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The Frequency of Osteoporosis in Patients with Liver Cirrhosis in Erzurum and Surrounding

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Abstract: Here**,** it was aimed to investigate whether the frequency of osteoporosis in patients with liver cirrhosis increases compared to the healthy population. DEXA (dual energy x-ray absorptiometry) test was applied to 50 patients with liver cirrhosis and 50 healthy people of similar age who were followed in the Gastroenterology Clinic of Atatürk University Medical Faculty Hospital. L1-L4 T scores were determined from the DEXA test results. In addition, age, gender, disease etiology, complications, Child-Pugh score, sodium-corrected MELD (Model for End-stage Liver Disease) score, serum vitamin D and corrected serum calcium levels were obtained from all patients. The mean age of the patient group with liver cirrhosis was 58.18±10.67 years. The mean age of the control group was 59.82±11.54. When compared in terms of age distribution, no statistically significant difference was found between the two groups ($p>0,05$). The mean DEXA test T scores of the patient group with liver cirrhosis was -1.58±1.44 SD and the mean of the DEXA test T scores of the control group was calculated as -1.01±1.32 SD. When compared in terms of T scores, a statistically significant decrease was found in the patients with liver cirrhosis compared to the control group ($p<0.05$). Liver cirrhosis may be a risk factor for the development of osteoporosis and these patients should be followed up for osteoporosis.

Keywords: Liver Cirrhosis; Osteoporosis; Chronic Hepatitis B Infection.

1. Introduction

Liver cirrhosis is one of the common causes of morbidity and mortality in developed countries. Liver cirrhosis is not considered as a disease alone today. It is accepted as a dynamic process that includes the treatment of clinical symptoms and complications (1). There is no single definition that includes all the details, but it is a definition in generally accepted morphological terms. Cirrhosis of the liver is considered to be a disease characterized by simultaneously developing parenchymal necrosis, regeneration and fibrosis resulting in lobular deterioration and nodules. As a result, liver cell failure and portal hypertension are the clinical definition of liver cirrhosis (2).

The main complications expected in liver cirrhosis are; portal hypertension, esophageal varicose bleeding, ascites, SBP (spontaneous bacterial peritonitis), splenomegaly and hypersplenism, HE (hepatic encephalopathy), hepatorenal syndrome, hepatopulmonary syndrome, malnutrition,

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coagulopathy, hepatic osteodystrophy, hematological disorders and hepatocellular carcinoma (3).

Hepatic osteodystrophy is a condition characterized by loss of bone mass and deterioration of its structure, especially in cholestatic chronic liver diseases. Although its pathogenesis is multifactorial, it is not clear. Its histological structure is similar to that of postmenopausal osteoporosis, and trabecular bone structure is more affected by cortical bone structure. Factors thought to be directly or indirectly effective IGF-1 (insulin-like growth factor-1) deficiency, hyperbilirubinaemia, hypogonadism (estrogen and testosterone deficiency), alcohol use, excessive iron accumulation, low vitamin D level, vitamin D receptor genotype, osteoprotegrin deficiency and immunosuppressive therapy before and after liver transplantation (4).

Osteoporosis is a condition that causes an increased risk of fractures, characterized by a decrease in bone mass and deterioration in microarchitecture. The World Health Organization defines osteoporosis as a BMD (Bone Mineral Density) of 2.5 SD or less than the average for a healthy young adult age group of the same age and gender. This value is also expressed as the T score of -2.5 SD or less (5).

DEXA is the most commonly used BMD measurement tool in the diagnosis of osteoporosis. Although any skeletal region can be measured, measurements on the lumbar vertebra and femur are generally evaluated. The T score is obtained by comparing the calculated amount of mineralization with the healthy young adult population (20-30 years old). The healthy young adult populations T score is considered to be 0 SD. T score of -1 SD and above indicates normal BMD. A range of -1 SD and -2.5 SD is considered osteopenia, while a value of -2.5 SD and below is considered osteoporosis (6).

2. Material and Methods

Between 01.07.2019 and 31.12.2019, 50 patients who were admitted to the gastroenterology outpatient clinic of Atatürk University Medical Faculty Hospital or hospitalized in our gastroenterology clinic were prospectively applied DEXA test. Fifty patients who were admitted to our general internal medicine and endocrinology outpatient clinics between 01.07.2019 and 31.12.2019 were included in the control group. DEXA test was performed in these patients and the frequency of osteoporosis was determined. In addition, age, gender, disease etiology, complications, Child-Pugh score, sodium-corrected MELD score, serum vitamin D and corrected serum calcium levels of all patients were determined through routine examinations requested from our patients. In the light of the data obtained, the frequency of osteoporosis in liver cirrhosis patients in and around Erzurum was analyzed and compared with the data of the healthy control group of the same age group.

2.1. Statistical analysis

All data obtained were recorded in Microsoft Excel 2013 program. SPSS (Statictical Package for Social Sciences) 20.0 statistics program was used in the analysis of the data. Group distributions were found to be normal with the Kolmogorov-Smirnov test in SPSS. Then, the data of the patient and control groups were analyzed with Student's t-test.

3. Results

Of the 50 patients diagnosed with liver cirrhosis included in our study, 32 (64%) were male and 18 (36%) were female. Of the 50 patients included in the control group, 15 (30%) were male and 35 (70%) were female. The mean age of the patient group diagnosed with liver cirrhosis was 58.18±10.67, while the mean age of the control group was 59.82±11.54. When compared in terms of age distribution, no statistically significant difference was found between the two groups (p: 0,463).

The mean BMI (Body Mass Index) of the patient group diagnosed with liver cirrhosis was calculated as 27.62±3.65 kg/m^2 and the mean BMI of the control group was calculated as 29.46±5.26 kg/m^2. When compared in terms of BMI, a statistically significant higher was found in the control group compared to the patient group (p: 0.045). In the patient group with a diagnosis of liver cirrhosis, 9 (18%) patients were smokers, 21 (42%) were non-smokers, and 20 (40%) patients became smokers at some point in their life, but quit before the last year. In the patient group with a diagnosis of liver cirrhosis, 7 patients (14%) were using alcohol, and 43 (86%) patients were not using alcohol. Of the 18 female patients in the liver cirrhosis group, 5 were in the premenopausal period and 13 were in the postmenopausal period. Of the 35 female patients in the control group, 6 were in the premenopausal period, while 29 were in the postmenopausal period.

When the patient group diagnosed with liver cirrhosis was examined etiologically, it was seen that 20 (40%) patients were most frequently due to chronic HBV infection, the second most common was 13 (26%) patients were considered cryptogenic, and the third most common was 4 (8%) patients due to chronic HCV infection. Detailed information about the etiological distribution of patients with liver cirrhosis is shown in figure 1.

In the liver cirrhosis patient group, 27 (54%) people were in the Child-Pugh A group, 14 (28%) were in the Child-Pugh B group, and 9 (18%) were in the Child-Pugh C group. When the new sodium-corrected MELD scores of the same group were calculated, it was found that 18 (36%) patients had a score of 9 and below, 24 (48%) patients had a score between 10 and 19, and 8 (16%) patients had a score between 20 and 29.

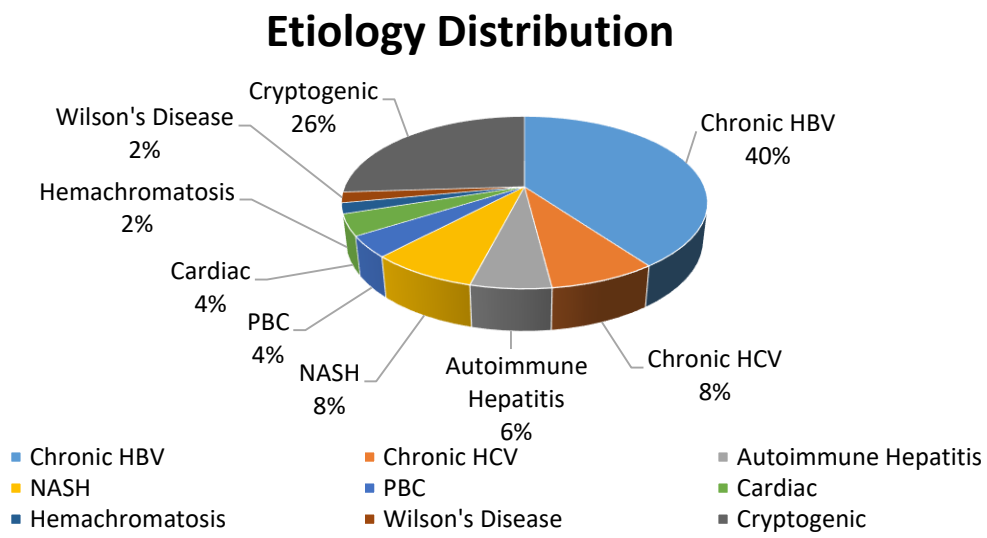

Figure 1: Etiological distribution of the patient group with liver cirrhosis.

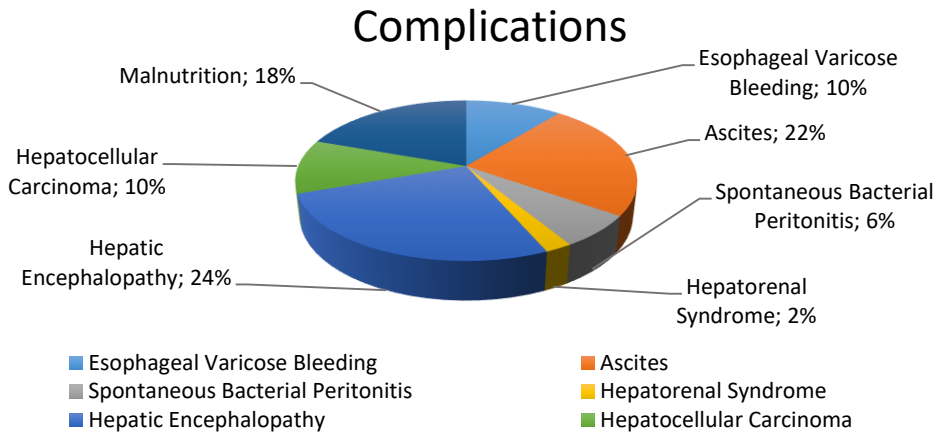

Figure 2: Complications in patients.

While the mean vitamin D level was 18.76±9.89 ng/ml in the group with liver cirrhosis, it was found as 23.48±14.15 ng/ml in the control group. When compared in terms of vitamin D levels, no statistically significant difference was found in both groups (p: 0.056). The mean corrected calcium value was 9.27±0.46 mg/dl in the patient group with liver cirrhosis and 9.29±0.59 mg/dl in the control group. When compared in terms of corrected calcium levels, no statistically significant difference was found between the two groups (p:0.83).

Complications developing in the patient group diagnosed with liver cirrhosis are shown in detail in figure-2. When the patients with EVB (Esophageal Varicose Bleeding) were examined, it was seen that 1 (2%) patient was male and 4 (8%) patients were female. When compared in terms of gender, the rate of EVB in sick females was found to be statistically significantly higher than in males (p: 0.031). In the patient group with a diagnosis of liver cirrhosis, 10 (20%) patients used entecavir, 8 (16%) patients used tenofovir, 8 (16%) patients used furosemide, and 1 (2%) patient used enoxaparin. When the T scores in the DEXA test of patients using entecavir and those not using it were compared, no statistically significant result was found (p:0.78). When the T scores of the DEXA test of the patients using and not using tenofovir were compared, a statistically significant low T score was found in patients who did not use the drug (p:0.008). When the T scores of the patients using furosemide and those who did not use the DEXA test were compared, no statistically significant difference was found (p:0.4).

In the liver cirrhosis group, patients with normal BMD, ostopenic and osteoporotic were grouped according to their etiology. 6 (12%) patients with liver cirrhosis due to chronic HBV infection had normal BMD and 14 (28%) patients had low BMD. The most common etiology in the group with low BMD was found to be chronic HBV infection.

When the DEXA test T scores of the patient group diagnosed with liver cirrhosis were examined, it was observed that it ranged from -4.4 to 3.1 SD. The mean of the T scores was calculated as -1.58±1.44 SD. When the DEXA test T scores of the control group were

examined, it was observed that it ranged from -3.6 to 1.6 SD. The mean of the T scores was calculated as -1.01±1.32 SD. When compared in terms of T scores, a statistically significant decrease was found in the patient group with a diagnosis of liver cirrhosis compared to the control group (p: 0.042).

4. Discussion

Osteoporosis is an important disease that causes an increase in mortality and morbidity, especially in postmenopausal women in our country and in the world. There are risk factors that should be evaluated before the diagnosis of osteoporosis. High BMI is considered to be a risk factor in many diseases other than osteoporosis. In contrast, one of the risk factors in osteoporosis is low body mass index (7). In our study, the mean BMI of the control group was found to be statistically significantly higher than the average BMI of the patient group with liver cirrhosis.

Many conditions such as chronic viral hepatitis, alcohol, hereditary metabolic diseases, NASH (Nonalcoholic Steatohepatitis), cardiac diseases, autoimmune diseases and toxic diseases can be involved in the etiology of liver cirrhosis. The most common etiology causes in the world and in our country are viral hepatitis and alcohol (8). In a study conducted by H. Enomoto et al in Japan on 48 621 cases, the most common (48.2%) chronic HCV infection in the etiology of liver cirrhosis, chronic alcohol use was the second (19.9%) and the third (11.5%) chronic HBV infection has been detected (9). In another study conducted by D. Kim et al. in the USA with 100 000 cases, the most common (32.1%) chronic HCV infection in the etiology of liver cirrhosis, chronic alcohol use was the second (61.1%) and the third (2.6%) chronic HBV infection has been found (10). In our study, in the etiology of liver cirrhosis, the most common (40%) chronic HBV infection, the second (26%) cryptogenic and the third (8%) chronic HCV infection were found. It was thought that the high rate of cryptogenic etiology in our study was due to the low number of patients in our study. In our study, liver cirrhosis due to chronic alcohol use was not detected. It was thought that the reason for this situation was the low alcohol consumption of our countries people due to religious and cultural reasons and the population of our study was limited to 50 patients.

Expected complications in patients with liver cirrhosis are portal hypertension, EVB, ascites, SBP, splenomegaly and hypersplenism, HE, hepatorenal syndrome, hepatopulmonary syndrome, malnutrition, coagulopathy, hepatic osteodystrophy, hematological disorders and HCC. Complications that develop may cause an increase in the mortality and morbidity of the patients. Sometimes patients can be diagnosed because of a complication that develops while living their lives without being aware of liver cirrhosis (11).

In the study conducted by J.C. Lai et al. on 1044 patients with liver cirrhosis, the frequency of HE was found to be 41% and the frequency of ascites as 36% (12). In our study, the frequency of HE was found to be the most common complication with 24% (12 patients) and ascites as the second most common complication with a frequency of 22% (11 patients). In our study, the frequency of HE and ascites development was lower than the study of J.C. Lai et al. The reason for this is thought to be the regular follow-up of the patients with liver cirrhosis in our region and the compliance of the patients with the recommendations and treatments.

