, and ARR in MS patients.

		Age	**EDSS**	**Disease duration**	**ARR**	**DMT***
MPO	**r**	-0.046	0.121	0.134	0.208	-
(*U/mg protein)*	**p**	0.651	0.402	0.354	0.147	0.558

Spearman's correlation, * Kruskal Wallis, **MPO:** Myeloperoxidase, **EDSS:** Expanded Disability Status Scale, **ARR:** Annualized relapse rate, **DMT:** Disease-modifying therapy.

Table 3: Comparison of disease type and serum MPO levels, demographic and clinical data in patients with RRMS and SPMS.

	RRMS (n=42)	**SPMS (n=8)**	**p**
Gender n (%)			
Female	31 (%73.8)	4 (%50)	0,178*
Male	11 (%26.2)	4 (%50)	
Age, years			
Mean±SD;	35.69±9.24;	39.62±12.62;	0.450**
Median (Min-Max)	35 (20-57)	39 (19-53)	
Disease duration,years			
Mean±SD;	4.85±4.02;	11.50±5.83;	**0.003****
Median (Min-Max**)**	4 (1-22)	12 (3-20)	
ARR			
Mean±SD;	0.97±0.50;	0,95±0,48;	0.915**
Median (Min-Max)	0.80 (0,42-2,0)	0.90 (0.41-1.60)	
EDSS			
Mean±SD;	1.86±0.89;	4.56±1.01;	**<0.001****
Median (min-max)	2 (0-4)	4.75 (3-6)	
MPO *(ng/ml)*			
Mean±SD;	8589.37±6767.34;	8725.95±6702.51;	0.968**
Median (Min-Max)	9423.07 (1038,46-40200)	7096.14 (1961.53-20192.30)	

*****Chi-squared test, ****** Mann-Whitney U Test, **EDSS:** Expanded Disability Status Scale, **ARR:** Annualized relapse rate, **MPO:** Myeloperoxidase.

4. Discussion

In our study, serum MPO levels were significantly lower in MS patients than in the healthy control group. There was no significant correlation between serum MPO levels and age, disease duration, ARR, EDSS scores in MS patients. Considering disease progression, there was no significant difference between RRMS patients and SPMS patients in terms of MPO levels.

MS is a neuroinflammatory autoimmune disease. In MS, inflammation, demyelination, and axonal damage of both the brain and spinal cord impair physical and cognitive abilities (16**).** In MS, some pathophysiological processes, including chronic inflammation of the CNS, oxidative stress, blood-brain barrier disruption, demyelination, axonal and neuronal damage, and remyelination, are observed (17).

Although the pathogenesis of MS is still not completely known, CNS axonal damage is directly associated with the intensification of inflammatory processes and oxidative stress (7).

Oxidative damage plays a role in cell degeneration in all stages of MS (18-20). Oxidative stress is caused by an imbalance between the production of free radicals and the antioxidant defense system. Increased free radicals, including reactive oxygen species and reactive nitrogen species, cause lipid and protein damage through peroxidation and nitration processes (21). MPO is one of the enzymes secreted by activated inflammatory cells and is produced by monocytes, macrophages, microglia, and neutrophils (8). In human neutrophils, it is part of the host defense system against microorganisms. The main function of MPO lies in the defense of the organism through production of HOCl, a powerful oxidant (9). Among different neurotoxic oxidants in the brain, HOCl is stable, highly reactive, and dominant. This acid plays a role in a number of neurodegenerative diseases, including multiple sclerosis, Parkinson's, and Alzheimer's diseases (22). Myeloperoxidase (MPO) plays a role in MS, with its presence in activated macrophages and an increased risk association of a -463 G/A promoter polymorphism (13). Also, MPO at 17q23.1 is within a region identified in genome scans as a MS susceptibility locus (23,24). In studies conducted considering the potential role of MPO in MS, high levels of MPO have been reported in activated microglia/macrophages at lesion sites in human MS plaques (13, 25). In addition, a significant correlation has been found between high MPO activity, demyelination, neuronal death, and ultimately neurodegeneration (26**).** Similar results have been shown in EAE, an animal model of MS (27-29).

Activated microglia/macrophages secrete MPO which generates cytotoxic HOCl and contributes to the myelin sheath damage surrounding axons (30). Contrary to what was expected in our study, MPO level was low in MS patients. There is evidence in the literature that MPO deficiency may play a role in the pathogenesis of MS. For instance, a study examining the incidence of EAE, an animal model of MS, in MPO knockout mice showed that MPO was detected in activated macrophages in the CNS of wild-type mice, yet unexpectedly, MPO mice had significantly increased incidence of EAE. It was shown that mice devoid of MPO had higher morbidity and mortality compared to their wild-type counterparts, and 90% of MPO

knockout mice developed complete hind limb paralysis 33% of wild-type mice (14). This is the first evidence that MPO plays an important role in EAE, consistent with its putative role in MS. In animals completely devoid of MPO, developmental upregulation of compensatory inflammatory molecules with biological functions different from MPO may play a role, possibly explaining the exacerbation observed in these mice. This concept is supported by the increased proliferation of antigen-specific T cells in mice devoid of MPO with EAE in the same study (14).

In our study, low levels of MPA in the serum of MS patients may play a role in the pathogenesis of MS with a similar mechanism. Similar to these results, considering the potential role of MPO in MS, leukocyte MPO activity has been investigated in patients with different MS subtypes and healthy controls, and low MPO activity has been shown in peripheral blood leukocytes in all MS types compared to healthy controls (31).

Experimental evidence demonstrating the significant degenerative role of MPO in MS disease progression suggests that pharmacological inhibition of extracellular MPO by 4-aminobenzoic acid hydrazide (4-ABAH) attenuates the severity of disease progression in an MS mouse model (26). When the correlation between serum MPO levels and disease progression was analyzed in MS patients in our study, MPO levels were slightly higher in SPMS patients compared to RRMS patients, but this was not statistically significant.

A decrease in myeloperoxidase and oxidative damage biomarker levels has been reported after 14 months of natalizumab treatment in MS patients previously compared to the pre-treatment period (32). In our study, it was investigated whether MPO levels in MS patients differ between DMTs used by patients. However, it is not known whether there is a difference between before and after treatment in our study. This is one of the limitations of our study, together with the small number of patients.

5. Conclusions

In conclusion, low serum MPO levels in MS patients may play a role in the pathogenesis of MS. There is a need for further studies on this subject to eliminate the inconsistency regarding MPO levels in MS.

Conflict of Interests

All authors declared that there is no conflict of interest.

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Compliance with Ethical Standards

The study was carried out in accordance with ethical standards in all aspects.

Author Contributions

Bilge N, Yevgi R, Kızıldağ N and Kızıltunç A contributed to the conception and design of the study. Bilge N, Yevgi R, Kızıldağ N and Kızıltunç A contributed to the collection of the data and statistical analysis and evaluation of the results. Bilge N and Yevgi R contributed to the creating and writing of manuscript. Bilge N contributed to revising the work and final approval of the version.

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A New Perspective to the Brucellosis From East of Turkey; Does the Infections Really Decrease Over the Years?

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Abstract: Brucella infection is a zoonotic disease caused by gram-negative bacteria in the structure of coccobacillus. The most frequently isolated species in our region are B. abortus, transmitted from, cows and B. melitensis, transmitted from sheep. The microorganism infects humans by direct contact with animal tissues or blood, and often via unpasteurized animal products. This study aimed to determine the prevalence of brucellosis in our region, its distribution according to years, seasons, age, and sex, and to observe the progress of brucellosis cases by years. Materials and Methods: The Rose Bengal agglutination test was used for screening. The BrucellaCapt test was applied to patients who were positive for Brucella screening test or who had clinically suspected brucellosis. We considered patients with 1/160 and above titers as positive. Results: The total number of samples that came to the laboratory from 2016 to 2019 was 20. 330, from which 19.595 were Brucella-negative, while 735 were Brucella-positive. The number of Brucella strains grown in the blood culture was determined as 12, and the Public Health laboratory identified all strains as Brucella melitensis. The distribution of patients according to age, sex, season, and branches were examined by the years. Conclusion: In conclusion, the incidence of Brucella cases in our region is a known fact. While it was expected that the cases would tend to decrease in recent years, it has been observed that it has increased slightly due to reasons such as imported livestock, insufficient sanitation conditions, and lack of awareness of the farmers.
Keywords: Brucella, Prevalance, Distrubution.