In the study conducted by T.W. Sherpa et al. on 50 patients diagnosed with liver cirrhosis, the incidence of malnutrition was found to be 74%, the incidence of EVB as 22%, and the incidence of SBP as 8% (13). In our study, the frequency of malnutrition in the patient group was found to be 18%, the frequency of EVB as 10% and the frequency of SBP as 6%. In our study, according to the study of T.W. Sherpa et al., the frequency of malnutrition and EVB is significantly lower, but the frequency of SBP development is almost similar.

62 patients (23%) were diagnosed with HCC in a study conducted by H. Oka et al. with 260 patients with liver cirrhosis (14). In our study, HCC developed in 10% of the patients. In our study, the frequency of HCC development is lower than that of H. Oka et al. In the study conducted by S.W. Lee et al with 97 patients with esophageal varicose bleeding, recurrent bleeding was detected in 14.4% (15). There was no statistically significant difference in gender between the groups with and without recurrent bleeding. In the study conducted by B. Kraja et al on 139 patients with esophageal varicose veins, 24% of the patients developed EVB (16). On the other hand, 14.7% of the patients with EVB are female and 85.3% are male. In our study, the frequency of EVB was found to be 10%. 80% of these patients are female and 20% are male. It was found that the EVB was statistically significantly higher in female patients compared to male patients.

Patients diagnosed with osteoporosis should be examined in terms of secondary causes. Oral nucleotide/nucleoside analogues, which are antiviral drugs, can be used to provide treatment in patients with chronic HBV infection. Tenofovir, one of these drugs, is thought to cause a decrease in BMD. Hydroxylation of vitamin D in the kidney is completed in the proximal tubule. Tenofovir is thought to cause abnormalities in the proximal tubule, leading to a decrease in vitamin D levels and, consequently, osteoporosis (5).

In the study conducted by M.T. Wei et al on 1224 Asian patients with chronic HBV infection, 276 patients used tenofovir, 335 patients used entecavir and 613 patients did not use either drug. In the 8-year follow-up, the frequency of osteopenia/osteoporosis was found to be 13.17% in the tenofovir group, 15.09% in the entecavir

group and 10.17% in the group not using drugs. There was no statistically significant difference between these 3 groups in terms of osteopenia / osteoporosis (17). In our study, the mean T score in the DEXA test was found to be -0.37±1.18 SD in patients using tenofovir and -1.81±1.38 SD in patients not using it. When the T scores in the DEXA test of the patients using and not using tenofovir were compared, a statistically significant low T score was found in patients who did not use the drug, contrary to the data in the literature. It was thought that this was due to the limited number of patients in our study and the weakness of the power to represent the general population. n the DEXA test, the mean T score was found to be -1.73±1.33 SD in patients using entecavir and -1.59±1.39 SD in patients not using entecavir. A statistically significant result was not found when T scores in the DEXA test of patients using entecavir and those who did not use it.

A study on osteopenia/osteoporosis was conducted by L.S. Lim et al on 6481 women using loop diuretic drugs and those who did not. A statistically significant decrease was found in the DEXA test T score levels in patients using loop diuretics compared to patients not using loop diuretics (18). In our study, when the T scores of the patients using furosemide and those not using it were compared, a statistically significant difference was not found in accordance with the literature.

Hepatic osteodystrophy is a bone metabolism disorder especially seen in those with cholestatic chronic liver disease. Factors thought to have an effect on osteoporosis are IGF-1 deficiency, hypogonadism (estrogen and testosterone deficiency), alcohol use, low vitamin D level, osteoprotegrin deficiency, and immunosuppressive drugs used before/after liver transplantation. The reasons for low vitamin D levels; disruption of hydroxylation in the liver, malabsorption, disruption of the enterohepatic cycle and increased urinary excretion. Osteoporosis that develops in patients with liver cirrhosis can cause an increase in morbidity and mortality with bone fractures (19).

In a study conducted by Y. Karoli et al on 72 patients with liver cirrhosis, the most common etiology in the group with low BMD was found to be chronic HCV infection (20). In our study, in contrast to the study of Y. Karoli et al, the most common etiology in the group with low BMD among 50 patients with liver cirrhosis was chronic HBV infection.

In a study conducted by V. Goral et al, when the DEXA test was compared in terms of T scores, a statistically significant decrease was found in the patient group with a diagnosis of liver cirrhosis compared to the control group (21). In our study, when the DEXA test T scores were compared, a statistically significant decrease was found in the patient group with a diagnosis of liver cirrhosis compared to the control group, similar to the literature data.

5. Conclusions

In our study, the number of patients considered cryptogenic was found to be higher when compared with the data in the literature. In our region, patients diagnosed with liver cirrhosis and whose etiology cannot be determined should be reviewed and carefully examined. It was observed that the vitamin D levels of the patients participating in our study were below the level considered optimal by the World Health Organization. Because of the long winter season in our region, wearing clothes that cover almost the entire body and the low number of sunny days throughout the year explain the low vitamin D level. We should follow the vitamin D levels of the patients in our region and arrange their treatment when necessary. In addition, we must inform the patients about the appropriate contact method with sunlight for vitamin D synthesis to occur. We should follow the patients who apply to our polyclinics closely in terms of complications. We should find and treat patients with HE, if any, by finding the underlying causes. When compared in terms of gender, the rate of EVB in sick women was found to be statistically significantly higher than the sick men. Female patients with esophageal varices should be followed up more closely in terms of EVB. The fact that our study was limited to 50 patients weakens the strength of this finding. Male and female patients can be compared in terms of EVB in future studies with higher populations. The DEXA test T scores of patients with entecavir, tenofovir and furosemide among patients with liver cirrhosis included in our study were analyzed. When compared with patients who did not use these drugs in the same group, their effects to increase the development of osteodystrophy were not observed. However, there are findings in the literature that they predispose to osteoporosis in larger studies on these drugs. Therefore, patients should be carefully monitored for osteoporosis while using drugs such as entecavir, tenofovir and furosemide.

Liver cirrhosis can predispose to the development of osteoporosis. These patients should be closely followed up for osteodystrophic complications and treated early. Studies with higher populations are required to clearly reveal the relationship between chronic HBV infection and osteodystrophy.

Conflict of Interests

Authors declare that they have no financial interests or personal conflicts that may affect the study in this article.

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Author Contributions

Concept- Menekşe B., Topdağı Ö.; Design-Menekşe B., Topdağı Ö.; Supervision-Topdağı Ö.; Data Collection and/or Processing-Menekşe B.; Analysis

and/or Interpretation - Menekşe B.; Literature Search- Menekşe B., Topdağı Ö., Sanalp Menekşe T.; Writing Manuscript- Menekşe B.; Critical Review- Topdağı Ö., Sanalp Menekşe T.

Ethical Approval

The Ethics Committee's approval of the Atatürk University Faculty of Medicine is obtained for the study. (Decision number 4-A of 24.05.2019 dated meeting no: 07).

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Characteristics of Post-Tonsillectomy Hemorrhage Patients and Our Approach to These Patients

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Abstract: Tonsillectomy is one of the most common operations performed by Otorhinolaryngologists in practice and can lead to important complications. Post-tonsillectomy hemorrhage is one of the major complications. The aim of the study was to present the characteristics and treatment approaches of patients who were admitted to our clinic with post-tonsillectomy hemorrhage, followed-up and treated. Patients who applied to Otorhinolaryngology Clinic of our hospital between January 2014 and December 2020 with complaints of bleeding from the tonsil after tonsillectomy were included in this study. Patient files and hospital automation system were reviewed retrospectively. A total of 634 tonsillectomy operations were performed in our clinic between 2014 and 2020. Of these, 34 patients had post-tonsillectomy hemorrhage. 20 (58.8%) patients were male and 14 (41.2%) were female, and the mean age was 20.5 years (7-40 years). Of the 34 patients who were intervened in our clinic, 14 (41.1%) were in the pediatric age group and 20 (58.9%) were in the adult age group. 7 (20.5%) of the cases were primary hemorrhage that occurred in the first 24 hours after the operation. In the present study, 14 patients had bleeding from both sides and 14 patients from the left side. Patients who present with post-tonsillectomy hemorrhage should be hospitalized, even for observation purposes, vascular access must be established, and both examination and vital signs and hematological parameters should be closely monitored, and life-threatening complications should be prevented by timely interventions.
Keywords: Tonsillectomy; Hemorrhage; Complication.

1. Introduction

Liver cirrhosis is one of the common causes of Tonsillectomy is one of the most common surgeries performed by Otorhinolaryngologists (ENT) (1, 2). Tonsillectomy was first described in the literature 3000 years ago (3). Obstructive sleep apnea and recurrent throat infections are the most common indications for tonsillectomy surgery (4). Most common complications after tonsillectomy are nausea, vomiting, respiratory problems, respiratory restriction, dehydration, fever and bleeding (5). Among these complications, tonsil bleeding is one of the most serious and common complications of tonsillectomy surgery (6). In various studies, the frequency of bleeding after tonsillectomy ranges between 0.8% and 18% (7).

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Primary hemorrhage occurs in the first 24 hours after the operation and can be more dangerous. Bleeding after 24 hours is called secondary hemorrhage (8).

Many studies have tried to reveal the factors associated with post-tonsillectomy hemorrhage (9). While investigating the causes of secondary hemorrhage after tonsillectomy, factors such as age and gender, surgical technique and experience, recent infections, and hematological parameters have been proposed. However, in many studies, the effects of very few of the factors mentioned above were found to be statistically significant on post-tonsillectomy hemorrhage (10, 11).

In this study, we aimed to present the characteristics and treatment approaches of patients who were admitted to our clinic with post-tonsillectomy hemorrhage, and followed-up and treated.

2. Material and Methods

The study was started after the approval of Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (access number: E.21142744-04/04). Patients who applied to Mengücek Gazi Training and Research Hospital Otorhinolaryngology Clinic and Dilber private clinic between January 2014 and December 2020 with complaints of bleeding from the tonsil after tonsillectomy were included in this study. Patient files and hospital automation system were reviewed retrospectively, 34 patients who presented with bleeding were identified and included in the study. Patients with missing information in their files and patients who could not be reached, and patients with only adenoid bleeding were excluded from the study. None of the patients with post-tonsillectomy hemorrhage were treated as outpatients. The patients were evaluated in terms of age, gender, time after tonsil surgery, and intervention for bleeding. In addition, cases that were hospitalized and treated with the complaint of PTH were evaluated for time of hemorrhage after tonsillectomy, how many days they were kept under observation, the severity of hemorrhage (minor/major bleeding), the procedure applied to stop the bleeding, whether there was a need for blood transfusion, and the presence of infection in the tonsillar bed.

All cases were hospitalized for routine follow-up at least one night regardless of the severity of bleeding. Laboratory tests included complete blood count, prothrombin time (PTZ), and activated partial thromboplastin time (aPTZ). After the physical examination, oral feeding was stopped in all patients and intravenous fluid supplementation was initiated. All clots detected in the tonsils were cleared. Patients gargled with cold water containing adrenaline and hydrogen peroxide, and gauze tampons soaked in 2 ml of local anesthetic containing 20 mg/ml lidocaine and 0.0125 mg/ml epinephrine were held with Allis forceps and compressed on the bleeding area. Cases that did not respond to this conservative treatment or had severe bleeding were intervened in operating room conditions under general anesthesia. Local compression, bipolar electrocauterization and/or suture-ligation were performed under general anesthesia. The cases were discharged according to their general conditions, bleeding parameters and hemoglobin values.

2.1. Statistical analysis

Statistical analysis was performed using SPSS (SPSS 20.0 for Windows, Inc. Chicago, IL, USA) package program. The data were evaluated using descriptive statistics and the Mann-Whitney U Test. $P < 0.05$ was considered significant in all analyses.

3. Results

A total of 634 tonsillectomy operations were performed in our clinic between 2014 and 2020. Of these, 34 patients had post-tonsillectomy hemorrhage. 20 (58.8%) patients were male and 14 (41.2%) were female, and the mean age was 20.5 years (7-40 years). Of the 34 patients who were intervened in our clinic, 14 (41.1%) were in the pediatric age group and 20 (58.9%) were in the adult age group. The onset of bleeding occurred anywhere from the first 24 hours to 14 days following surgery (mean: 6.5 days) (figure I). 7 (20.5%) of the cases were primary hemorrhage that occurred in the first 24 hours after the operation. 27 (79.5%) of the cases were secondary hemorrhage that occurred more than 24 hours after the operation. In 22 (64.7%) of the patients who were treated in our clinic, bleeding was controlled with cold application, local compression and topical hemostatic agent application after clearing the clot in the field under local anesthesia. Surgical treatment in operating room conditions was preferred in patients whose bleeding could not be controlled with conservative methods under local anesthesia. Hemorrhage intervention was performed under general anesthesia in 12 (35.3%) patients whose bleeding could not be controlled by local intervention, and suture-ligation and coagulation methods with bipolar cautery were primarily preferred.

Table 1: Time of hemorrhage according to gender.

Gender	N	Mean ±SD	p
Male	20	5.8±3.5	0.31
Female	14	7.5±4.5	

N: Number; SD: Standard deviation.

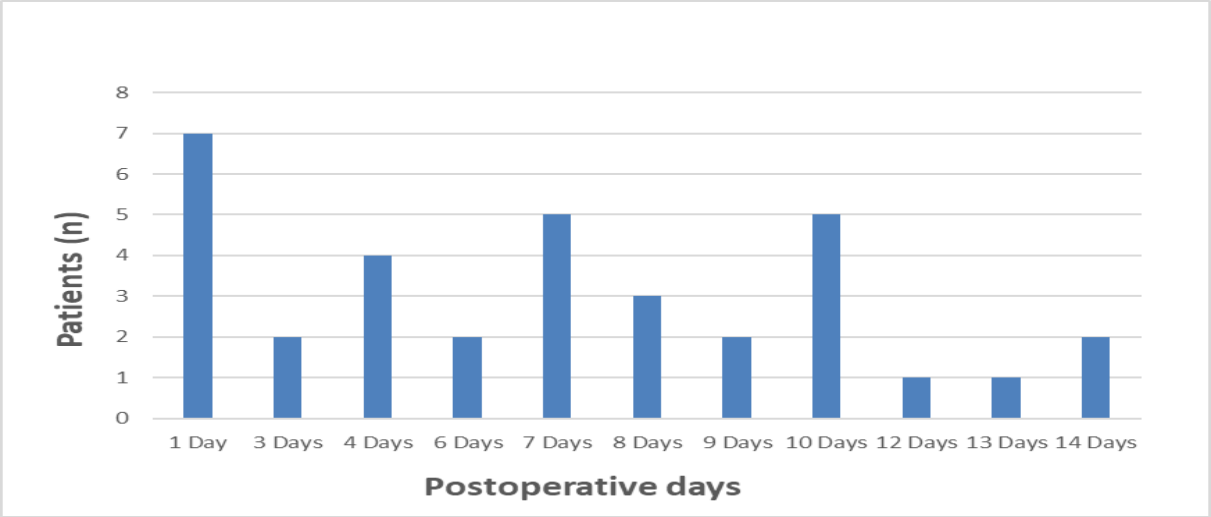

Figure 1: Distribution of the number of bleeding after tonsillectomy by days.

In the present study, 14 patients had bleeding from both sides, 14 patients from the left side and 6 patients from the right side. No significant difference was found between bleeding times according to gender (p: 0.31) (Table 1). In the complete blood count performed during the evaluation phase of the patients, hemoglobin values were within normal limits and none of the patients required blood transfusion. No pathological indications were found in the blood analysis performed for bleeding diathesis investigation.

4. Discussion

Although the incidence of complications after tonsillectomy has decreased thanks to surgical techniques developed in recent years, post-tonsillectomy hemorrhage is still the most common and life-threatening complication of this operation[7]. In many studies, the frequency of post-tonsillectomy hemorrhage has been reported to vary between 0.8% and 18% (12-14). In the present study, the frequency of bleeding was 5.36%, with 34 cases observed after 634 tonsillectomy operations.

Postoperative bleeding may lead to negative consequences such as re-hospitalization, emergency reoperation, and death (5). It has been suggested that primary bleeding occurs due to acute vascular injuries during surgery, whereas secondary bleeding is associated with dissolution of clot in previously coagulated foci and fibrinolysis, which can sometimes be seen due to surgical wound infection (1). A bleeding complication can lead to readmission after discharge from the hospital. Patients presenting with bleeding complications may be followed only with close clinical observation initially, or in more severe cases, they may require surgical intervention under local or general anesthesia due to bleeding (1-15). Furthermore, post-tonsillectomy hemorrhage may result in mortality, albeit rare. Post-tonsillectomy hemorrhage is more common in adults than in children (15-18). In our clinic, postoperative hemorrhage rate was calculated as 41.1% in the pediatric group and 58.9% in the adult group. While hemorrhage seen in the first 24 hours after surgery is evaluated as primary, hemorrhage after 24 hours is evaluated as secondary (1, 14, 15, 19). The rate of primary hemorrhage was 20.5%, while the rate of secondary hemorrhage was 79.5%. Mortality was not observed in the present study.

Although there is no clear relationship between sex and bleeding, many studies have reported more post-tonsillectomy hemorrhage in men than in women (16, 20-22). Similarly, in our study, the rate of men was higher than women but they are dissimilar to those reported by Carmody et al and Myssiorek et al (23, 24). In the study conducted by Taşlı H. et al., when the time of admission with post-tonsillectomy hemorrhage was examined, the average time of admission was 7.6 (1-16 days) after surgery (25). In the study conducted by DO. Francis et al., When the time of admission with secondary post-tonsillectomy hemorrhage was examined, the average time of admission was 5.5 (3-10 days) after surgery (4). Similarly, the average time of admission in our clinic was determined to be 6.5 days after surgery (1-14 days).

In most clinics, during tonsil surgery, intraoperative bleeding foci are intervened by suturing or ligation after 5-7 minutes of tamponade containing adrenaline, and sometimes cauterization with bipolar cautery is applied. In 22 (64.7%) of the patients who were treated in our clinic, bleeding was controlled with cold application, local compression and topical hemostatic agent application after clearing the clot in the field under local anesthesia. Surgical treatment in operating room conditions was preferred in patients whose bleeding could not be controlled with conservative methods under local anesthesia. In the present study, hemorrhage intervention was performed under general anesthesia in 12 (35.3%) patients whose bleeding could not be controlled by local intervention, and suture-ligation and coagulation methods with bipolar cautery were primarily preferred.

5. Conclusions

Patients who present with post-tonsillectomy hemorrhage should be hospitalized, even for

observation purposes, vascular access must be established, and both examination and vital signs and hematological parameters should be closely monitored. Life-threatening complications should be prevented in these patients by timely interventions

Conflict of Interests
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Evaluation of the Effectiveness of our Intraoperative Pathology Consultations in a Five-Year Period: A Total of 2.179 Cases

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Abstract: Intraoperative consultations (IOCs), which meet the urgent need for diagnosis during an operation, are widely used for the management of surgical operations. Inquiries that are most frequently made in IOCs include the presence/absence of a mass, malignant-benign differentiation of the mass. IOC is the most urgent and difficult area of pathology. Accurate diagnosis rates and sensitivity and specificity values are important clinical quality indicators. We aimed to evaluate the IOC data of our clinic in light of the literature. The reports of IOCs requested from the Department of Pathology of Ataturk University Faculty of Medicine between 2016 and 2020 and paraffin section (PS) reports of these tissues were retrospectively analyzed. Both reports were compared. The results were classified as age, gender, organ distribution, reason for IOC request, disagreement between IOC and PS diagnoses, and paraffin follow-up. A total of 2.179 cases, 70.03% female and 29.97% male, were included in the study. In 94.81% of the cases, diagnoses made during IOCs were confirmed by the PS examination, 3.62% were deferred, and disagreement was observed between IOC and PS examination in 2.5% of cases. In our study, the sensitivity was 95.7%, specificity 99.3%, positive predictive value 99.1%, and negative predictive value 97.2%. The high agreement between IOC and PS diagnoses is an important quality indicator. However, it is inevitable to avoid certain technical and interpretation mistakes due to the nature of the process. In our study, high accuracy, sensitivity and specificity values were observed in accordance with the literature.

Keywords: Intraoperative Consultation; Frozen Section; Sensitivity; Specificity; Accuracy.

1. Introduction

Tissue samples taken from the body are usually diagnosed in the pathology laboratory after detection, sampling, processing and staining for at least one day. However, when surgeons need urgent diagnosis to guide the operation, diagnostic methods called intraoperative consultation (IOC) or frozen section (FS) have been developed, which are based on freezing the tissues during the operation and taking rapid sections. FS was first applied by Welch in 1891, and with the introduction of cryostat in 1959, it is now widely used in clinical practice (1). IOC is one of the most pressing and difficult areas of pathology practice. It is generally used to determine the presence/absence of a lesion in the tissue to be operated, the nature of the lesion (malignant-benign, if any), the presence of lesion in the surgical margins, whether the sample taken is sufficient for diagnosis and the depth of invasion of the lesion. IOC is a challenging process that requires a multidisciplinary approach, involving an absolute clinic-pathological evaluation with the patient's clinical, laboratory and radiological findings. In routine surgical practice, IOC should ideally be requested in 5-15% of the operated patients, although this number varies according to the availability of hospital beds, clinics and surgeons (1, 2). However, purpose of FS examination requests are sometimes abused for various

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reasons. Dehner and Rosai reported that the cases were urgently directed to treatment in 44% of the FS requests while the operation was completed without waiting for the FS results in 42% (3).

The necessity of making a definitive diagnosis in a short time, limited sampling, freezing, staining and folding artifacts, insufficiency of histological sections compared to routine sections, and freezing difficulties are disadvantages of the method and may lead to diagnostic errors. In studies conducted in many centers including different numbers of cases, it has been stated that the agreement rate between IOC and permanent paraffin section examination ranged from 89.1% to 99.3%. Error rates can vary according to organ type, tumor type, tumor heterogeneity, sampling adequacy, and patholog experience (1, 2, 4-7).

The agreement between IOC and the final pathology report has the potential to change treatment decisions and affect patient care. Determining this agreement is an important component of laboratory quality assurance. Therefore, we aimed to retrospectively evaluate the requested IOCs from our clinic and their results over a five-year period, compare them to the PS results in light of the literature, and identify problems encountered in practice to guide the development of measures.

2. Material and Methods

This study evaluates the IOC's requested by various surgical branches of our hospital from Atatürk University Faculty of Medicine, Department of Pathology between January 1, 2016-December 31, 2020. The areas representing the lesion were sampled from the tissues sent to our laboratory by the relevant surgical branch. After the samples taken from the tissues were frozen in Thermo Scientific Cryotome and Leica 3050 devices, at least four sections of 4-8 μm thickness were taken and fixed with 80% alcohol over 1 minute. Then, the tissues were hematoxylin-stained for 1 minute, rinsed with distilled water, differentiated with acid alcohol for 5 seconds, rinsed again, eosin-stained for 30 seconds, rinsed and dehydrated with alcohol. The samples obtained were evaluated with a light microscope. The results were reported to the relevant surgical branch. Then, the routine processing of the same tissue samples was performed after formaldehyde fixation. These tissues were re-evaluated and the results were reported after processing.

For this study, the reports of IOCs made from 2016 to 2020 and the reports of the PS examination of the same tissues were screened from the archive of our department using the automation system of our hospital and listed. Then, the diagnoses made during IOC and after PS examination were compared. The cases were classified according to year, age, gender, affected organ, benign-malignant nature, whether the diagnosis was deferred, and agreement between the IOC and PS diagnoses. Furthermore, the cases reported to be malignant in IOC and benign in PS examination were evaluated as false positive while those reported to be benign in IOC and malignant in PS examination were evaluated as false negative. Accordingly, the accuracy rate, sensitivity, specificity, positive and negative predictive values of the IOC results were calculated and compared with the literature.

This study was carried out in Atatürk University Faculty of Medicine, Department of Pathology and was approved by the Ethics Committee of the same faculty with the decision numbere 07 and dated 29.11.2018

2.1. Statistical Analyses

All measures of agreement for the entire series are reported with 95% confidence intervals (95% CI). The values and confidence intervals were calculated. Data then analysed statistically to determine overall accuracy, sensitivity and specificity of each benign and malignant group of tumours.

3. Results

The study included a total of 2.179 cases, 1.526 (70.03%) female and 653 (29.97%) male, diagnosed over a five-year period. The mean age was 49.98 ± 17.40 years, with the youngest patient being a 1-month-old male and the oldest being a 90-year-old female. In the distribution of cases according to affected organs and systems, the female genital system ranked first place with 639 (29.3%) cases. This was followed by brain and nervous system lesions with 453 (20.8%) cases, breast lesions with 352 (16.1%) and others (Table 1).

In 1.553 (71.27%) of the cases, IOCs were requested to determine the presence/absence of a lesion and make a malignant-benign differentiation of the lesion if present. This was followed by IOCs requested to inquire about the continuity of the lesion at the surgical margin (n = 349, 16.01%) and the presence/absence of a tumor in the sentinel lymph node (n = 337, 15.46%) (Table 2).

Of our cases, 93.88% of our cases received a definitive diagnosis by IOC, with the diagnoses being benign in 1.251 (57.41%) of the patients and malignant in 795 (36.48%), and these diagnoses were confirmed by PS examination.

In 54 (2.47%) cases, there was a disagreement between the diagnoses of IOC and PS examination, with brain and other nervous system lesions ranking first among these cases (n=13/54, 24.07%), followed by breast sentinel lymph node examinations (n=12/54, 22.22%) and female genital system lesions (n=8/54, 14.81%) (Tables 3 and 4).

In the remaining 79 of the 2.179 cases (3.62%), it was determined during IOC that a diagnosis would be made after the PS examination. According to the organ distribution of the cases deferred until PS examination, brain and other nervous system lesions ranked first with 32/79 cases (40.50%) and female genital system lesions ranked second with 20/79 (25.31%) cases and other systems (Figure 1).

Table 1: Number of Intraoperative Consultation Requests and Organ Distribution by Years.

Organ	**2016**	**2017**	**2018**	**2019**	**2020**	**Total**
Female genital system	107	101	127	152	152	639
Brain and other nervous system	65	78	87	116	107	453
Breast	31	61	77	89	94	352
Head and Neck	28	32	46	57	51	214
Thyroid-Parathyroid	23	25	25	41	29	143
Liver, Gallbladder	18	22	16	28	31	115
Lung, Pleura, Mediastinum	10	13	25	14	12	74
Bone and soft tissue	5	8	18	21	9	61
Intestine	4	6	9	7	5	31
Peritoneum	6	4	8	6	5	29
Stomach, duodenum	3	3	3	8	5	22
Pancreas	4	1	2	9	6	22
Kidney	6	2	2	5	2	17
Testis	2	1	1	1	2	7
Total	312	357	446	554	510	2,179

Table 2: Reasons for Intraoperative Consultation Requests by Years.

Organ	**2016**	**2017**	**2018**	**2019**	**2020**	**Total**
Presence/absence of a mass	215	268	297	397	376	1,553
Evaluation of surgical margin	47	46	72	95	89	349
Evaluation of sentinel lymph node	25	51	69	94	98	337
Thyroid-parathyroid differentiation and other lesion	23	22	34	40	28	147
Presence/absence of ganglion cells	4	7	9	8	5	33
Organ transplant	8	3	4	8	4	27
Total	322	397	485	642	600	2,446

Table 3: Comparison of IOC and PS Diagnoses by Years.

	Agreement between IOC and PS diagnoses		Deferred until PS diagnosis	Disagreement between IOC and PS diagnoses	Total
	Malign	Benign			
2016	102	201	4	5	312
2017	155	178	13	11	357
2018	164	255	14	13	446
2019	186	327	25	16	554
2020	188	290	20	12	510
Total	**795**	**1.251**	**79**	**54**	**2.179**

IOC, intraoperative consultation; PS, paraffin section.

Table 4: Organ Distribution of disagreement between IOC and PS Diagnoses.

Organ	False Positive	False Negative	Different Diagnoses	Total
Central nervous system	3	3	7	13
Breast	2	10	0	12
Uterus	1	4	3	8
Ovary	1	5	0	6
Lung	0	4	2	6
Thyroid	1	4	0	5
Tongue	0	1	0	1
Parotid	0	1	0	1
Skin	0	1	0	1
Testis	0	1	0	1

Total	**8**	**34**	**12**	**54**

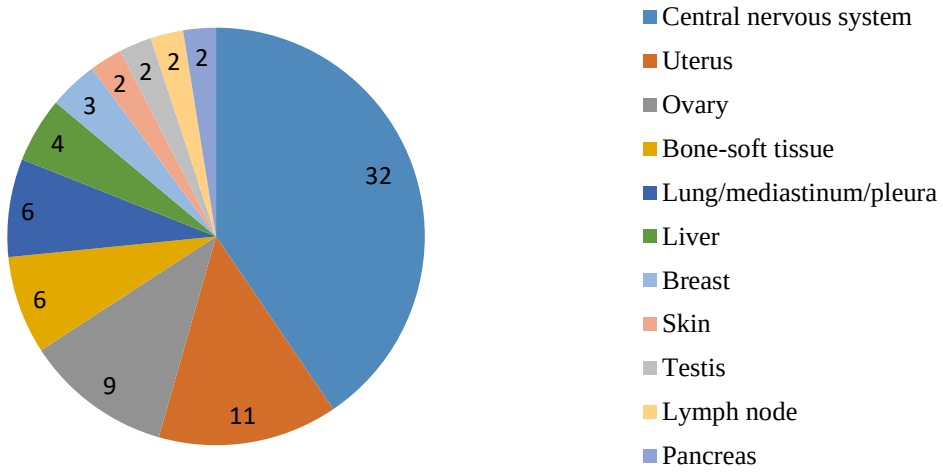

Figure 1: Organ distribution of IOCs postponed to paraffin section.

4. Discussion

IOC is one of the most important diagnostic methods in surgical case management. However, achieving an accurate diagnosis using this method requires multidisciplinary teamwork in an environment including the necessary equipment. The most important stakeholders of this team are surgeons, pathologists, and surgical and pathology technicians. Responsibility for establishing a definitive diagnosis and guiding the operation in a limited time increases the probability of error. Therefore, it is necessary to inform the pathology team to perform IOC about the case in advance and undertake an adequate preoperative clinic-pathological evaluation.