1. Introduction

Brucella infection is azoonotic disease caused by Brucella bacteria in the structure of gram-negative coccobacillus (1). From the bacteria in the group, *B. abortus* is transmitted from cows, *B. melitensis* and *B. ovis* from sheep, *B. suis* from pigs, *B. canis* from dogs, and *B. neotomae* from rats. Approximately 300 million of the 1.4 billion sheep in the world are thought to be infected with Brucella. While these bacteria cause abortions by attaching to a carbohydrate substance called erythritol found in the uterus of animals, it is believed that it has no such effect in humans (1-3). Nevertheless, abortions in animals cause severe damage to the economy (4).

Brucella can be transmitted to humans by direct tissue contact with animals or by blood, as well as often by non-pasteurized products (cheese, milk, poorly boiled yogurt, etc.). Brucella dies when exposed to 60 °C for 10-15 minutes. The recommended boiling method is to keep the milk product at the same temperature for about 15 minutes at the boiling temperature. Microorganisms

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can live for 6 weeks in animal-habited barn dust, 10 weeks in water, 30 days in ice cream made from raw milk, 4-5 months in salt-free cream oil made from raw milk in a refrigerator, and 45 days in brined cheese containing 10% salt (5).

The main symptom Brucella produces in humans is joint pain and suddenly rising fever (undulant fever). Chronic brucella can progress to arthritis, hepatitis, encephalitis, endocarditis, and orchitis, causing infertility in men. It has been shown in some studies that chronic cases may cause even depression (6).

Also, Brucella is among the category B bioterrorism agents. It is not seen in developed countries because of the industrial processing of dairy products and attention to the vaccination of animals. As in Turkey, it continues to exist frequently in developing countries such as the Middle East, Asia, Africa, and South America due to the insufficient sanitation conditions and failure to take precautions (7, 8).

This study aimed to determine the Brucella prevalence in our region and to determine the distribution according to years, seasons, age, and sex. It was intended to demonstrate the Brucella infection progress, which is thought to decrease, with the data of our region.

2. Material and Methods

Our study includes the results of patients admitted to our hospital between 2016 and 2019, 20330 patients sample was examined. Ethical clearance was obtained for study. The Rose Bengal test is a screening test for B rucella with a sensitivity of 96-100% and a specificity of 91-100% in acute cases (9). Rose Bengal lam agglutination test (Seromed, Turkey) was used as the screening test. In this test, the Rose Bengal test antigen (*B. abortus* S99 strain) was mixed with patient serum and stirred by hand with rotation movements for 4 minutes to see if there were any signs of agglutination. Samples with agglutination were considered positive, while those without agglutination were accepted as negative. The Brucellacapt test (*B. abortus* S99 strain and *B. melitensis* biotype-3) was applied to the serums of the patients with positive screening tests for *B. abortus*, *B. melitensis*, and *B. suis*, as well as patients with clinically suspected brucellosis despite a negative Rose Bengal test (10). The specificity and sensitivity of the Brucellacapt test is 98-99% and 94-95%, respectively. The IgG, IgM, IgA, and non-agglutinin, which are produced in brucellosis, reveal IgG and IgA's. The test results were assessed between 1/20-1/5120 titers. Although some sources accept Brucellacapt sample positivity as 1/160, while others suggest 1/320, we classified patients with 1/160 and above as positive (11, 12). If symptoms were present, confirmatory blood was drawn from the relatively low-titered patients. Blood cultures were incubated for 1 week and bone marrow cultures for 21 days using the BD BACTEC 9120 system (Becton Dickinson-Spain, Madrid, Spain). Blood and bone marrow cultures were taken into blood and chocolate agars and kept in aerobic and waxed jars. The culture-positive samples demonstrated gram-negative coccobacillus with gram staining; agglutination was performed with positive patient sera, and the samples were sent to the reference laboratory of the Turkish General Directorate of Public Health.

2.4. Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc, Chicago, IL, USA). Numbers (n), percentage (%), mean, and standard deviation (SD) were given for the descriptive statistics. The independent samples t-test or Mann-Whitney U test were used to compare numerical variables. Pearson Chi-Square or Fisher's exact tests were used to analyzing categorical data. Pearson correlation was used to search for relationships between scale variables. Agreements of the two laboratory tests to identify Brucella positivity were calculated by Cohen's Kappa Coefficient. The level of significance, p, was set at 0.05.

3. Results

The total number of samples coming to the laboratory between 2016-2019 was 20330. While the number of Brucella negative patients was 19.595, the number of Brucella positive patients was 735. The number of Brucella strains grown in the blood cultures was determined as 12, and all isolates were identified as *Brucella melitensis* by the Turkish General Directorate of Public Health laboratory.

According to Cohen's Kappa test the Rose Bengal and Brucella tube agglutination test was almost equally accurate. Kappa=0.987, p=0.000. According to the Chi-Square test there is no statistically significant difference between Brucella positivity according to the seasons. x^2:2.834, p=0.116. There is a statistically significant difference between the units and Brucella positivity. Infectious diseases, Internal medicine, Pediatrics are most common units. x^2:44,652, p=0.007. There is no statistically significant difference between gender and brucella positivity. x^2:0,797, p=0.213.

Table 1 shows the distributions of sex, mean age, Rose-Bengal positivity, and the number of positive cases as to the 1/160 threshold. The number of positive patients by years is given in Figure 1.

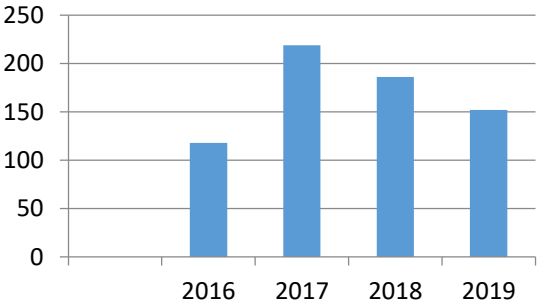

Figure 1: Number of positive patients through the years 2016-2019.

Figure 2 shows the positive patient distribution and percentage slices according to the seasons, while Table 2 demonstrates the number of positive cases according to the departments.

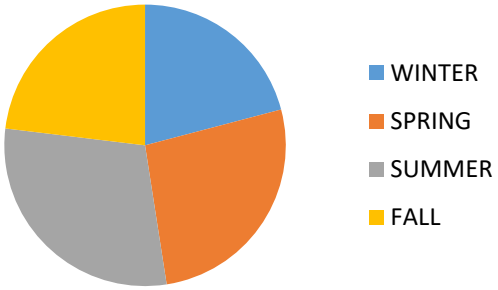

Figure 2: Distribution of positive patients by seasons (2016-2019).

According to Mann Whitney U test there is a statistically significant difference between age and Brucella positivity average age is between 37.42±19.62. z:-3,115, p=0.002. There is a statistically significant difference between Brucella positivity by years in there is a peak in 2017. z:-2,147, p=0.032.

4. Discussion

In our study, serum MPO levels were significantly lower in MS patients than in the healthy control group. There was no significant correlation between serum MPO levels and age, disease duration, ARR, EDSS scores in MS patients. Considering disease progression, there was no significant difference between RRMS patients and SPMS patients in terms of MPO levels.

MS is a neuroinflammatory autoimmune disease. In MS, inflammation, demyelination, and axonal damage of both the brain and spinal cord impair physical and cognitive abilities (16). In MS, some pathophysiological processes, including chronic inflammation of the CNS, oxidative stress, blood-brain barrier disruption, demyelination, axonal and neuronal damage, and remyelination, are observed (17).