IOC's are usually made in emergency situations, such as malignant-benign mass differentiation, presence of tumor at the surgical margin, presence of sentinel lymph node metastasis, and tumor invasion depth, but they may also be needed if an unexpected situation occurs during the operation. Generally, it is accepted that IOC is required at a rate of 5-15% in surgical operations performed (1, 2, 4-7). A total of 2.179 IOCs performed in our hospital over a five-year period were included in our study. Our cases corresponded to 3.05% of the total 71.430 surgical operations undertaken in our hospital between 2016 and 2020, and this rate is consistent with the literature data.

In the literature, it is stated that the agreement rate between the IOC and PS diagnoses varies between 89.1 and 98.4% in various series including all systems (1, 2, 4-7). It has been reported that 0.1% of the errors made cause a great change in treatment (1, 2) . In IOCs applied to surgical specimens, the accurate diagnosis rates are determined by the structure of the treated organ, type of tumor, size of lesion, number of samples, and most importantly clinic-pathological correlation (2). Many reasons such as limited sample size, freezing difficulty of fatty and necrotic tissues, poor quality of sections, freezing and folding artifacts, inadequate cellular details, presence of bleeding, mucus, inflammation, and insufficient clinical information and surgeon-pathologist communication are among the diagnostic difficulties that lead to errors. Other important causes of diagnostic error are interpretation and inexperience (1, 2, 4). In some studies, tissue sampling errors (45%), interpretation errors in microscopic examination (40%), technical reasons (18%) and insufficient clinical knowledge of the patient (14%) are stated to be the most common causes of errors in IOC (1, 2).

In our study, 57.4% of the cases were diagnosed as benign and 36.48% as malignant in IOC, and these diagnoses were confirmed by PS examination. Thus, the rate of PS confirmation of the IOC diagnoses was 93.89%. When we included 12 cases with a diagnostic disagreement between IOC and PS examination but an agreement in terms of malignant-benign differentiation in this evaluation, the accurate diagnosis rate of IOC increased to 94.81%. It is clear that this rate indicates a high accuracy value when compared with the literature data. It has been reported that the accuracy rate is reduced to 89.1% in various series (5-9). Kösem et al.(8) reported the accuracy rate as 92.7% and Arora et al. (9) as 98.48%.

The diagnosis of 3.6% of the cases in our series was deferred until PS examination, while this rate was reported to range from 0.2 to 7.56 % in different series (8-12). In a study by Hwang et al. including 4.434 cases, it was reported that 2.17% of the cases were deferred until PS examination (10) . This rate was determined as 4.7% by Wen et al (11). 6.6% by Kösem et al (8) and 7.56% by Alabalık et al.(12). It is generally stated that mostly the diagnoses of central nervous system and female genital system lesions are deferred until PS examination (8, 10-14). In our series, consistent with the literature, the most common tissue samples in which the definitive diagnosis was left to PS examination belonged to the central nervous system

lesions (n = 32) and female genital system lesions (n = 20).

In 54 (2.47%) cases in our study, there was a disagreement between the IOC and PS diagnoses. Eight of these cases were defined as false positive and 34 as false negative. Based on these values, the sensitivity of IOC was determined to be 95.8%, specificity 99.3%, positive predictive value 99.0%, and negative predictive value 97.3%. In various series, it has been reported that the sensitivity and specificity rates range from 86.9 to 100% (9) and 57.1 to 98.9% (13) respectively. In our 12 cases, the IOC and PS diagnoses were consistent in terms of benign/malignant differentiation but inconsistent in terms of histopathological type. Our sensitivity and specificity rates are in agreement with the literature (1, 10).

In the current study, central nervous system lesions constituted most of the false positive cases while female genital system lesions constitutes most of the false negative cases. There were three false positive and three false negative cases of brain and other nervous system lesions. Among the false positive cases, FS defined low-grade astrocytoma in one case and low-grade glial tumors in two, but these tissues indicated as gliosis, normal glial tissue, and abscess wall in PS examination. Many studies have demonstrated that abscesses of neuroglial tissue and gliosis areas can cause diagnostic problems and lead to errors (12, 15) . Among our false negative cases, one that was interpreted as benign in FS was reported as ependymoma in PS examination while the remaining two were defined as low-grade glial tumors in FS but diagnosed as gliosarcoma and oligodendroglioma in PS examination. In a study by Alabalık et al., it was stated that the highest disagreement between the FS and PS diagnoses was observed in central nervous system lesions (17.65%) (12). In another study evaluating intracranial tumors, Tofte et al. reported that the rate of disagreement between the IOC and PS diagnoses was 9.7% (15). Therefore, we consider our rate of 2.86% disagreement in nervous system cases to be in compliance with the literature. Especially in tumors of the central nervous system, insufficient sampling, freezing artifacts, and presence of gliosis, as well as inexperience and interpretation difficulties lead to erroneous evaluation.

We determined that one female genital system lesion was diagnosed to be positive for malignancy in the ovary without specifying the nature in FS but reported as a mucinous cyst in PS, and one case interpreted as an endometrial carcinoma focus (endometrial intraepithelial neoplasia) in the uterus in FS was tumor negative in PS examination. The 10 false-negative cases of the female genital system were generally related to benign-borderline tumors of the ovary and lesions evaluated as hyperplasia in the endometrium, which were reported to be carcinomas in PS examination. Problems arising from the nature of ovarian tumors and sampling inadequacy lead to significant FS errors. Especially borderline tumors constitute a common problem. There are studies indicating that the specificity of FS in borderline tumors can decrease to 31% (16). In a series of 792 cases including gynecological materials, Wang et al. reported the sensitivity of IOCs as 86.95% and specificity as 57.1% (17). In another study, Göl et al. stated that there were fewer false positive cases while a false negativity was more common (18). In a study by İlker et al. it was determined that the false-negative rate in ovarian tumors was 3.8% (19). In our study, the rate of disagreement between the IOC and PS diagnoses was 3.12%, which is compatible with the literature, and similar errors were determined as reasons for this disagreement.

In our study, sentinel lymph nodes represented another important group showing inconsistency between the FS and PS diagnoses, with two false positive and 10 false negative cases being observed in this group. In the evaluation of lymph nodes, in addition to interpretation errors concerning cells of many different structures, there are also freezing and sectioning difficulties. Many studies have been conducted on this subject, often reporting false negative results, varying between 10 and 60% (20-23). In a meta-analysis, the intraoperative FS sensitivity of breast cancers was investigated and the accurate detection rate was determined as 62-76% (24). The failure of FS in routine intraoperative evaluation can be attributed to this method not being able to detect micrometastatic disease (25) as in our cases.

Thyroid tissue is among the most problematic areas of IOCs. In our study, while parathyroid diagnosis was made in FS in one of the tissues sent for thyroid-parathyroid differentiation, this area disappeared in PS examination. Other false negative tissues occurred due to the inadequacy of sampling, as well as inappropriate freezing, especially for diagnoses based on nuclear properties. Studies suggest that in some lesions of the thyroid, it is not appropriate to perform FS examination due to its inability to detect lesions smaller than 1 cm or show signs of surrounding capsule invasion. It is emphasized that while FS is beneficial in 3% of the follicular lesions of the thyroid, it causes erroneous diagnoses in 5% of cases, and therefore it is not recommended to perform FS examination in follicular lesions of the thyroid (26, 27).

It has been reported that significant problems can be experienced in the detection of tumors and lymphoma/carcinoma differentiation in pulmonary, pleural and mediastinal lesions, differentiation of lepidic in situ, minimally invasive and invasive carcinomas, and differentiation of small cell carcinomas (28). In a study by Liu et al., the rate of FS/PS diagnosis agreement in early stage lung adenocarcinomas was found to be 84.4% (29). In our study, similar to the literature, three cases that were reported as lung, pleura and mediastinal tissues with benign lymphoid areas in FS were diagnosed as Hodgkin lymphoma (n=2) and epithelial tumor

metastasis to the lymph node (n=1) in PS. In another case, the result of the FS evaluation was the suspicion of insitu/minimally invasive carcinoma while it was diagnosed as invasive carcinoma in PS.

5. Conclusions

IOC is still a very important and valuable diagnostic method in terms of providing surgical guidance during the operation. A high rate of agreement between the IOC and PS diagnoses is an important quality indicator. This is even more important considering the possibility of irreversible procedures that can be performed on patients based on false positive diagnoses. Although false negative diagnoses and deferral lead to secondary operative interventions in patients, they seem to be rectifiable. On the other hand, it is inevitable that certain technical and interpretation errors will be experienced. The high rates of accurate diagnosis, sensitivity, specificity, positive predictive value and negative predictive value in our series are very important quality indicators for our clinic and consistent with the data reported in the literature.

Conflict of Interests

The authors declare that they have no affiliation with any private or legal entity that would result in conflict of interest.

Financial Support

We do not have financial resources to declare.

Author Contributions

Arslan R, Ceylan O and Esin K.Ü originally conceived the idea and hypothesis. Arslan R designed the study. Arslan R made the research organization. Esin K.Ü collected the data. Ceylan O interpreted the results. Ceylan O and Arslan R drafted the manuscript. All authors reviewed and approved the manuscript.

Ethical Approval

This study was carried out in Atatürk University, Faculty of Medicine, Department of Pathology and was approved by the Ethics Committee of the same faculty with the decision numbere 07 and dated 29.11. 2018.

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Antimicrobial Activity of Metallic Nanoparticles: Their Implications for Multidrug Resistance Acinetobacter baumannii

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Abstract: In recent years, the demand for biocides from alternative compounds has increased due to reasons such as multi-drug resistant bacteria and limited antibiotic approval to prevent their resistance, and the inability of existing antibiotics to fully combat bacterial infections. With this in mind, we examined the effect of silver and zinc oxide nanoparticles on Acinetobacter baumannni, which has multi-drug resistance. The blood sample was taken and placed in the Vitek II device. A. baumannii was examined by molecular method and the presence of multi-drug resistance gene was investigated. Anitibiogram sensitivities were examined by disk diffusion method (bioMerieux; Durham, NC). It was interpreted according to the Current Clinical and Laboratory Standards Institute (CLSI) criteria. It was determined that the gene region of A. baumnannii with multi-drug resistance was blaOXA-51. A. baumannii with multi-drug resistance showed only Ampicill's sensitivity to sulbactam (SAM) and Getamycin (GN), and its antimicrobial effect against Silver (AgNPs) and Zinc oxide nanoparticles (ZnONPs) was studied. It is very important not to form an inhibition zone against AgNPs at a concentration of only 16 µg/disc from nanomolecules prepared at a concentration of (1.024-16 µg/disc). The effect of ZnO-NPs was determined as 1.024 µg/disc. NPs with important applications in biomedicine provide hope for the development of effective antimicrobial agents in the future. However, it is a known fact that it has toxic effects on human and animal health. In order to reduce these effects, concrete methods should be developed by expert authors in the first place.
Keywords: Nanoparticle; A. baumannii; Multi Drug Resistance.

1. Introduction

Acinetobacter baumannii has been identified as a nosocomial red alert pathogen contributing to increased morbidity and mortality (1-3). *A. baumannii* is a pathogen resistant to many classes of antibiotics, causing many infections including hospital-acquired pneumonia, respiratory tract infection, urinary tract infections, surgical site and bloodstream infections (4, 5). Worldwide, due to *A. baumannii*, especially intensive care units (ICUs); Outbreaks have occurred in surgical wards, burn units, and general medical wards (6-11). As a result, active surveillance studies have been initiated in high-risk patients to prevent the spread

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of *A. baumannii* (12). In addition, the "super bacteria" resulting from the unconscious and misuse of antibiotics have developed resistance to almost all known antibiotics. The level of antibiotic resistance they show is attributed to the presence of a super-resistant gene called 'New Delhi metallo-beta-lactamase 1' (13). With its enzymes capable of breaking down antimicrobial agents such as aminoglycosides and beta-lactamase, many develop resistance to antimicrobials through many mechanisms, such as the carbapenemases enzyme, ribosomal mutations, pump systems (14). With all these conditions, factors such as limited approval of antibiotics have drawn attention to alternative antimicrobials. Nanoparticles (NPs) have found a place in the medical field with their use in cancer treatments and their ability to inhibit the formation of advanced glycation end products (16). Thanks to these molecules that we have combined, the treatment can be reduced in dose and antimicrobial activities can be increased. Combined use of conjugated antimicrobial agents and NPs improves their ability to kill isolates that develop antimicrobial resistance, increases antimicrobial concentrations at bacteria-antibiotic interaction sites, and helps to bind antimicrobial agents to bacteria (17). Today, many studies have been carried out with NPs and its antimicrobial effect and mechanism have been investigated. Silver (Ag) shows a broad spectrum antimicrobial effect against bacteria, fungi and viruses. This effect is called oligodynamic activity. Ag and its compounds interact with bioactive Ag+, proteins and amino acids by ionizing in body fluids or water. Microorganisms are highly sensitive to the toxic effects of Ag+ and Ag compounds. It has been determined that Ag NPs have mechanisms of action such as affecting the cell membrane, disrupting DNA damage and electron transport, superior antimicrobial activities mediated by the synthesis of reactive oxygen species (ROS) and killing biofilm-forming isolates. They provide all these effects by having a larger surface-to-volume ratio. Thus, they interact more with the cell membrane and penetrate the cell easily (18, 19). ZnO (Zinc Oxide) NPs, on the other hand, have been reported to have antimicrobial properties such as disrupting the cell membrane of pathogens, accumulating in the cell and producing toxic H_2O_2 (hydrogen peroxide) (20). In the light of this information presented to the literature, we planned to examine the antimicrobial effect of Ag NPs and ZnONPs against *A. baumannii*, which has multi-drug resistance.

2. Material and Methods

2.1. Sample Collection and Colony Identification

The blood sample of a patient treated in the intensive care unit was taken under aseptic conditions and placed in the VItek II device. After 48 hours, the sample, which we obtained with the button indicating growth, was cultivated on 5% sheep blood agar and McConkey agar media and incubated at 37 °C under aerobic conditions. The growth characteristics of the media were examined according to their macroscopic appearance, colony and gram staining characteristics. Microorganism was identified by conventional methods. Oxidase negative colonies morphologically similar to Acinetobacter were identified using Vitek II (bioMerieux; Durham, NC) (21).

Determination of Antibiotic Resistance Gene: The resistance gene of *A.baumannnii* was investigated using the Multiplex PCR technique. For the PCR reaction, the method kit consisting of Taq PCR master mix (New England Biolabs, Beverly, MA), sterile RNase-free water, primer and DNA template was made according to the method. And it was examined in a 2% agarose gel (22, 23).

2.2. Ethics Committee Approval

Since this sample was collected as part of routine infection control surveillance, individual informed consent and ethics committee report were not obtained prior to including the sample in the study.

2.3. Sensitivity Tests

Antibiogram tests were performed using Disk diffusion method and E-tests (bioMerieux; Durham, NC) and were interpreted according to the current Clinical and Laboratory Standards Institute (CLSI) criteria (24). Acinetobacter isolates were tested against imipenem, doripenem, meropenem, ertapenem, sulfamethoxazole-trimethoprim, ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, cefepime, ciprofloxacin, amikacin, gentamicin, polymycline, and tigesycline. Multidrug resistance was defined as susceptible to two or less antibiotics without polymyxin B and tigecycline (25).

2.4. AgNPs Preparation

AgNPs were purchased without sigma. Nanoparticles in distilled water (ddH2O). a stock suspension was prepared by resuspension. AgNPs were absorbed in 100 µl on 6mm sized disks from the final solution (1-1.024 µg/disk).

2.5. Preparation of ZnONPs

The nanoparticle was purchased from sigma. The final concentration of the suspended solution was prepared. 100 µl of the final solution (1-1.024 µg/disk) was impregnated onto 6mm disks.

2.6. NPs by Disk Diffusion Method

The bacterial suspension was adjusted to the turbidity of McFarland standard solution 0.5 and an inoculum containing approximately 1×108 CFU/mL was prepared.

Table 1: Multi-drug resistant A. baumannii profile: [30 µg/disc].

IPM	R
DORI	R
MER	R
ERT	R
SXT	R
SAM	S
AMK	R
GEN	S
TZP	R
CIP	R
CAZ	R
FEP	I
CT	R

IPM Imipenem, DORI Doripenem, MER Meropenem, ERT Ertapenem, SXT Sulfamethoxazole-Trimethoprim, SAM Ampicillin-Sulbactam, Amk Amikacin, GENgentamicin, TZP Piperacillin Tazobactam, CIP Ciprofloxacin, CAZ Ceftazidime, FEP Cefepime, CT Colistin, MIC Minimum Inhibitory Concentration, R Resistant, S Sensitive, no NS Sensitization, I Medium Susceptible.

It was inoculated on the whole surface of Müller Hinton Agar medium. 6 mm sized discs impregnated with nanoparticles, which we previously recalled and sterilized, were placed in the medium. Microorganism petri dishes and nanodisks were incubated at 37 °C for 24 hours. The antimicrobial activity of the nanodisks was determined by measuring the zone of inhibition around each disc (mm). Each test was repeated 2 times.

3. Results

Antibiotic Resistance Gene: DNA bands in *A. baumannii* 2% agarose gel analyzed with multiplex pcr were separated using the CHEF DR III system (Bio-Rad, Nazareth, Belgium). And the resistance gene was determined to be blaOXA-51.

3.1. Antimicrobial Activity

AgNPs showed an antibacterial effect against the agent. The zone diameters formed by the molecule prepared in different concentrations are shown in Table 2.

Table 2: Zone diameters obtained with different AgNPs concentrations against *A. baumannii* strain.

Zone of inhibition (mm)	AgNPs (µg/disc)
16	1.024
15	512
13	256
12	128
11	64
8	32
NI	16

NI: no inhibition, AgNPs: Silver nanoparticle. All experiments were repeated twice. Standard deviations were not significant.

The zone diameters formed by the molecule prepared in different concentrations against the ZnONPs agent are shown in Table 3.

Table 3: Zone diameters obtained with different concentrations of ZnONPs against *A. baumannii* strain.

Zone of inhibition (mm)	ZnONPs (µg/disc)
4	1.024
2	512
1	256
NI	128
NI	64
NI	32
NI	16

ZnONPs: Zinc Oxide Nanoparticle, NI: No Inhibition. All experiments were repeated twice. Standard deviations were not significant.

4. Discussion

A. baumannii is a Gram-negative microorganism with the ability to develop and accumulate multidrug resistance. The leading factor in hospital infections is very capable of causing morbidity and mortality (26). Alternative searches have begun to eradicate this isolate, which has the property of escaping the mechanism of action of most drugs. The major concern with the development of multidrug resistance is the spread of resistant organisms. In this respect, the idea of replacing traditional antimicrobials with new technology has emerged to prevent antimicrobial resistance. Nanotechnology-driven innovations are starting to show promise for patients and practitioners to tackle the problem of drug resistance. Ultimately, biocides that were in harmony with the ecosystem attracted attention (27). Among these, a bactericidal effect of nanomolecules with very small fragments on microorganisms was determined. Zinc oxide 12 nm, silver (5, 9, 10, 12, and 13.5 nm) shows the highest antibacterial activity (28). The use of silver is very common, especially in wound healing and burns. Bactericidal properties due to high surface areas shows (29). In our study, we examined AgNPs in different concentrations (1.024-16 µg) by disk diffusion method. Silvernano molecules (30), which have a greater effect on the gram-negative cell wall structure, did not form a zone diameter only at a concentration of 16 µg/disc. It has been reported that its effect on Gram-negative microorganisms is related to the thinness of the peptidoglycan, which is a wall component (31). These zone diameters we saw in our study give hope that AgNPs molecules will be an alternative in isolates with multi-drug resistance and especially in *A. baumannii* factor. ZnO-NPs show bactericidal effects due to reasons such as destruction of cell integrity and release of antimicrobial ions, Zn^{2+} ions. Zinc, which has many activities, is capable of producing ROS, hydrogen peroxide (H2O2) and superoxide ions ($O_2^{\bullet-}$) used to target microorganisms when exposed to UV radiation in aqueous solution and prepared an aqueous solution (31, 32). In a study by Navale et al., ZnO-NPs have been shown to have strong bactericidal and antifungal

properties against *S. aureus, S. typhimurium*, and *Aspergillus flavus* and *fumigatus* pathogens (33). In another study conducted with ZnO-NPs, it was reported that Campylobacter jejuni showed a bactericidal effect by disrupting the cell membrane structure. And with *E. coli* O157: H7 it has been reported to show antimicrobial effects against *Salmonella enterica* serotype Enteritidis (34). In our study, the effect of ZnO-NPs on multi-drug resistant *A. baumannii* isolate was determined as 1.024 µg/disk. We hope that an anti-mulrabial effect will occur with the nanparticle concentration we have determined at higher dose ranges. Morones J.R. at all. In a study (0, 25, 50, 75 and 100 µg/ml-1), AgNPs determined in concentration ranges were *E. coli, P. aeruginosa, V. cholera, S. typhus, Acinetobacter baumannii, Enterococcus faecalis, Klebsiella pneumoniae, Listeria. monocytogenes, Micrococcus luteus, Proteus mirabilis, Salmonella typhi, Enterobacter aerogenes, Bacillus subtilis, Brucella abortus, Moraxella catarrhalis, Proteus mirabilis, Streptococcus viridans, Streptococcus pneumonia, Streptococulans, Streptococcus mucosa*. In our study, *A. baumanni* did not create an inhibition zone only at a concentration of 16 (µg/disc). The AgNPs particle, which has a bactericidal effect against S. mutans, is used in dental treatments (36). In addition, many studies showing that it is effective in invasive fungal species in immunosuppressive patients have been presented in the literature (37-40). All these studies show that Ag particles are effective in most microorganisms in different sizes and concentrations. Our study reflects results consistent with this information presented in the literature. The antibacterial effect against our multi-drug resistant isolate gives us hope in our future studies. We believe that it will be an alternative antimicrobial by revealing the MIC (Minimal Inhibition Concentracion) and MBC (Minimal bactericial Concentracion) values. In addition, we hope that a response will be obtained with much lower doses and times with antibiotics and nanoformulation in order to prevent this drain. The US Food and Drug Association has listed the ZnO 'nanoparticle as "generally recognized as safe" (GRAS) (41). This has enabled the use of these particles, which are in harmony with the ecosystem, in nanomedicine. Most Gram-positive and Gram-negative bacteria, and especially foodborne pathogens, are susceptible to ZnONPs (42). In a study with ZnONPs, antibacterial activity was reported against E. coli, Listeria monocytogenes, Salmonella and Staphylococcus aureus (43). Pati et al. In his study, he talked about its antimicrobial activity against S. aureus (44). Reddy et al. He reported that for E. coli (~13 nm) it produced inhibition by ZnONPs at a concentration 3.4 mM and for *S. aureus* at a concentration 1 mM it was completely inhibited (45). Also in another study, the MIC of ZnONPs for Campylobacter jejuni was reported at a concentration of 0.05 to 0.025 mg/mL (46).

5. Conclusions

In our study, it was determined as ≥1.024 µg/disk. We believe that ZnONP will have an effect against *A. baumannii* in higher concentrations.

Limits of the study and Perspectives

We believe that it will be more comprehensive to work with more than one nanoparticle and compare it with each other. In addition, our next goal will be to determine the MIC (Minimum Inhibition Concentration), MBC (Minimum Bactericidal Concentration) values and the effective duration of all these particles. In addition, we aim to conduct antimicrobial studies of plant and nanomolecular compounds (bio-nano) and antibiotic nanocompounds (antimicro-nano).

Meanwhile, although nanomedicine creates a new alternative field, we are also aware of the existence of toxic effects and negative processes. Our only wish is to create guidelines that will overcome all these negativities and to work on new antimicrobials.

Conflict of Interests

There is no conflict of interest between authors.

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Author Contributions

DÇ: Writing, analysis and statistics, ÖÇ: Literature review, laboratory analysis and statistics

Ethical Approval

Since this sample was collected as part of routine infection control surveillance, individual informed consent and ethics committee report were not obtained prior to including the sample in the study

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ALDH Expression in Hematopoietic Stem Cells Derived from Cord Blood: Effect of Transfer Time

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Abstract: The human aldehyde dehydrogenase (ALDH) activity measurements have recently been considered a quick and accurate quality control test used to determine the function of cord blood cells. The relationship between high ALDH activity of hematopoietic stem cells and engraftment is also known. However, there is limited data on the relationship between ALDH expression and transfer time of the cord blood to the laboratory in cord blood banking.

The aim of this study is to investigate whether the transfer time has an effect on ALDH expression. 20 volunteers were included in the study. After collection of the cord blood, transfer times to the laboratory were calculated. Subsequently, CD34+ cell count, Total Nucleated Cell (TNC) count, and ALDH expression were analyzed. ALDH expression was found to be high in cord blood containing a high number of CD34+ cells. Similarly, a positive correlation was detected between TNC count and ALDH expression. There was no correlation between the transfer time, which is an important parameter in cord blood banking, and ALDH expression.

The findings of the present study show that ALDH test can be used in cord blood banking, and it reveals for the first time that it can be used safely regardless of the transfer time.

Keywords: ALDH; Cord Blood; Hematopoietic Stem Cell.

1. Introduction

The human aldehyde dehydrogenase (ALDH) superfamily consists of 19 known functional genes. It is classified into 11 families and 4 subfamilies in different chromosomal settlements (1-2). ALDH enzymes can also be found in cytosol, nucleus, mitochondria, and endoplasmic reticulum. Enzyme levels of ALDHs may vary in human tissues and organs depending on the enzyme family and subfamily (3). ALDH has been defined as an important enzyme for preserving normal hematopoietic stem cells (4) and is used as a marker to identify and isolate various types of stem cells (5-6). Stem cells are defined as cells that are capable of self-renewal and differentiation into mature cells that form certain tissues and organs (7). Since stem cells are usually found in small numbers in tissues and organs, different strategies are needed at the stages of their isolation and enrichment. The presence of single or multiple biomarkers is important for their use in both research and therapeutic areas (8). Cord blood is a very rich source of hematopoietic stem cells. It has been successfully used as a source of hematopoietic stem cell in bone marrow transplantation since 1988. The number of transplants performed has exceeded 35.000 units (9). Collection and transfer of cord blood are the major limitations. Unfortunately, not all blood collected can be used in hematopoietic stem cell transplantation and/or regenerative and reparative medicine applications.

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One of the most important reasons for this is bacterial or fungal infection (10) and the presence of too few cells due to the inadequate volume of blood collected during the blood collection stage (11). In addition, factors such as the storage method, loss of cells during freezing and thawing, and exposure to temporary warming determine the quality of cord blood (12). Recently, ALDH activity measurements have been considered a quick and accurate quality control test used to determine the function of cord blood cells (13-14) The relationship between high ALDH activity of hematopoietic stem cells and engraftment is also known (15) However, there is limited data on the relationship between ALDH expression and the transfer time of the cord blood to the laboratory in cord blood banking. The aim of this study is to investigate whether the transfer time has an effect on ALDH expression.

2. Material and Methods

2.1. Study Population

Approval was obtained from the Ethics Committee of Akdeniz University Faculty of Medicine (Decision No: 147, Date 21.02.2018) for the study. After the approval was obtained, families who agreed to participate in the study among those who applied to Akdeniz University Technopark Babylife Cord Blood and Human Cell-tissue production center were included in the study. Samples were taken from 20 cord blood units for the study. The remaining parts of the samples were used, and no additional samples were taken for the quality control tests.

2.2. Cord Blood Collection

Cord blood was collected from the umbilical vein. Before collecting the blood, the area was cleaned with a disinfectant containing iodine. Cord blood was then taken into the blood collection bag containing CPDA. Collection time and reception time at the laboratory were recorded. The transfer time of blood was calculated using these data.

2.3. Total Nucleated Cell (TNC) count

TNC count was carried out with a fully automatic cell counting (Swelab alpha-no-111-450) device in our laboratory.

2.4. CD34 Count

CD34 cell count was carried out according to ISHAGE protocol (16). Live cells were detected by 7-AAD (BD Pharmingen 7-AAD- 559925), and CD34 (mouse anti-human CD34 -345802 BD) and CD45 (mouse anti-human CD45-345808 BD) monoclonal antibodies were used. Flow cytometric analyses were performed with BD Facs Calibur.

2.5. ALDH Assay

Aldehyde dehydrogenase (ALDH) assays (AldeRed™ ALDH Detection Assay SCR150 Sigma-Aldrich) were performed according to the manufacturer's instructions. After the samples were prepared, flow cytometric analysis was performed (with the BD Accuri C6 flow cytometer). ALDH values were calculated as Mean Fluorescence Intensity (MFI).

2.6. Statistically Analysis

SPSS 21 software was used. Mann–Whitney U test and Pearson correlation test were carried out. $P < 0.05$ was considered statistically significant

3. Results

When the relationship between CD34+ cell counts and ALDH expressions of cord blood samples included in the study was evaluated, ALDH expression of samples containing a high number of CD34+ cells was also found to be high (Figures 1, 2).

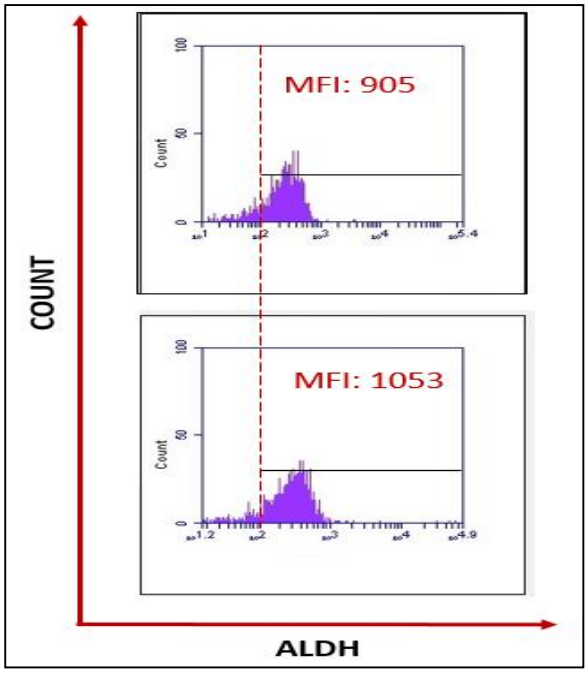

Figure 1: ALDH Flow Cytometry Histogram. Comparison of a sample containing a low CD34+ cell count with a sample containing a high CD34+ cell count. (MFI: Mean Fluorescence Intensity n=20 p<0.05 value according to the Mann-Whitney U test).