When the Public Health data were examined, it was evident that, although at a low level, there was an increase in the number of Brucella cases since 2015 (Figure 4). Additionally, our region is also within the area marked dark, where Brucella cases are prevalent (Figure 3) (13, 14). Brucella is a serious infection, especially in less developed or developing countries such as our area, where animal farming is highly prevalent but not industrialized. The lack of vaccination, disadvantaged animal care conditions, as well as the presence of Brucella bacteria, appears as the main reasons for not being able to cope with the disease. In this context, the general aspect of the Brucella cases in recent years in our study area is presented. According to the 2017 data of the Turkish General Directorate of Public Health, the incidence of Brucellosis cases in our city was detected as 82.4%. The 2018 and 2019 data are not announced yet (13). The outcome of our study is parallel to Turkey's data. After 2016, Brucella analysis and detections were clearly increased in the area. Although verbal communications with local animal owners indicated that the situation might be caused by the altering of the domestic animal race and the absence of vaccination, no supporting scientific data could be found. The surveys on the people who engage in husbandry showed that 66% of them had knowledge about Brucella, whereas 84.5% used unpasteurized dairy (15). As the surveys indicated, the fundamental reason for the high prevalence is that even though the disease is known, there isn't adequate prevention.

In our research, a significant difference was found in the patients submitted from especially infectious diseases, internal medicine, and pediatrics policlinics and clinics compared to other services (p=0.007, Chi-Square) (16). Again, as it is stated in our research, people admitted to the hospital with any symptom might be diagnosed with Brucella. Thus, it might be indicated to execute a routine screening for brucella in our area.

A significant difference was observed when data were analyzed per the years (p=0.032, Mann Whitney U). Brucella cases increase gradually throughout the years. Particularly after 2015, an apparent rise was also observed in our country (17). According to the data for 2015 from the Turkish General Directorate of Public Health, 4173 positive patients were diagnosed, whereas, in 2016, this number increased to 5148 (18). After 2016, these numbers show a tendency to grow more. Considering that 2019 is not yet passed, it should be recognized that the numbers in our research will continue increasing further.

Again between Brucella positivity and age, there was a substantial difference. The mean age of all patients was 37.42±19.62, which indicates that middle-aged people engage in husbandry. Engagement and contact with animals and the consumption of dairy are less common among the young generations. Despite the frequency of positive patients in pediatric clinics, when the entire sample is considered, there is no correlation between age and the number of Brucella patients; however, there is an increase in the prevalence with rising age. Also, a thesis study conducted in Erzincan shows that there is no statistical difference in the infection according to the age groups. However, the prevalence is higher in the group of people who are older than 45 (19). When seasonal factors were considered, besides the fact that more Brucella positive patients were diagnosed during the summer, a significant difference was not found. In other studies conducted in our country, parallel to ours, Gültepe and his friends discovered no distinct difference between gender and age groups in their research in Van (20). When positivity ratios are analyzed per the monthly distributions, they appear to be increasing as from March, reaching the highest level in August, and as from October, they are observed to decrease to the prior ratios.

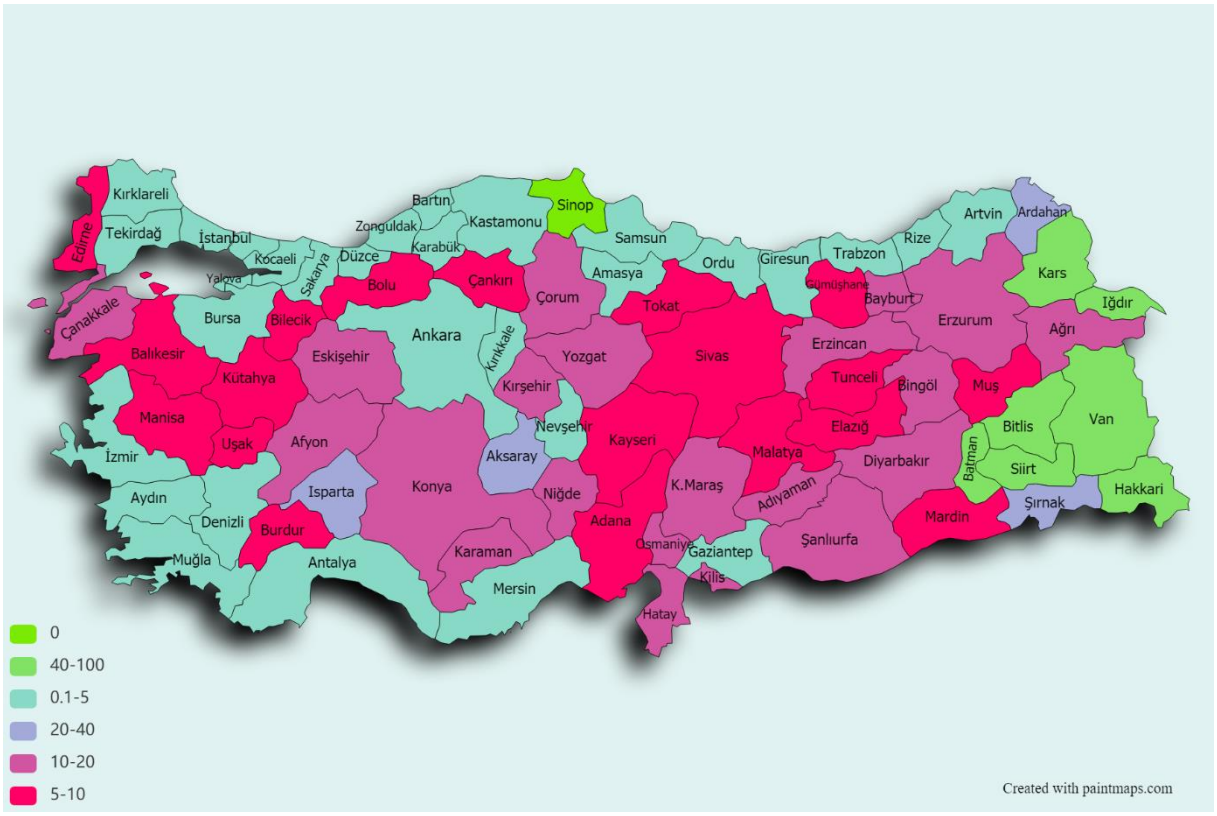

Figure 3: Brucella distrubution (map by 2017 Public Health Data) (13).

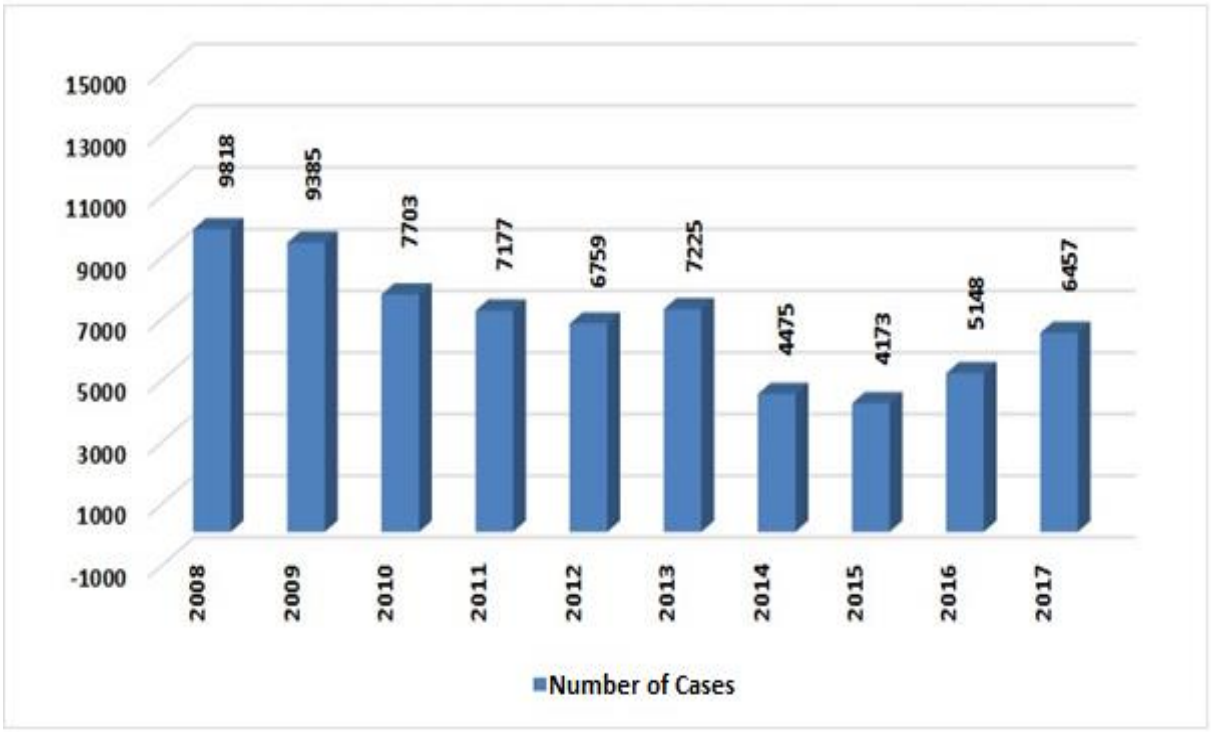

Figure 4: Distribution of Brucella-positive cases by year (Turkish Public Health Data) (14).

Table 1: Demographic Data of the Positive Patients in Table.

Sex	**Female**		**Male**
n:735	n:299 (%40.7)		n:436 (%59.3)
Age average		37.42±19.62	
Rose	**Negative**		**Positive**
n:20330	n:19 595		n:735
1/160 limit	**Under 1/160**		**Over 1/160**
	n: 129 (17.6%)		n: 606 (82.4%)

This outcome brings the rise of cheese consumption in summer months and thecommonly consumed herby cheese to mind, which is made in Van without boiling the milk. Whereas in our area, the reason for not having a seasonal difference might be related to the consumption of foods that were not cooked well, bacteria which stay alive almost 4 months in butter or 2 months in brine cheese, and the time for fresh animal diary being roughly equal throughout the year. Besides, the different milking periods for sheep and cows, which are the hosts for Brucella abortus and Brucella melitensis, may have contributed to the lack of seasonal difference. The milking period for cattle is more extended than that for the sheep. Additionally, a few months of milking time difference exists between warm cities like Iğdır and colder districts like Selim and Sarıkamış.

In our research, no difference was found between gender and Brucella positivity. This outcome was attributed to the almost equal contributions of the middle-aged women and men in milking and nurturing processes; they have nearly the same contact with animals.

Table 2.: Distribution of positive patients by services (in decreasing order).

	n/%
Infectious diseases	267/36
Internal medicine	264/36
Pediatrics	131/17
Orthopedics	15/2
Family Medicine	9/1
Urology	8/1
Physical Medicine and Rehabilitation	8/1
General Surgery	7/1
Neurosurgery	6/1
Neurology	5/1
Biochemistry	5/1
Emergency medicine	4/0.5
ENT	2/0.3
Anesthesia	2/0.3
Cardiology	1/0.1
Dermatology	1/0.1

5. Conclusions

Consequently, the frequency of Brucella cases in our region is a well-known fact. Despite the expected decrease in the cases during the recent years, with the lack of sanitation and the inadequate awareness for the people working in husbandry and animal import business, even if small, an increase was observed. We consider that raising the awareness primarily among the people working in animal farming, educating the consumers to not consume the dairies unless they are sure of its pasteurization, and generalized application of dairy food processing rules are some of the fundamental preventive measures for the decrease in the prevalence of this disease.

Conflict of Interests

None

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Author Contributions

Conception; Constructing an idea or hypothesis for research and/or manuscript; Balkan Bozlak ÇE. Design; Planning methodology to reach the conclusion; Balkan Bozlak ÇE, Çelebi Ö. Supervision; Organising and supervising the course of the project or the article and taking the responsibility; Balkan Bozlak ÇE, Çelebi Ö. Fundings; Providing personnel, environmental and financial support and tools and instruments that are vital for the project; Balkan Bozlak ÇE. Materials; Biological materials, reagents and referred patients; Balkan Bozlak ÇE. Data Collection and/or Processing; Taking responsibility in execution of the experiments, patient follow-up, data management and reporting, Balkan Bozlak ÇE. Analysis and/or Interpretation; Taking responsibility in logical interpretation and presentation of the results; Balkan Bozlak ÇE., Çelebi Ö. Literature Review; Taking responsibility in this necessary function; Balkan Bozlak ÇE. Writer; Taking responsibility in the construction of the whole or body of the manuscript; Balkan Bozlak ÇE. Critical Review; Reviewing the article before submission not only for spelling and grammar but also for its intellectual content; Balkan Bozlak ÇE, Çelebi Ö.

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The Renoprotective Effects of Desflurane and Sevoflurane in Lower Limb Ischemia-Reperfusion Injury on Streptozotocin-Induced Diabetic Rats

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Abstract: The study aims to determine the protective effects of sevoflurane and desflurane on the kidneys in lower limb ischemia-reperfusion injury (IRI) streptozotocin-induced diabetic rats. Thirty Wistar rats were randomly divided into equal five groups. The control (group C), diabetes-control (group DC), Diabetes-I/R (group DIR), Diabetes-I/R-sevoflurane (group DIRS), Diabetes-I/R-desflurane (group DIRD). 55 mg/kg Streptozotocin was administered intraperitoneally to diabetic groups as a single dose. 72nd hours were considered diabetic blood glucose 250 mg/dl or above, and at the end of 4 weeks, all groups underwent laparotomy. In groups C and DC, there was no further action. In the group, DIR was performed 2 hours in the infrarenal abdominal aorta in order clamped be put and remove. Sevoflurane and desflurane were administered so that the minimum alveolar concentration was one during the ischemia and reperfusion periods. At the end of the reperfusion period, kidney tissues were taken for biochemical and histopathological examinations. In the DIR group, Nitric oxide synthases (NOS) enzyme activity was observed significantly higher than C and DC groups. In DIRS and DIRD groups, it was significantly lower than DIR. TBARS level is significantly higher in all groups compared to group C. In the DIR group, the TBARS level was significantly higher than in the DC group. In DIRS and DIRD groups, it was significantly lower than DIR. DIR group Superoxide dismutase (SOD) enzyme activity was observed significantly higher than C and DC groups. In DIRS and DIRD groups, it was significantly lower than DIR. These results demonstrate that sevoflurane and desflurane have protective effects on the kidneys in lower limb IRI on streptozotocin-induced diabetic rats.

Keywords: Ischemia-Reperfusion, Sevoflurane, Desflurane, Kidney.

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1. Introduction

Nowadays, ischemia-reperfusion injury (IRI) is occurred routinely with different methods such as vascular clamps or tourniquet applications to provide bleeding control in many surgeries from major vascular surgeries such as aortic dissection (1) to extremity surgery (2). Besides, IRI occurs in many clinical conditions such as myocardial and cerebrovascular infarctions, thrombolytic therapy, cardiopulmonary resuscitation, and hemorrhagic shock.

Ischemia-reperfusion injury is a paradox with cellular dysfunction and death following blood flow restoration to ischemic tissues (3). Restoring blood flow is essential for the recovery of tissues from ischemia. Following reperfusion, do not return to standard terms. Instead, it also increases damage by activating various immune system responses and cell death programs (4). It can lead to multi-system organ failure by causing systemic damage to the ischemic organ itself and distant organs such as the kidney (5).

Diabetes mellitus (DM) is a metabolic disease with high levels of blood glucose. Nephropathy and vasculopathy are common in the long period of the disease. It is also an independent risk factor among the causes of acute kidney injury (AKI) (6). It was increased in diabetic patients undergoing surgery (7), with sepsis/septic shock (8), and even without precipitating events (9). It has also been clearly shown to aggravate IRI-induced kidney damage (10).

Reno-protective effects of various agents such as xenon (11), statin (12), lithium (13) in IRI have been investigated. Although anesthetic agents such as dexmedetomidine (14) and isoflurane (15) have been studied, there is no standardized anesthesia protocol.

This study aimed to investigate the renoprotective effects of desflurane and sevoflurane in lower limb IRI on diabetic rats.

2. Material and Methods

This study was performed after the Experimental Animals Ethical Committee approve of Gazi University, dated 27.11.2013, and code number "G.Ü. ET-13.074".