When the relationship between the total nucleated cell count and ALDH expression was examined, samples containing high TNC count were also found to have high ALDH expression (Figure 3). In order to determine whether ALDH expression was affected by transfer time, the relationship between the ALDH expression levels and transfer times of samples that reached the laboratory within different periods of time was examined. Although CD34+ cell count showed a positive correlation with TNC count, it was found to have no correlation with transfer time (Figure 4). This result revealed that ALDH can be safely used as a cellular quality control parameter in autologous cord blood banking without being affected by transfer time.

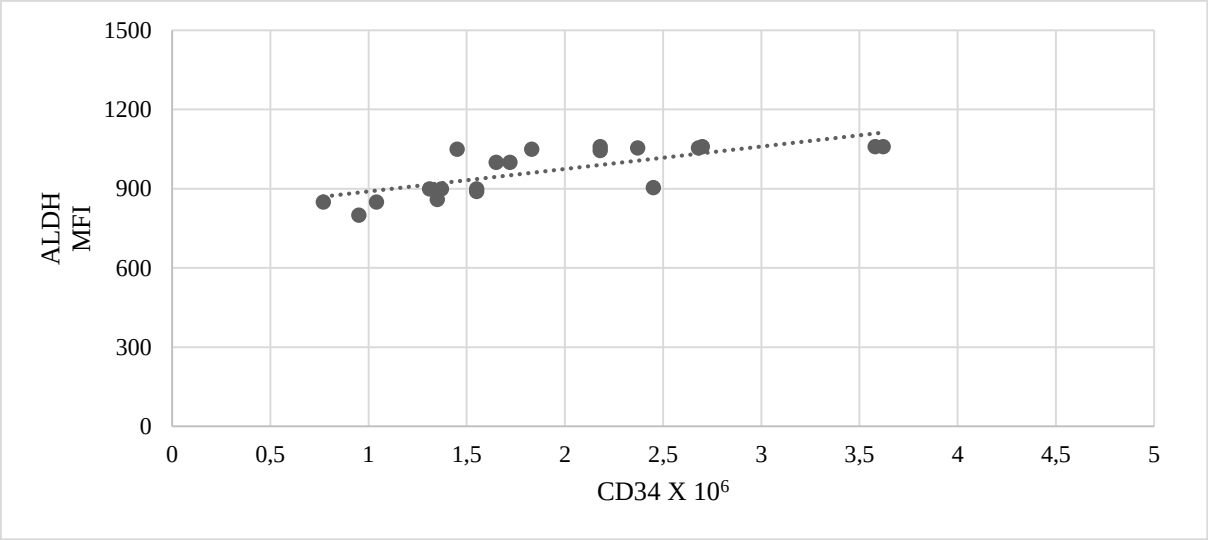

Figure 2: The relationship between ALDH expression and the CD34+ cell count (MFI: Mean Fluorescence Intensity n=20 $p<0.05$ value according to the Pearson correlation test).

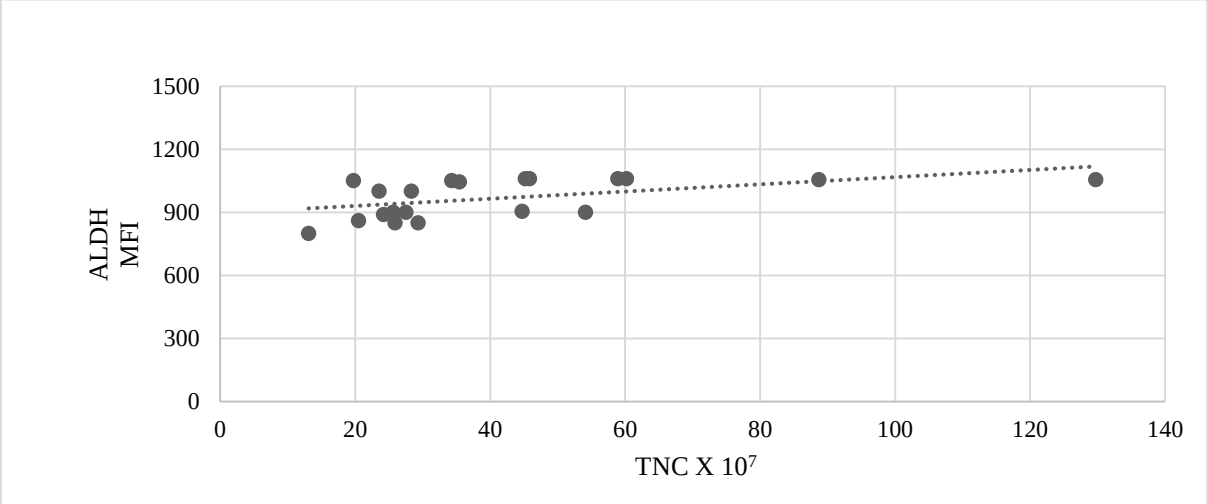

Figure 3: The relationship between ALDH expression and TNC count (MFI: Mean Fluorescence Intensity, TNC: Total Nucleated Cell, n=20 $p<0.05$ value according to the Pearson correlation test).

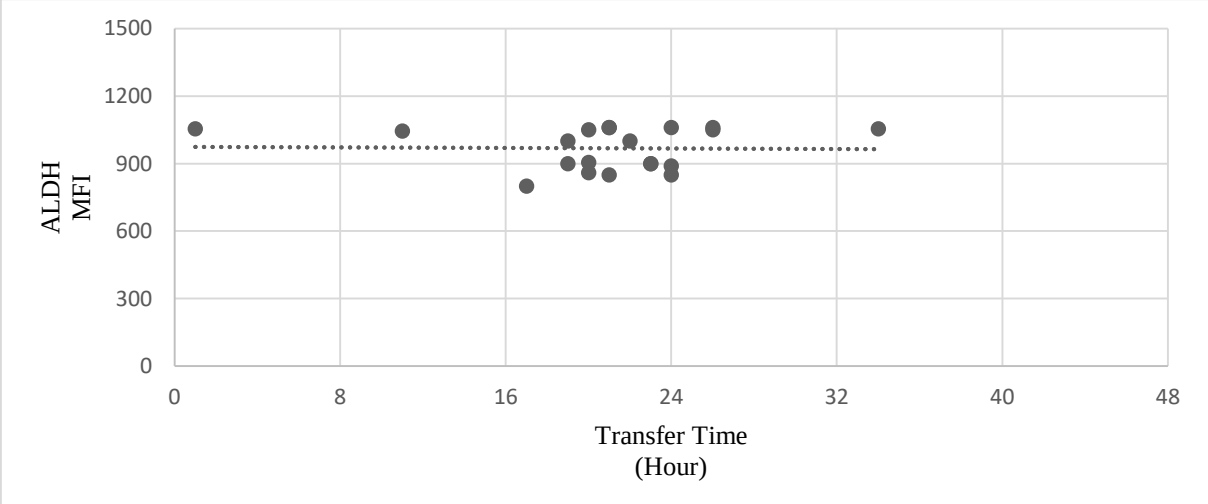

Figure 4: The relationship between ALDH expression and transfer time (MFI: Mean Fluorescence Intensity n=20 $p>0.05$ value according to the Pearson correlation test).

4. Discussion

Hematopoietic stem cells ensure maintenance of the blood tissue under physiological conditions and bone marrow restructuring after bone marrow transplantation (17). It uses its two important features when performing these functions: self-renewal and differentiation (18-19). The cord blood banking system enables the hematopoietic stem cell to acquire the characteristics of a ready-to-use product with a shelf life. Although cord blood is an important source, due to the low number of stem cells contained in it, issues such as delayed engraftment and primary graft failure may occur after transplantation (20). In such cases, quick and reliable tests to assess the potency and quality control parameters of stem cells contained in cord blood both before freezing and at the time of thawing for transplantation are even more important. Today, the number of granulocyte-macrophage colony-forming unit (CFU-GM) is the best indicator of neutrophil engraftment and overall survival of the recipient (21). However, colony-forming unit (CFU) testing is expensive, time-consuming, and difficult to standardize. Due to these characteristics of CFU, there is an increasing need for alternative approaches. An alternative and promising approach is the analysis of ALDH, which is based on increased enzyme activity in hematopoietic stem cells (22). It has been revealed that ALDH can also be used in cord blood banking, and particularly, it remains reliable after freezing-thawing (23). In the present study, the time needed to deliver the blood to the laboratory after it is collected, which is an important restrictive factor in cord blood banking, and the effect of this period of time on ALDH expression was examined. ALDH expression was determined flow cytometrically in these samples obtained from 20 units of cord blood in total. A positive correlation was found between CD34+ cell count and TNC count and ALDH expression in support of the literature. However, it has been found that the transfer time does not have a negative effect on ALDH expression. The cord blood samples included in the study were selected from blood samples collected within the first 48 hours in accordance with the Cord Blood Banking Regulation published by the Ministry of Health of the Republic of Turkey.

5. Conclusions

The data we obtained support that ALDH test can be used in cord blood banking and reveal for the first time that it can be used safely regardless of the transfer time.

Conflict of Interests

The authors report no conflicts of interest

Financial Support

None

Author Contributions

D.B: Designed and performed experiments, analysed data and wrote the manuscript.

Ethical Approval

Approval was obtained from the Ethics Committee of Akdeniz University Faculty of Medicine (Decision No: 147, Date 21.02.2018) for the study.

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A Comparison of Vitamin D Levels and Also Monocyte/HDL Cholesterol Ratios of Adults with Different Body Mass Index Who Consulted Diet Outpatient Clinic

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Abstract: Monocyte/HDL-Cholesterol ratio is an inflammatory marker. In this study, it was aimed to see if there was a difference in vitamin D levels and also monocyte/HDL-Cholesterol ratio among underweight, healthy, overweight and obese groups. The medical records of people who attended to the outpatient nutrition clinic for the first time at the İstinye State Hospital within the month of April were reviewed retrospectively. The attendees were grouped to underweight, healthy, overweight and obese groups, then the obese group was divided into 4 subgroups according to their body mass index. Monocyte counts, HDL-Cholesterol and vitamin D levels were found from the records. Monocyte/HDL-Cholesterol ratio was calculated for each person. Descriptive analyzes, Kolmogorov-Smirnov, Shapiro-Wilk normality test, Mann-Whitney U and Kruskal Wallis H tests were used. The data were analyzed using SPSS 25.0. Approval of the Regional Clinical Research Ethics Committee was obtained (July 23, 2019, 1364). Informed consent form was taken from the patients. The HDL-Cholesterol means of healthy group were significantly higher than the groups with higher body mass index ($p<0.05$). Monocyte means of men and HDL-Cholesterol means of women were significantly higher than the other gender ($p<0.05$). There was no statistically significant difference in monocyte/HDL-Cholesterol ratio means between body mass index groups ($p>0.05$). Vitamin D means of morbid obese and super obese people were significantly lower than the means of normal and overweight groups ($p<0.05$). In this study, monocyte/HDL-Cholesterol ratio doesn't seem as an inflammatory marker for obesity. It should be keep in mind that vitamin D may be low in obese people.

Keywords: Obesity; Vitamin D; Monocytes; Monocyte/HDL-Cholesterol ratio; HDL-Cholesterol.

1. Introduction

Obesity is defined as excessive accumulation and/or storage of fat in the body. Overweight and obesity are worldwide problems that affect 39% of adults aged 18 years and over (39% of men and 40% of women). Common health consequences of overweight and obesity are cardiovascular diseases, diabetes,

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musculoskeletal disorders and some cancers (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon) (1).
As elevated BMI, increased mortality and reduction in quality of life are observationally associated (2). Obesity is associated with chronic inflammation (3).
The frequency of obesity is increasing rapidly in our country. In TURDEP-II Study, the frequency of obesity was found 35% in the general population (44% in women and 27% in men) (4).
It is known that obesity activates macrophages, mast cells and T lymphocytes; and low-grade inflammation accompanies obesity (3, 5).
The main functions of monocyte include chemotaxis, phagocytosis, endocytosis, secretion of factors that modulate inflammatory responses and microbial killing; all of which are integral to immune defence (6). Monocytes are one of the most important cell types for the secretion of pro-inflammatory and pro-oxidant cytokines in the inflammation zone (7).
High HDL-C levels are associated with less low-grade inflammation (8). One of the functions of HDL involves the modulation of inflammation. This function appears to have evolved as part of the innate immune system.The immunomodulatory effects of HDLs are mainly mediated via lipopolysaccharide binding and neutralization, the HDL-associated enzymes, plasma paraoxonase (PON1) and platelet-activating factor acetylhydrolase (PAF-AH), inhibition of the expression of endothelial cell adhesion molecules and release of proinflammatory cytokines, and stimulation of the expression of endothelial nitric oxide synthase (9). In healthy individuals, HDL is anti-inflammatory (10). It was demonstrated in an experimental study that HDL exhibits an anti-inflammatory effect on human monocytes by inhibiting the activation of CD11b (11). Monocyte / HDL-Cholesterol ratio (MHR) is defined as an indicator of inflammation and is thought to predict metabolic syndrome (12) and to be associated with obesity and polycystic ovarian syndrome (13).
Vitamin D is a hormone obtained through dietary consumption and synthesized in the skin on sun exposure. The conversion of 7-dehydrocholesterol to previtamin D in the skin occurs with the effect of Ultraviolet B radiation. This previtamin D is converted to vitamin D by heat isomerization. Vitamin D is converted to 25-hydroxyvitamin D in the liver and then to the biologically active form 1,25-dihydroxyvitamin D in the kidneys. It binds to the vitamin D receptor, a hormone receptor located in the nucleus of the cell. Vitamin D plays important role in metabolism. TEMD Osteoporosis and Metabolic Bone Diseases Working Group considers a level of Vit D between 30-50 ng/ml sufficient for its extra-skeletal effects (14). Vitamin D contributes to regulate the proliferation, differentiation, and function of immune cells such as dendritic cells, macrophages, T cells and B cells. Vitamin D exerts immunomodulating effects (15). When initial vitamin D levels are low, in highly inflammatory conditions, increasing vitamin D levels tends to reduce markers of inflammation (16).
Obesity has been identified as a risk factor for vitamin D (vit D) deficiency (17, 18). Some studies show low vit D levels in obese people (19, 20).
Body mass index (BMI) began to be widely used in the world after the National Institutes of Health Consensus recommendation to use in obesity in 1985(21). The formula for BMI is weight in kilograms divided by height in meters squared.
In the diet and nutrition outpatient clinic of İstinye State Hospital, the diet plans for both the patients referred from other outpatient clinics and the patients who decide to lose weight are given. There are 250-350 new patient attendances per month. In this study, it was aimed to categorize the people who consulted to the diet outpatient clinic for the first time within the month of April according to BMI and to see if there is a difference in vit D levels and also MHR between these groups.