The research was done in Gazi University Experimental and Clinical Research Center (GUDAM). For this research, in the range of 250 and 350 g, 30 Wistar albino rats were used, which are raised under the same environmental provisions. The rats were exposed to cycles of 12h daylight and 12h darkness and reached food until 2h before anesthesia was given. Six healthy rats were a control group (C). The other rats were split as diabetic control (DC), diabetic ischemia-reperfusion (DIR), diabetic ischemia-reperfusion, and sevoflurane (DIRS), diabetic ischemia-reperfusion, and desflurane (DIRD) into four groups randomly. Streptozotocin (STZ) was used for treatment and prepared just before the treatment. After three days of applying STZ, the glucose levels were evaluated, and the rats were classified as diabetic, which glucose (FBG) levels are over 250mg/dl. Before applying sevoflurane and desflurane, the rats were observed for four weeks to have chronic diabetes after the STZ injection (16). The vaporizers were adjusted to be desflurane 6% and sevoflurane 2% for the target minimum alveolar concentration (MAC) 1. The anesthesia protocol was administered in a wide transparent plastic box. The box was integrated into a semi-open anesthesia device with static hoses. Anesthetics were driven into the box with 100% oxygen flow of 6 lt/min for 4 hours. The control group (C), the Diabetic control group, and the DIR group had no administration. After shaving the abdomen, all rats were positioned supine on the operating table. A median laparotomy was applied after cleaning the abdomen region with 1% polyvinylidene and covered with a drape. The infrarenal aorta blood flow stopped with an atraumatic clamp for two hours. Afterward, the clamp was removed, and blood flow was maintained for 2 hours. Inhalation anesthesia was maintained during the ischemia and perfusion periods. After the reperfusion period, histopathological and biochemical assessment of kidney specimens were completed.

2.1. Histopathological Evaluation

Histopathological assessment was studied in the Department of Histology at Kirikkale University. After the fixation process, specimens were prepared with paraffin blocks. Tissue sections of 5 μ were stained via hematoxylin and eosin (H&E). As defined by Bostan et al. (17), the histopathological assessment and scoring were performed under light microscopy. Tubular cell spillage (TCS), tubular dilatation (TD), lymphocyte infiltration (LI), Bowman space dilatation (BSD), tubular cell degeneration and necrosis (TCDN), tubular hyaline cylinder (THC), vascular vacuolization and hypertrophy (VVH), and Glomerular vacuolization(GV) were classified via a scoring system: 0: no change; +1: minimal; +2: medium; +3: severe change.

2.2. Biochemical Evaluation

The biochemical examination was conducted in the Department of Medical Biochemistry at Gazi University. The level of malondialdehyde (MDA), an indicator of lipid peroxidation and oxidative stress in kidney tissue, was evaluated by measuring the Thiobarbituric acid reactive substance (TBARS) level. Besides, Nitric oxide synthase (NOS), Glutathione transferase (GST), Catalase (CAT), and Superoxide Dismutase (SOD) enzyme activities measurements were performed. NOS, GST, CAT, and SOD assays were administered as defined by Aebi, Habig, and Durak (18-20). $NBTH_2$ occurs with the reduction of NBT. The amount of enzyme that caused 50% inhibition of NBTH2 absorbance at 560 nm was defined as SOD activity. The decrease in absorbance at 240 nm due to the consumption of H2O2 forms the basis

of the CAT activity method. The GST activity is assessed by measuring the GSH-CDNB complex at 340 nm. Sulfanilic acid is diazotized with nitric oxide at acid pH and binds to N-(1-naphthyl-ethylene diamine). The empty tube absorbance at 540 nm is compared with the sample tube's absorbance, thereby measuring the NOS activity. The TBARS examination was performed via the thiobarbituric acid (TBA) method to establish lipid peroxidation (21). TBARS determine carried out based on the TBA-MDA reaction, which constitutes a pink pigment with maximum absorption at 532 nm in acid pH. The 1,1,3,3-tetra ethoxy propane was defined as a standard MDA solution. TBARS levels and enzyme activities were monitored using a Shimadzu UV-1601 spectrophotometer based on endpoint change in absorbance at 25 °C. Results were announced U/mg protein and mIU/mg protein for SOD and GST, respectively. NOS and CAT results were expressed IU/mg protein. Results of TBARS levels were as nmol/mg protein.

2.3. Statistical analysis

We performed the statistical analysis with the SPSS 20.0 (IBM, Armonk, New York, USA) packet program. P values less than 0.05 were considered statistically significant. Data were presented as Mean±Standard Error Mean. Kruskal–Wallis variance analysis was used for the evaluation of data. The Mann-Whitney U test with Bonferroni correction was used in the analysis of significant variables.

3. Results

3.1. Histopathological Findings

In histopathological examination showed a significant difference in GV level between all study groups (p=0.022). GV was more common in DIR groups compared to the control group (p=0.001). The decrease in GV in the DIRD group was significant compared to the DIR group (p=0.023) (Table 1, Figure 1-5).

The TD difference between the groups was significant (p=0.007). The increase in TD was significant in the DIR group compared to the control group and the DC group (p<0.0001, p=0.034, respectively) (Table 1, Figure 1-5).

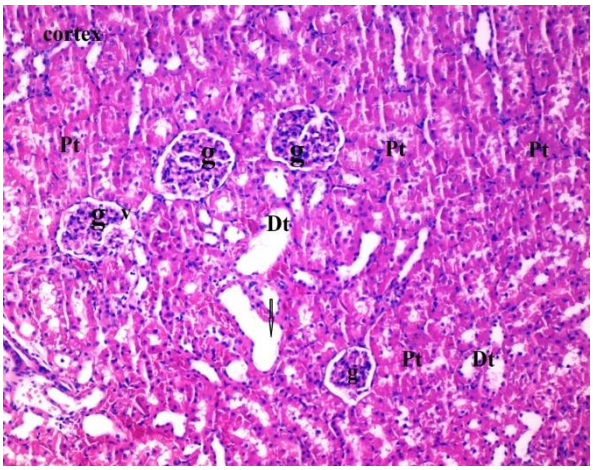

Figure 1: Control group kidney tissue (Pt: Proximal tubule, Dt: Distal tubule, g: glomerule, arrow: dilated tubule, v: vacuole) (H&EX10).

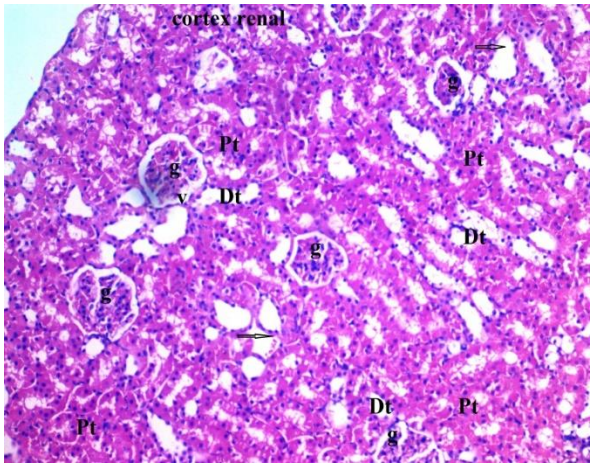

Figure 1: Diabetes Control group kidney tissue (Pt: Proximal tubule, Dt: Distal tubule, g: glomerule, arrow: dilated tubule, v: vacuole) (H&EX10).

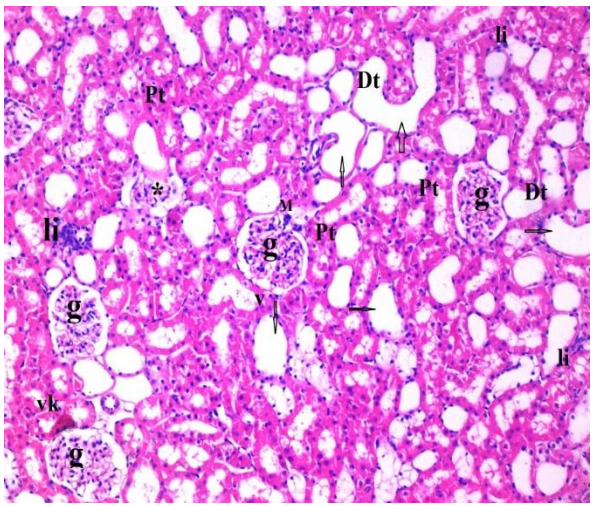

Figure 2: Diabetes ischemia reperfusion group kidney tissue (Pt: Proximal tubule, Dt: Distal tubule, v: vacuole, Li: Lymphocyte infiltration, g: glomerule, *: degenerate glomerule, arrow: dilated tubule, vk: vascular congestion) (H&EX10).