2. Material and Methods

Medical records of people who attended to İstinye State Hospital's nutrition and diet outpatient clinic in the month of April were reviewed retrospectively. Who consulted for the first time were included in the study; those with chronic inflammatory disease, cancer, pregnancy status were excluded. There were 301 people, 249 women (82.7%) and 52 men (17.3%). The mean age of women was 42.31±14.58 and the mean age of men was 38.00±15.97 years.
On medical history, the presence of accompying diseases such diabetes mellitus (dm), hyperlipidemia (hl), hyperuricemia (hu) was learned, body mass index (BMI) was calculated and waist circumference (wc) was measured. The wc was measured by performing a normal expiration on the skin in the plane passing through the middle of the distance between the bottom of the lower costa and the top of the spinal iliaca anterior superior. BMI was calculated as body weight in kilograms divided by squared body height in meters. According to Turkish Endocrinology and Metabolism Association (TEMD) Obesity Diagnosis and Treatment Guidelines criteria (22), patients were divided into 4 categories according to BMI: Underweight <18.50, healthy 18.5-24.99, overweight 25.00-29.99, obese ≥30,00. The Obese category was divided into 4 groups: Mildly obese 30.00-34.99, moderately obese 35.00-39.99, morbid obese 40.00-49.99, super obese ≥50,00.
Mean age, wc, rates of accompanying diseases (dm, hl, hu) were found in each category according to men and women. Means of monocyte, HDL and MHR were found in each group categorized according to BMI.
The results of hemogram, HDL and vit D were taken from the medical records.

2.1. Biochemical and Hormone Analysis

Fasting blood samples were collected in gel tubes that did not include anticoagulants to measure cholesterol.

An additional blood sample was collected in an EDTA tube and used to measure the hemogram. All the blood samples were collected after 12 h overnight fasting and centrifuged at 1800 ×g for 15 min before analyzing. Biochemical parameters were measured colorimetrically using Abbott original reagents in an Abbott Architect c8000 autoanalyzer. The total cholesterol and triglicerid levels in the serum were measured enzymatically colorimetrically. After precipitation of apoB containing lipoproteins, HDL was measured. Vit D concentration was measured using Roche Cobas 8000 by immunoassay electrochemiluminescence binding assay) method. The complete blood count analysis were performed in Mindray Auto Hematology Analyser BC-6800 model, The targeted blood cells undergo 3D analysis using information from scatter of laser light. Original kits of the manufacturer were used in haemogram assays.

Approval of the Regional Clinical Research Ethics Committee was obtained (23/07/2019, 1364). Informed consent form was taken from the patients. Helsinki criteria were applied within the scope of the research. There is no conflict of interest and financial support regarding the article.

2.2. Statistical Analyses

In this study, frequency and percentage statistics were used to determine the distribution of the participants according to their demographic characteristics. Descriptive analyzes were applied for age, wc and BMI values. Cross tables were used to determine the distribution of concomitant diseases in their medical resumes by gender.

Monocyte, HDL, MHR and vit D distributions were examined by Kolmogorov-Smirnov and Shapiro-Wilk normality tests. Measurement scores did not show normal distribution. Mann- Whitney U and Kruskal Wallis H tests were applied to compare the measurement scores by BMI category and gender. The data were analyzed using SPSS 25.0.

3. Results

The Means±Standart Deviation (SD) of wc of women and men were 102.61±15.05 cm and 106.85±14.10 cm, respectively. Their BMI was 32.31±7.22 kg/m^2 for women and 30.66±5.65 kg/m2 for men. When the presence of diseases such as dm, hl, hu in these people who applied to the diet outpatient clinic was questioned, it was seen that 61.9% of women and 72.5% of men have dm; 79.1% of women and 79.1% of men have hl; 8.6% of women and 14.3% of men have hu.

According to the BMI, mean age, gender and wc of the participants and the accompanying disease information obtained from the medical resume of these groups are shown in Table 1.

In table 2, HDL levels of the BMI groups were compared with each other. HDL means of patients with normal weight were higher than those of overweight, mildly obese, moderately obese, morbid obese and super obese patients. HDL means of overweight patients were higher than those of moderately obese and morbid obese patients. HDL means of the mildly obese patients were higher than the means of morbid obese patients ($p<0.01$).

In table 3, the comparison of monocyte counts and MHR of the BMI groups can be seen. There wasn't a statistically significant difference in monocyte counts and MHR according to the BMI categories ($p>0.05$). Table 4 shows the comparison of monocyte, HDL and MHR by gender. When the table was analyzed, no statistically significant difference was observed in the means of MHR by gender ($p>0.05$). However, there is a significant gender-dependent difference in mean monocyte and HDL levels ($p<0.05$). The means of monocyte counts of men and HDL of women were significantly higher than the other gender. Table 5 shows the comparison of vit D levels by BMI category. A statistically significant difference was observed in vit D means according to the BMI category ($p<0.05$). Vit D means of the healthy weight and overweight groups were higher than the means of morbid obese and super obese groups.

4. Discussion

The difference in the number of male and female patients is too great, 82.7% of 301 people are female and 17.3% were male. In a study made in İzmir in 2019 on obese and overweight individuals over the age of 18 who applied to an obesity counseling unit in primary care, the rate of women was found to be 82.9% and the rate of men was 17.1%. In Turkey obesity in women is more common than in men, women are more likely to go to a dietician than men (23).

Mean wc of women and men in the healthy category was 83.16±7.60 cm and 86.20±10.16 cm, respectively. According to the TEMD Lipid Obesity and Hypertension Working Group's Metabolic Syndrome Study (24); if wc is >80 cm for women and >94 cm for men it is abdominal obesity. In this study women who have healthy BMI, have abdominal obesity.

As the obesity category increases in this study, the incidence of dm increases. In the mildly obese group, dm was present at a rate of 58.1%. The presence rates of dm in the moderetely, morbid and super obese groups, were 73.5%, 85.7% and 100%. This situation is compatible with the literature (25).

In a study conducted in the USA in 2011, it was found that the number of monocytes increased significantly as the BMI increased (26). In a study examining 15.654 people in Korea in 2008, there was no difference in terms of monocyte count between those with and without metabolic syndrome (27). In our study, no statistically significant difference was observed in the means of monocyte count according to the BMI category ($p>0.05$).

Table 1: Means of the age and waist circumference, and percentage of the gender and accompanying diseases according to BMI.

BMI Classification	%	Gender	n	Age ($\bar{x}$ ±SS) (years)	Weist Circumference ($\bar{x}$ ±SS) (cm)	DM %	HL %	HU %
Underweight	2.7	Female	6	18.50 ± 3.62	67.00 ± 6.20	0.0	33.3	0.0
		Male	2	15.00 ± 0.00	70.00 ± 0.00			
Healthy	12.0	Female	31	35.35 ± 16.35	83.16 ± 7.60	50	57.1	0.0
		Male	5	33.00 ± 18.23	86.20 ± 10.16			
Overweight	24.6	Female	57	39.21 ± 15.08	95.55 ± 7.31	65.3	76.6	5.9
		Male	17	45.24 ± 12.10	102.71 ± 5.02			
Mildly obese	32.9	Female	81	43.85 ± 11.62	104.34 ± 7.39	58.1	82.3	14
		Male	18	34.39 ± 16.81	111.22 ± 4.52			
Moderately	18.3	Female	47	46.47 ± 13.94	111.39 ± 6.90	73.5	85.4	18.8
Obese		Male	8	43.13 ± 15.20	121.50 ± 6.63			
Morbid	7.6	Female	21	49.10 ± 12.80	122.90 ± 8.68	85.7	78.9	0.0
Obese		Male	2	24.00 ± 1.41	132.50 ± 12.02			
Super Obese	2.0	Female	6	54.17 ± 10.98	137.17 ± 20.27	100	100	0.0
		Male	0	-	-			

BMI: body mass index (kg/m^2), DM: diabetes mellitus history, HL: hyperlipidemia history, HU: hyperuricemia history wc: waist circumference (cm).

Table 2: Comparison of HDL levels of the BMI groups.

BMI Group	HDL($\bar{x}$ ±SS) (mg/dl)	Difference (p<0.01)
Underweight (1)	55.50 ± 12.15	
Healthy (2)*	59.43 ± 12.65	2>3, 2>4, 2>5, 2>6, 2>7
Overweight (3)**	52.28 ± 12.92	3>5 3>6
Mildly obese (4)***	50.75 ± 12.59	4>6
Moderately obese (5)	47.65 ± 8.99	
Morbid obese (6)	43.94 ± 7.51	
Super obese (7)	46.20 ± 9.52	

BMI: body mass index (kg / m^2), HDL: high density lipoprotein.
*There is a significant difference between Healthy group and Overweight, Mildly obese, Moderately obese, Morbid obese and Super obese groups. **There is a significant difference between Overweight group and Healthy group, Moderately obese and Morbid obese groups. ***There is a significant difference between Mildly obese and Morbid obese groups.

Table 3: Comparison of monocyte counts and Monocyte/HDL ratio of the BMI groups.

B	Underweight	Healthy	Overweight	Mildly obese	Moderately obese	Morbid obese	Super obese
Monocyte Count	468.75± 134.85	497.00± 167.03	446.82± 106.47	472.26± 155.95	470.57± 124.55	450.00± 166.40	521.67± 189.46
Monocyte/HDL Ratio	8.28± 4.62	9.33± 3.72	9.30± 3.56	9.65± 4.43	10.10± 4.54	9.12± 3.10	11.14± 5.08

BMI: body mass index (kg/ ^{2}).

Table 4: Comparison of monocyte counts, HDL (mg/dl) and monocyte/HDL ratio according to gender.

Variables	Gender	Means ± SS	p
Monocyte	Female	455.50 ± 135.94	<0.01
	Male	526.96 ± 148.82	<0.01 p=0.051
HDL	Female	52.67 ± 12.05	
	Male	42.46 ± 8.34	
	Female	9.29 ± 3.92	
Monocyte /HDL	Male	10.70 ± 4.40	

HDL: High Density Lipoprotein

Table 5: Comparison of vitamin D levels (ng/mL) according to BMI category.

BMI Groups	Vit D (means ± SS)	Difference (p=0.01)
Underweight (1)	15.00 ± 4.12	2>6
Healthy (2)*	21.29 ± 9.82	2>7;
Overweight (3)**	19.66 ± 9.87	3>6
Mildly obese (4)	17.78 ± 13.31	3>7
Moderately obese (5)	15.86 ± 10.94	
Morbid obese (6)	10.60 ± 4.06	
Super obese (7)	10.00 ± 0.00	

BMI: body mass index (kg / m^2), Vit D: Vitamin D. *There is a significant difference between Healthy group and Morbid obese and Super obese groups. **There is a significant difference between Overweight group and Morbid obese and Super obese groups.

Monocytes are involved in systemic inflammation (28). In different studies, the number of monocytes of men was found to be significantly higher than that of women ($p<0.01$) (29, 30). In our study, the number of monocytes of men was found to be significantly higher than that of women ($p<0.01$).

HDL is low in obesity (31). There are cohord studies showing that HDL decreases with increasing BMI (32). In our study, a statistically significant difference was observed in HDL means according to the BMI category ($p<0.05$). HDL means of patients with normal weight higher than those of overweight, mildly obese, moderately obese, morbid obese and super obese patients. HDL means of overweight patients were higher than those of moderately obese and morbid obese patients. HDL means of the mildly obese patients were higher than the means of morbid obese patients.

The HDL of women was found to be significantly higher than men and is compatible with the literature (33).

Considered as an inflammatory marker, MHR has been studied and found high in metabolic syndrome (34), dm (35), obese people who have polycystic ovary syndrome (36). In our study, no statistically significant difference was observed in the means of MHR according to the BMI category ($p>0.05$). MHR means according to gender were (9.29±3.92) and (10.70±4.40) in females and males, respectively, this difference was not statistically significant ($p>0.05$).

In a study of 196 patients, a significant relationship was found between vit D deficiency and obesity (37). There are other studies in which obese people have low vit D (19). In a study where it was determined that vit D elevation occurs less in obese people after vit D supplementation and skin irradiation than non-obese people, the hypothesis has been suggested that the storage of vit D in body fat tissues and low bioavailability (19). There are studies showing that 25-hydroxylation and 1-α hydroxylation are impaired in obesity (38). There are studies suggesting that vit D is low in obese people (20, 39). In our study, a statistically significant difference was observed in vit D means according to the BMI categories ($p<0.05$). Vit D means of patients with normal weight and overweight appear to be significantly higher than the means of morbid obese and super obese patients.

5. Conclusions

MHR may not be suitable for assessing inflammation in obesity. It should be keep in mind that vit D may be low in obese people. In a study with more people, more comprehensive findings can be detected.

Limitations of the Study

It is our limitations that it is a retrospective study, the number of cases is low, and it covers only those who attended to the diet outpatient clinic. The small number of men and the number of people in the underweight

group are insufficient for comparison, so they are the limitations of the study.

Conflict of Interests

The authors declare that they haven't any real or potential conflicts of interest, including financial, personal or other relationships with other persons or organizations that may inappropriately influence the work.

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Author Contributions

Conceived and designed the analysis: Aynur Arslan (AA), Hediye Nur Ataç (HNA), Collected the data: AA, HNA, Contributed data or analysis tools: AA, HNA, Performed the analysis: AA, HNA, Wrote the paper: AA

Ethical Approval

Approval of the University of Health Sciences, Okmeydani Education and Research Hospital Clinical Research Ethics Committee (July 23, 2019; Number 1364).

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Clinical Presentation of Neuropsychiatric Systemic Lupus Erythematosus and Demographic and Radiological Characteristics of Patients

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Abstract: Systemic lupus erythematosus (SLE) is a vasculitis that may affect numerous systems such as the kidneys, skin, joints, heart, lungs and nervous system. The purpose of our study is to evaluate patients with SLE in whom central nervous system involvement is monitored. Files of 1028 patients who were followed up with (SLE) diagnosis was examined Demographic, clinical and radiological characteristics were recorded for patients with a final diagnosis of neuropsychiatric systemic lupus erythematosus (NPSLE) with central involvement. Among 1028 patients diagnosed with SLE, 1.07% had NPSLE. Mean age was 37±5.3. 90.9% of the patients (n=10) were female, while 9.1% (n=1) were male. From a clinical aspect, 45.4% complained from hemiparesis, 27.3% from headache, 18.2% from psychiatric complaints and 9.1% complained from impairment of consciousness. From a radiological aspect, 45.4% (n=5) were consistent with subcortical plaque, 36.4% (n=4) with ischemic stroke, 9.1% (n=1) with cerebral venous thrombosis, and 9.1% (n=1) appeared consistent with posterior reversible encephalopathy syndrome (PRES). Mortality rate was 9.1% (n=1). The central involvement type that caused mortality was ischemia. Since magnetic resonance imaging (MRI) is not sufficient for showing microvascular involvement in NPSLE patients, it is possible for NPSLE diagnosis to be delayed despite consistent clinical characteristics. In case of clinical suspicion, other imaging methods should be applied apart from MRI. This is because early diagnosis is an important factor that reduces morbidity and mortality.