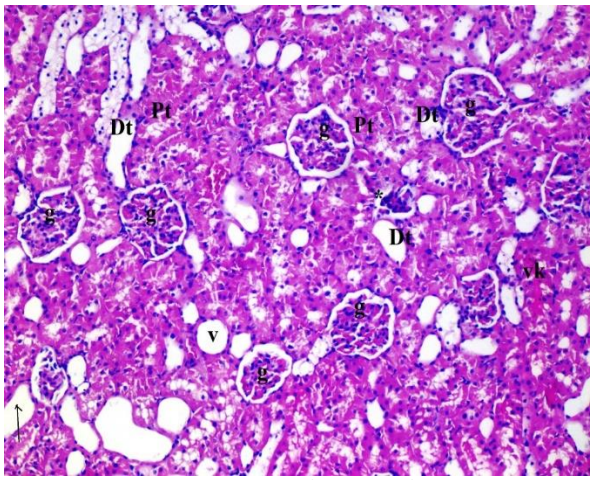

Figure 4: Diabetes ischemia reperfusion sevoflurane group kidney tissue (Pt: Proximal tubule, Dt: Distal tubule, g: glomerule, v: vacuole, arrow: dilated tubule, *: degenerate glomerule, vk: vascular congestion) (H&EX10).

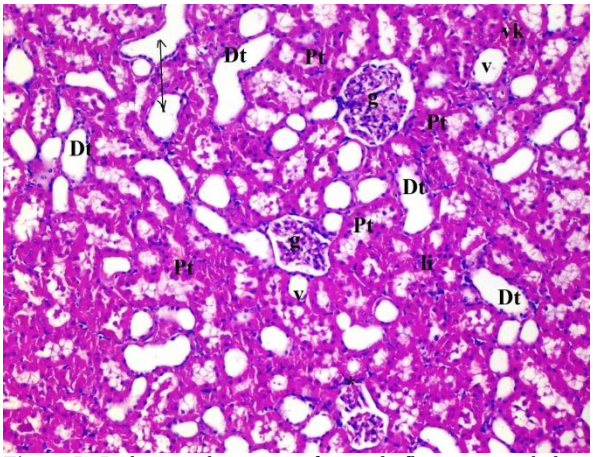

Figure 5: Diabetes ischemia reperfusion desflurane group kidney tissue (Pt: Proximal tubule, Dt: Distal tubule, g: glomerule, v: vacuole, arrow: dilated tubule, *: degenerate glomerule, vk: vascular congestion) (H&EX10).

There was a significant difference in the VVH level between the groups (p<0.021). VVH was more common in the DC and DIR groups than the control group (p=0.030, p=0.008, respectively). Besides, it was significantly lower in the DIRD group than in the DIR group (p=0.008) (Table 1, Figure 1-5).

THC was found to be significantly different between groups (p=0.013). THC was more common in the DIR group than the control and DC groups (p=0.001, p=0.006, respectively). Besides, it was found that the DIRS and DIRD groups were significantly lower than the DIR group (p=0.025, p=0.006, respectively) (Table1, Figure 1-5).

The LI difference was significant between the groups (p=0.046). It was more common in the DIR group than in the control group (p=0.008). It was also significantly lower in the DIRS group than in the DIR group (p=0.008). There was a significant difference in LI between the groups (p=0.046). LI was more common in the DIR group than in the control group (p=0.008). It was also significantly lower in the DIRS group than in the DIR group (p=0.008) (Table 1, Figure 1-5).

THDN, BSD and THD were similar between groups (p=0.108, p=0.113, p=0.097, respectively), (Table 1, Figure 1-5).

3.2. Biochemical Findings

In the biochemical examination, when the groups were compared in terms of serum GST enzyme activity, there was significant (p<0.0001). GST enzyme activity was significantly higher in DC, DIR, DIRS, and DIRD groups than in the C group (p<0.0001, all). Similarly, it was significantly higher in DIR groups than in the DC group (p<0.0001, p=0.001, p=0.006, respectively). Also, it was found to be significantly lower in the DIRS and DIRD groups compared to the DIR group (p=0.030, p=0.006, respectively) (Table 2).

There was a significant difference in serum CAT enzyme activity between the groups (p=0.003). CAT enzyme activity was significantly higher in the DIR group than the C and DC groups (p<0.0001, p=0.024, respectively). It was also found to be significantly lower in the DIRS and DIRD groups compared to the DIR group (p=0.003, p=0.006, respectively) (Table 2).

When the groups were compared with each other in terms of serum NOS enzyme activity, there was a significant difference between them (p=0.043). NOS enzyme activity was significantly higher in the DIR group than the C and DC groups (p=0.006, p=0.021, respectively). Besides, it was found to be significantly lower in the DIRS and DIRD groups compared to the DIR group (p=0.027, p=0.017, respectively) (Table-2).

There was a significant difference in serum TBARS level between the groups (p<0.0001). TBARS level was significantly higher in all groups than in group C (p=0.002, p<0.0001, p=0.009, p=0.042, respectively). Similarly, it was significantly higher in the DIR group than the DC group (p=0.004). Also, it was found to be significantly lower in the DIRS and DIRD groups compared to the DIR group (p<0.0001, p=0.001, respectively) (Table 2).

When compared in terms of SOD enzyme activity, there was a significant difference between the groups (p<0.0001). SOD enzyme activity was significantly higher in the DIR group than the C and DC groups (p<0.001, p=0.003, respectively). Besides, it was found to be significantly lower in the DIRS and DIRD group compared to the DIR group (p<0.0001, p<0.0001, respectively) (Table 2).

4. Discussion

Ischemia/reperfusion injury can describe as restriction of blood flow to a tissue followed by restoration of blood supply and re-oxygenation. The fatal damages can occur during organ transplantation, sepsis, and infarctions. Under these conditions, tissue injury increases via inflammation cascade elements such as over leukocytes activation, cytokines, and reactive oxygen species (22, 23). IRI's effects on the kidney can be explained by AKI, which can result in rapidly progressive dysfunction and result in mortality (24, 25). It has been reported that more than 10 million patients develop AKI each year, and nearly 2 million of them die (26).

There is a direct relationship between the severity of IRI and the levels of antioxidant defense system elements such as SOD, CAT, GST, MDA, and NOS. Oxidoreductases constitute the most important free radical scavenging systems exemplified by CAT, SOD, and Glutathione peroxidase (GSH-Px) and play a cell-protective role beyond antioxidant function (27). High blood levels of CAT show antioxidant activity (28). High GST activity is considered a marker for the elimination of metabolites associated with peroxidation (29). Malondialdehyde is a stable product created by the peroxidation of polyunsaturated fatty acids. It shows the peroxidation of the cell wall. The tissue and plasma MDA levels are well-known indicators of systemic response and oxidative stress after the IRI (30). An idea about the degree of membrane damage can be obtained by measuring the MDA level (31).

Table 1: Histopathological findings of kidney tissue (Mean±SEM).

	Group C (n=6)	Group DC (n=6)	Group DIR (n=6)	Group DIRS (n=6)	Group DIRD (n=6)	P**
Glomerular vacuolization (GV)	0,33±0,21	0,83±0,17	1,33±0,21*	0,83±0,17	0,67±0,21&	0,022
Tubular dilatation (TD)	0,33±0,21	1,00±0,00	1,83±0,31*,?	0,83±0,31&	0,67±0,33&	0,007
Vascular vacuolization and hypertrophy (VVH)	0,33±0,21	1,00±0,00*	1,17±0,17*	0,83±0,31	0,50±0,21&	0,021
Tubular cell degeneration and necrosis (TCDN)	0,33±0,21	0,67±0,21	1,00±0,00	0,50±0,22	0,33±0,21	0,108
Bowman space dilatation (BSD)	0,00±0,00	0,33±0,21	0,67±0,21	0,17±0,17	0,50±0,21	0,113
Tubular hyalin cylinders (THC)	0,17±0,17	0,33±0,21	1,17±0,17*,?	0,50±0,22&	0,33±0,21&	0,013
Lymphocyte infiltration (LI)	0,33±0,21	0,67±0,21	1,17±0,17*	0,67±0,21	0,33±0,21&	0,046
Tubular cell spill (TCS)	0,50±0,22	1,00±0,00	1,00±0,00	0,67±0,21	0,50±0,23	0,097

Table 2: Oxidant state parameters (Mean±SEM).

	Group C (n=6)	Group DC (n=6)	Group DIR (n=6)	Group DIRS (n=6)	Group DIRD (n=6)	P**
GST (mIU/mg.protein)	1,29±0,11	10,09±0,71*	12,55±2,34*	8,24±0,53*,&	6,96±1,42*,&	<0,0001
CAT (IU/mg.protein)	5439,40±1436,19	11661,60±1368,07	19054,83±3311,60*,?	9253,50±1301,51 &	10120,33±2068,71 &	0,003
NOS (IU/mg.protein)	89,82±34,53	162,72±31,54	408,90±129,07*,?	172,75±55,00&	152,22±50,67&	0,043
TBARS (nmol/mg.protein)	11,15±1,91	23,61±2,71*	34,72±3,05*,?	21,61±2,89*, &	19,04±0,98*, &	<0,0001
SOD (U/mg protein)	78,85±27,34	209,60±28,03	493,78±122,96*,?	111,75±11,50&	145,70±37,98&	<0,0001

P**: Kruskal-Wallis test - Significance level p<0.05, *p<0.05: Compared to group C; &p<0.05: Compared to group DIR; ?p<0.05: Compared to group DC.

Malondialdehyde is measured as TBARS. Although MDA is not specific, it correlates well with the degree of lipid peroxidation. Several studies have been published showing the beneficial effects of different agents on the kidney in IRI. Ascorbic acid acts via free radical scavenging systems and shows antioxidant activities (32), and iloprost inhibits lipid peroxidation (33). Leptin increases nitrite and decreasing tumor necrosis factor-alpha (TNF-α) levels (34). The antioxidant effect of levosimendan is owing to the NO-related mechanisms (35). Doxycycline decreases the level of proinflammatory cytokines (36).

Volatile anesthetics, one of the main components of general anesthesia, also have significant effects, such as immune system modulation (37-39). It is thought that trifluoro carbon in molecular structures is responsible for the immune-modulatory effect, and lipid solubility is related to renal protection (40). They have a protective effect in renal tubules through externalization of membrane phosphatidylserine and the release of transforming growth factor (TGF)-β1 in the proximal renal tubule (41).

In experimental studies published in recent years, desflurane preconditioning has been shown to protect the kidney against IRI by regulating the pathway of Nrf2-Keap1-ARE signal, inhibiting oxidative stress, and inflammation (42)]. Sevoflurane has been reported to exert a protective effect against kidney damage by

reducing the expression of TNF-α and NF-κB in renal IRI (43). It demonstrated that upregulation of HIF-2α through sevoflurane pretreatment could improve the renal dysfunction caused by IRI (44).

A randomized clinical study performed on renal transplantation showed that renal protective effects of sevoflurane and desflurane were demonstrated by similar preoperative and postoperative serum creatinine, Interleukin (IL)-2, and TNF-α levels (45).

In our study, SOD, CAT, GST, NOS enzyme activities, and TBARS levels as IRI biochemical markers significantly increased in diabetic rats compared to the control group. SOD, CAT, and GST enzyme activities and TBARS levels were lower in the sevoflurane and desflurane groups than the DIR group without medication.

As histopathological markers, VVH, GV, THC, LI, BSD, TD, and THDN levels were significantly increased in the DIR group. On the other hand, these parameters were significantly decreased in the desflurane group compared to the sevoflurane group, while the BSD was significantly increased in the desflurane group. All these data were interpreted as that sevoflurane and desflurane reduced the effects of lower extremity IRI on the kidney, created by clamping the abdominal aorta.

5. Conclusions

These biochemical and histopathological findings indicate partial renoprotective effects of sevoflurane and desflurane at the second hour of reperfusion. More clinical and experimental studies are needed to prevent and treat IRI-induced AKI effectively.

Conflict of Interests

The authors declare that there is no conflict of interest.

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Author Contributions

Aydin ME and Arslan M contributed to the conception and design of the study. Sezen CS contributed to the collection of the data and statistical analysis and evaluation of the results. Bayraktar AC contributed to the creating and writing of the manuscript. Erbatur ME and Kavutcu M contributed to revising the work and final approval of the version.

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An Uncommon Cause of Intestinal Obstruction: Paraduodenal Hernia

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Abstract: Internal hernias are defined as the herniation of the internal organs from a congenital or acquired cavity in the abdominal cavity. Paraduodenal hernias are a rare subgroup of internal heanias and causes 1% of small bowel obstruction. Paraduodenal hernias seen due to congenital malrotation of intestines. Herniated abdominal content locates in right or left retrocolic area. Altough paraduodenal hernias seen rare, they should be kept in mind in the manner of differential diagnosis. In this article, we present case of a 52-year-old male patient who was admitted to the emergency department with the complaints of ileus and diagnosed as left paradudenal hernia. © 2021 NTMS.

Keywords: Instestinal Obstruction, Small Bowel, Paraduodenal Hernia, Hernia.

1. Introduction

Paradudenal hernias are rare causes of small bowel obstruction that constitutes approximately half of the internal hernias. Left paradudenal hernias are three times more common than the right ones (1). Computed tomography (CT) is an important diagnostic tool in the preoperative evaluation of patients. Although it is difficult to consider paraduodenal hernias among the differential diagnosis, it is an important clinical presenation that may eventually lead to necrosis of the intestines. Contrasted abdominal tomography is highly effective in aiding the diagnosis. In this article, we present case of a 52-year-old male patient who was admitted to the emergency department with the complaints of ileus and diagnosed as left paradudenal hernia.

2. Material and Methods

2.1. Case

A 52-year-old male patient with abdominal pain, dyspepsia and vomiting symptoms exists for the last two days was admitted to the emergency service. He had previously undergone laparoscopic cholecystectomy and had long-term complaints of bloating and vomiting. On physical examination, there was minimal distension in the abdomen, without any muscular defense or rebound. In the laboratory tests white blood cell count was 7000/μL hemoglobin 15 g/dL. routine biochemistry and bleeding time values were within normal limits. Direct abdominal x-ray showed small bowel-type air-fluid levels. Contrast abdomen tomography showed that the small intestine was leveled and collected in an area such as in a sac (Figure 1a).Vascular structures in hernia sac extends to the hernia neck (Figure 1b).

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Patient was hospitalized with the sac diagnosis of intestinal obstruction. During follow-up, his complaints did not regress and he was taken to the operation. The operation revealed that all small intestines were covered with a peritoneal membrane and herniated into the left paraduodenal space (Figure 2). The hernia sac was opened and intestines were mobilized. The blood supply to the intestines was normal. Peritoneal gap was closed (Figure 3). The patient was discharged on the postoperative 4th day.

3. Discussion

Internal hernias are defined as the herniation of the internal organ from a congenital or acquired cavity in the abdominal cavity. According to Hansmann and Morton's studies on 967 cases, the internal hernias are divided into 7 main types by their location: paraduodenal, foramen of Winslow, pericecal, intersigmoid, transmesenteric, transomental and retroanastomotic (2). In addition, Liew and colleagues identified 25 different internal hernias according to their anatomical location (3).

Paraduodenal hernias are a subgroup of internal hernias, constitute 50% of internal hernias and 1% of small bowel obstruction. Firstly Andrews pointed out the congenital malrotation occurred in paraduodenal hernias (4). Paraduodenal hernias are divided to two groups as right and left. In paraduodenal hernias, herniated abdominal content in retrocolic area named as fossa of Waldeyer at right, fossa of Landzert at left. Paraduodenal hernias are generally seen in the 4th and 5th decades of life, more frequent in males and occurs three times more commonly on the left side (5).

Clinical manifestations include cramp-style pain, nausea, vomiting, distension, and clinical conditions ranging from ileus to shock due to herniated bowel segment. Until 50% of the patients are diagnosed, there is a history of intermediate ileus and the other 50% group is diagnosed incidentally (6).

Radiological evaluation is very important in the diagnosis. Air-fluid levels can be seen at direct X-ray. In the barium examinations, dilated loops of small intestine at the proximal of the obstruction can be seen and slowed barium passage in the obstructed area can be noted. Arteriographs can show left deviation of jejunal or splenic artery. Ultrasonography may show an increase in thickness in small bowel walls or intraabdominal fluid. On computed tomography, the findings can be detected such as collecting the intestines form as mass without capsule and the right or left deviation of the veins at the level of the hernia neck (7). Although the imaging modalities are helpful, the findings of paradudenal hernia were shaped within the framework of the 'Classic Empty Abdomen Sign' which was defined by Kummer in 1921 as the well-defined mass formed small intestines without passage of intestines to the real pelvis standing at upright position (8).

As surgical approach, open surgery technique is mostly used, but there are studies about laparoscopic approach (9). In operation, dilatation of the hernia neck or emptying of the content within the hernia sac with closure of the hernia neck can be applied. Additional surgical procedures may be required depending on the condition of the hernia. Since the hernia neck may be adjacent to right / left colic arteries, celiac artery and celiac vein, attention should be paid to the vascular structures mentioned above during the surgery (9).

Left paraduodenal hernias should be treated surgically as soon as they are diagnosed since they have the risk of intestinal ischemia associated with obstruction and strangulation (10, 11).

1a 1b

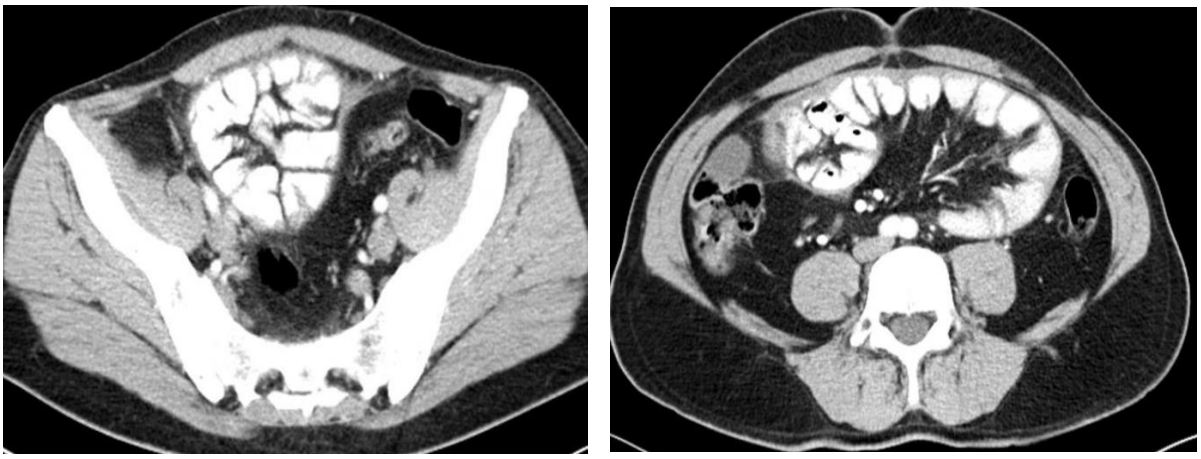

Figure 1: Contrast abdomen tomography showing (a) leveling, enlargement of the small intestine and collection of the intestines in an area such as in the sac.,(b) vascular structures extending to hernia neck.

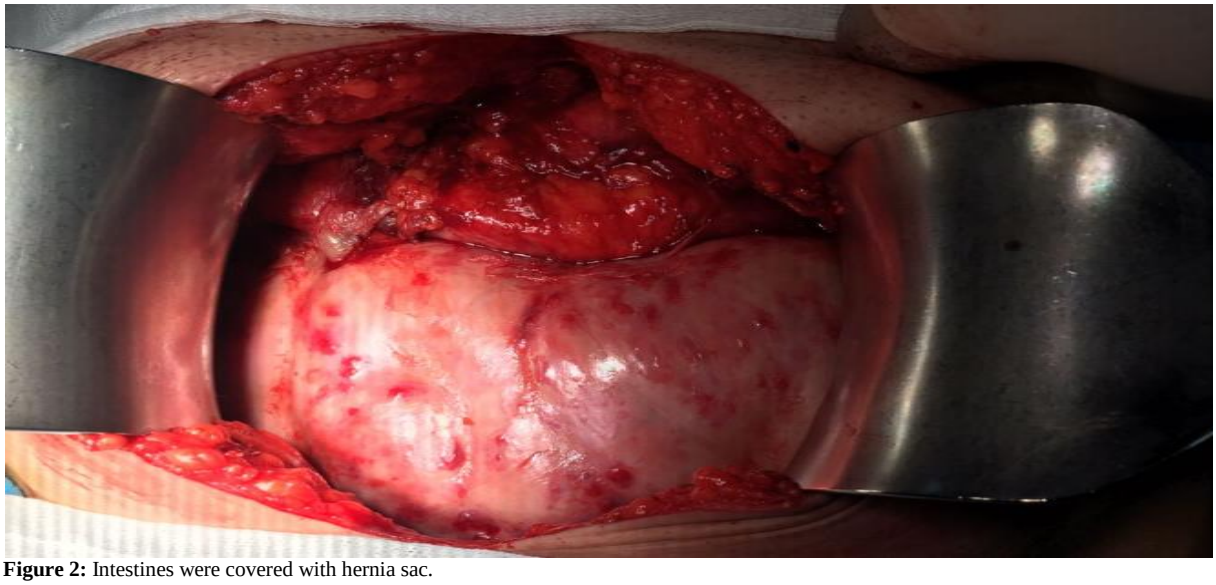

Figure 2: Intestines were covered with hernia sac.

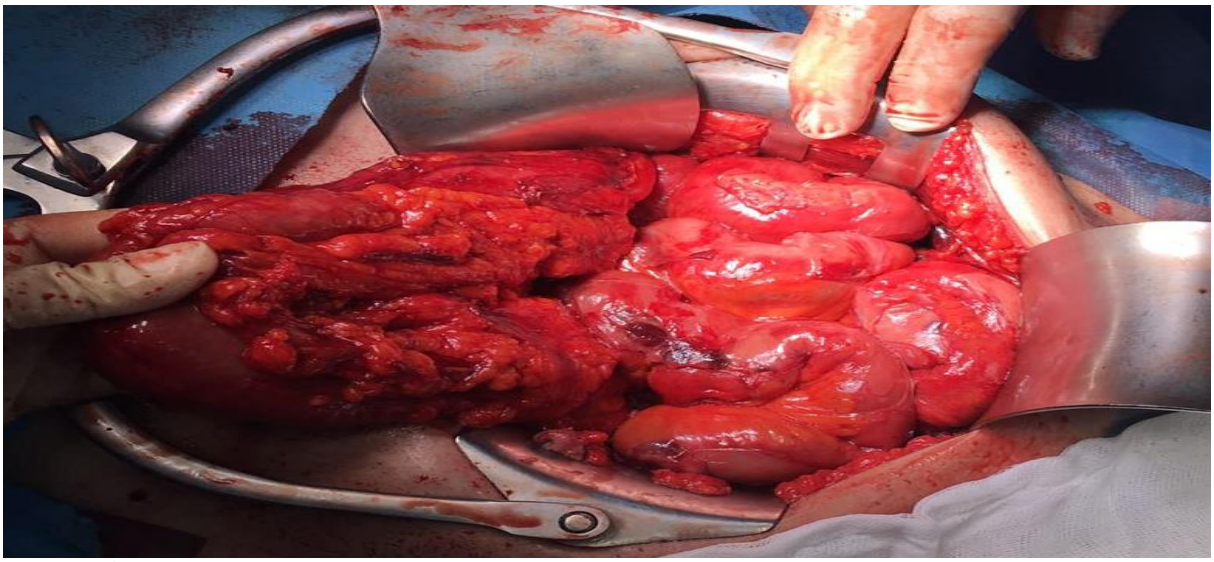

Figure 3: After the hernia sac opened and intestines mobilized.

Conflict of Interests

The authors have no conflicts of interest to declare.

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Author Contributions

Disci E, Yıldırgan Mİ. Albayrak Y. and Memis U. contributed to the conception and design of the study. Disci E and Memis U. contributed to data collection, literature review and writing of manuscript. Disci E, Yıldırgan M.İ., Albayrak Y. and Memis U. contributed to revising the work and final approval of the version.

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