Keywords: Systemic Lupus Erythematosus; Central Nervous System; Magnetic Resonance Imaging.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that may affect the kidneys, skin, joints, heart, lungs, hematopoietic system and nervous system. SLE mostly affects women between ages 16-55 (1), and the highest age ranges from 45 to 69 in women while it ranges between 40-89 in men (2).
Nervous system involvement is called neuropsychiatric SLE (NPSLE) in SLE (3), and it is observed in 10% to 80% of patients (4). Furthermore, central nervous system (CNS) involvement is associated with high morbidity and mortality (5). NPSLE may affect peripheral and central nervous system. Meanwhile, the incidence of cerebrovascular system involvement varies between 3% and 20% in SLE (6). In NPSLE, neuroinflammation and cerebral ischemia occurs with the effect of genetic, environmental and

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neuroendocrine factors (7). One of the first important assumptions in NPSLE is that impaired blood-brain barrier (BBB) allows autoantibodies and immune peripheral blood components to enter CNS and results in inflammation and damage (8).

The difficulties often encountered by clinicians in the diagnosis and management of NPSLE patients are caused by the variety of clinical manifestations of patients from common and nonspecific characteristics such as headache, cognitive impairments and mood disorders to rare and complicated conditions such as Guillain – Barré syndrome and autonomic dysfunction (9). NPSLE may be presented as a clinically common disease (e.g., psychosis, anxiety or depression) or a focal disease (e.g., stroke or transverse myelitis) (3, 10). The most common magnetic resonance imaging (MRI) anomalies observed in these patients are small subcortical hyperintense lesions and infarctions (11). NPSLE treatment may be applied with corticosteroids alone, or in combination with other immunosuppressive drugs including cyclophosphamide for remission induction or azathioprine for maintenance treatment (12). Early diagnosis and treatment are important since it reduces morbidity and increases the quality of life. Antimalarial drugs (e.g., hydroxychloroquine) are recommended in SLE patients in order to prevent NPSLE (13).

The purpose of this study is to evaluate the clinical presentation, and demographic and radiological characteristics of NPSLE patients followed up in our region.

2. Material and Methods

The data of 1028 patients, who have applied to our center between the dates January 2012- June 2019 and diagnosed with SLE, were examined retrospectively from the hospital's automation system. Clinical characteristics and radiological data of the patients were recorded. Patients under age 18 and patients with missing data in their file have been excluded from the study. Final diagnosis of patients with SLE according to the criteria of American College of Rheumatology were included in the study. From these patients, the clinical results, the age of diagnosis, medical treatments and radiological findings belonging to 11 patients diagnosed with NPSLE were recorded. Patients with headache and psychiatric clinical complaints and normal MRI results were not included in NPSLE patient group. In order to exclude other reasons that may explain the current clinical status, oral contraceptive use, pregnancy status, concomitant infections, familial history of thrombosis, homocysteine levels, antithrombin III, protein C and S deficiency, and gene mutations (methylenetetrahydrofolate reductase, prothrombin II, Factor V Leiden mutation) were recorded. Ethics committee approval was taken for the study (06/17/26.09.2019).

2.1. Statistical analysis

For the statistical analyses SPSS 22.0 was used. Categorical variables are demonstrated in number and percentage. Continuous variables were presented as mean ± standard deviation. Numerical data were checked for normal distribution by Kolmogorov-Smirnov test. $p<0.05$ value was recognized to be statistically significant.

3. Results

Eleven of 1028 SLE patients (1.07%) had NPSLE. Mean age was 37±5.3. The age interval of patients diagnosed with NPSLE was between 22-46, and only one female patient was diagnosed simultaneously with SLE and NPSLE at age 82. 90.9% of the patients (n=10) were female, while 9.1% (n=1) were male. 27.3% (n=3) of patients diagnosed with NPSLE had SLE diagnosis before central involvement, while 72.7% (n=8) was diagnosed with SLE after central involvement. Our patient group consisted of recently diagnosed patients and patients diagnosed in the past with a disease period ranging from 6 months to 5 years. Neurological symptoms, radiological involvement types and concomitant antibody positivity's are presented in Table 1, and clinical data is presented in Table 2. In one of patients, the first neurological attack was ischemic stroke, and the second neurological attack was demyelination syndrome. This patient was diagnosed with SLE and NPSLE after the second attack. The rate of mortality was 9.1% (n=1) in our patients diagnosed with NPSLE, and the mortal type of central involvement was ischemic stroke. With regard to concomitant secondary risk factors, one patient was pregnant. Central involvement of the pregnant patient was ischemic stroke. Other patients did not have any risk factors (oral contraceptive use, concomitant infections, familial history of thrombosis, high homocysteine levels, antithrombin III, protein C and S deficiency, gene mutations) in their etiology apart from SLE. One patient had low complement 3 and 4 (C3, C4) levels, while it was normal in other patients.

4. Discussion

The cases with SLE were 1.07% NPSLE. The female rate was higher. Clinically, most of the cases were hemiparesis. Radiologically, most of the cases were subcortical plaques. The mortality rate was 9.1%.

NPSLE has a wide variety of symptoms ranging from headache, anxiety disorder and mild cognitive impairment to severe neurological manifestations such as transverse myelitis, Guillain Barre Syndrome and ischemic stroke. Diagnosis may be delayed rarely due to variable clinical symptoms. The disease often affects patients of female gender (1). In our study, most of our patients were women, and the distribution of age and gender was similar to literature.

NPSLE is the most common presented with cerebrovascular disease, seizures, acute confusional state and neuropathy (14, 15). Similar to literature, the most common presentation type was cerebrovascular disease. 36.4% of patients had ischemia (one patient had ischemia and demyelination syndrome in different periods), 9.1% had cerebral venous thrombosis and 9.1% had PRES as involvement. Focal NPSLE generally represents localized CNS involvement in the form of venous thrombosis or arterial ischemia. These are considered to constitute about 20% of NPSLE cases (14, 16). This rate was higher in our study with 45.5%, and there was 36.4% arterial ischemia and 9.1% cerebral venous thrombosis. The fact that this rate is higher than the values in literature is attributed to ethnic differences. These patients did not have secondary risk factors, such as contraceptive use, concomitant infections, familial history of thrombosis, high homocysteine levels, antithrombin III, protein C and S deficiency and gene mutations, apart from SLE that could lead to ischemia and venous thrombosis. A patient with multiple arterial ischemia areas in her brain had pregnancy as a risk factor, and the pregnancy resulted in intrauterine death of the fetus. SLE and NPSLE diagnosis was determined simultaneously while determining the etiology of ischemia in this patient who did not have any known systemic disease in the past, and the patient had negative antiphospholipid antibodies. It has been shown in different studies that arterial ischemia and venous thrombosis rate varied between 3% and 43% (15, 17). Arterial ischemia and venous thrombosis mostly depend on thromboembolic phenomena that appear in hypercoagulability conditions associated with SLE and associated with the presence of antiphospholipid antibodies (15, 18, 19). Antiphospholipid antibodies are Lupus Anticoagulant (LA), Anticardiolipin Antibody and Anti β 2-glycoprotein I Antibodies. One of our patients had anticardiolipin antibody positivity and borderline elevated lupus anticoagulant, and this patient had clinical migraine headache and radiological involvement in subcortical plaque form. One of the other two patients with borderline elevated lupus anticoagulant level had clinical cerebral venous thrombosis, and the other patient had ischemic stroke. Other three patients with clinical ischemic stroke had no antiphospholipid antibody positivity. In our study group, 40% of patients with ischemic stroke and venous thrombosis had accompanying antiphospholipid antibody positivity while 60% had no antiphospholipid antibody positivity. This suggests that NPSLE patients have high ischemia risk even in the absence of antiphospholipid antibodies. Increased ANA serum titers are determined in more than 95% of patients (20). Positive dsDNA antibodies are determined between 37%-80% (20) and interpreted as an indicator of disease exacerbation (21). All of our patients diagnosed with NPSLE had positive anti-dsDNA. The patients with subcortical plaque had slightly higher antibody titers while patients applying with clinical stroke had significantly higher antibody titters, and this finding supports the fact that high antibody titers demonstrate disease exacerbation. More than 50% decrease in antibody titers also supports this result in the controls of patients with stroke that were performed 3 months later.

Ischemic (4%-40%) or hemorrhagic (6%-20%) stroke, which are observed in SLE patients as a complication of reversible cerebral vasoconstriction syndrome (RCVS), may result in persistent sequelae, and even death. Non-aneurysmal subarachnoid hemorrhage and posterior reversible encephalopathy syndrome (PRES) are other reported complications (22, 23).

Table 1: Neurological symptoms, radiological involvement patterns and concomitant antibody positivity at the time of diagnosis of NPSLE.

Neurological symptoms %(n)	
Hemiparesis	45.4 (5)
Headache	27.3 (3)
Psychiatric findings	18.2 (2)
Consciousness disturbance	9.1 (1)
Radiological involvement %(n)	
Ischemia	36.4 (4)
Cerebral veneus thrombosis	9.1 (1)
PRES	9.1 (1)
Subcortical plaque	45.4 (5)
Antibody positivity %(n)	
ANA	100 (11)
Anti-dsDNA	100 (11)
P-ANCA	18.2 (2)
SS-A	9.1 (1)
Anticardiolipin antibody	9.1 (1)

PRES: Posterior reversible encephalopathy syndrome, ANA: Antinuclear antibody, p ANCA: perinuclear antineutrophil cytoplasmic antibody, SS-A: Sjogren syndrome antibody.

Table 2: Clinical data of NPSLE patients.

Patient	Gender	AGE		SLE symptom	NPSLE symptom	Antibody positivity	Radiological involvement	Treatment
		SLE	NPSLE					
1	F	41	41	Proteinuria Anemia Lymphopenia Arthritis	Hemiparesis	ANA anti ds-DNA	Subcortical plaques and acute infarct	Hydroxychloroquine, Deltacortril
2	F	46	46	Arthritis Anemia Proteinuria	Headache	ANA anti ds-DNA	Subcortical plaques	Hydroxychloroquine, Azathioprine
3	F	14	19	Proteinuria Arthritis C3-C4 Levels↓	Anxiety	ANA anti ds-DNA	Subcortical plaques	Deltacortril Antidepressant
4	F	37	37	Anemia Proteinuria Arthritis Abortion	Headache	ANA anti ds-DNA SS-A Anticardiolipin antibody Lupus anticoagulant borderline high	Subcortical plaques	Azathioprine
5	F	33	33	Arthritis Photosensitivity	Headache	ANA anti ds-DNA	Subcortical plaques	Hydroxychloroquine
6	F	42	43	Anemia Thrombocytopenia Proteinuria Abortion	Hemiparesis	ANA, anti ds-DNA	Multiple acute infarct	Deltacortril
7	F	20	20	PhotosensitivityAnemia Thrombocytopenia	Hemiparesis Epileptic seizure	ANA anti ds-DNA Lupus anticoagulant borderline high	Cerebral veneus thrombosis	Hydroxychloroquine Azathioprine Warfarin sodium Levetiracetam
8	F	82	82	Discoid rash Photosensitivity Arthritis	Right hemiparesis	ANA anti ds-DNA p-ANCA	Subcortical and periventricular contrast retaining plaques (Demiyelinating syndrome) Acute infarct	Hydroxychloroquine Acetylsalicylic acid
9	M	38	38	PhotosensitivityDiscoid rash Arthritis Anemia Proteinuria	Hemiparesis	ANA anti ds-DNA p-ANCA Lupus anticoagulant borderline high	Subcortical plaques and acute infarct	Rituximab Deltacortril Acetylsalicylic acid
10	F	23	26	Anemia Thrombocytopenia Arthritis Discoid rash	Psychosis	ANA anti ds-DNA	Subcortical plaques	Azathioprine Antidepressant
11	F	22	22	Arthritis Proteinuria	Consciousness disturbance (somnolance)	ANA anti ds-DNA	PRES	Hydroxychloroquine Calcium channel blocker

In SLE patients, it is important to differentiate RCVS from cerebral vasculitis since they have different treatments; and while cerebral vasculitis responds to high dose corticosteroids and aggressive immunosuppression, RCVS responds to calcium channel blockers (24). RVCS is one of the rare clinical manifestations in SLE patients. Similar to literature, we had a patient with a clinical manifestation of PRES in our study.

The most common MRI anomalies observed in these patients are small subcortical hyperintense lesions and infarctions (11). The most common type of radiological involvement was subcortical plaques (45.4%) in our patients, similar to literature.

MRI is especially sensitive in the determination of hemorrhagic and ischemic infarction and transverse myelitis; however, it does not currently have the spatial resolution required for detecting microvascular involvement (it is known that 42% SLE patients with neurological symptoms have microvascular involvement) (25, 26). Most of the neuropsychiatric events associated with SLE appear at the initial of disease or in the first 1-2 years after the diagnosis (12,17). In our study, 27.3% (n=3) of patients diagnosed with NPSLE had SLE diagnosis before central involvement and SLE diagnosis period varied between 1 and 5 years, while 72.7% (n=8) was diagnosed with SLE after central involvement. Eleven of 1028 SLE patients (1.07%) had NPSLE diagnosis. In our SLE patient group, NPSLE rate was lower than the levels in literature (12). The reason of that was considered to be: 1) Headache, mild anxiety disorders and mood disorders were not reported as complaints by some patients, 2) The deficiencies in complaint recording for these patients due to inadequate questioning, 3) MRI being evaluated as normal in complaining patients and the possibility of missing microvascular involvement. MRI is one of the common used imaging methods with a relatively easy access that contributions in diagnosis and differential diagnosis of NPSLE. However, it is not a sufficiently reliable method in determining NPSLE diagnosis since it does not show microvascular lesions. In cases that conventional MRI remains insufficient in determination of the lesion, the use of advanced imaging methods is recommended such as SPECT or PET (12). Since the cost of these tests is higher, they should be used in cases with SLE diagnosis and NPSLE suspicion, in which MRI remained to be insufficient for diagnosis. This is because central involvement is the most important risk factor affecting morbidity and mortality in SLE.

High dose steroids (methylprednisolone) are used in SLE treatment, and an immunosuppressive agent should be included in treatment after the patient goes in remission. There is no standard treatment regimen in NPSLE, and a treatment protocol that is similar to SLE is applied. At least 5 years of immunosuppressive treatment is recommended to SLE patients with neurological involvement (27). Medicines such as steroids, cyclophosphamide, mycophenolate mofetil and azathioprine are used as immunosuppressive agents. The immunosuppressive agent to be selected is evaluated according to the experience of the clinician and the severity of disease. It was shown in a study that a much better response is obtained with cyclophosphamide use in the treatment of patients diagnosed with severe NPSLE compared to the use of methylprednisolone (28). In addition, symptomatic treatments are applied on the patients, in which antiepileptic agents are administered to patients with seizures, and antidepressant and anxiolytic agents are administered to patients with psychiatric complaints. Life-long anticoagulation with warfarin is recommended in all thrombosis cases associated with antiphospholipid antibody. Different combinations were used in our patients in the form of monotherapy and polytherapy according to their clinical status. Although one of the most effective drugs was cyclophosphamide, none of the patients diagnosed with NPSLE used cyclophosphamide in our clinic. As immunosuppressants, deltacortril and azathioprine use were higher in our patients. Hydroxychloroquine was started in 54.5% of our patients who were diagnosed with NPSLE since it has an effect known to reduce central nervous system involvement. Rituximab and deltacortril were used in the male patient with poor clinical status and wide ischemic area, but the patient passed away 2 months after the treatment. In a retrospective study, it was shown that rituximab was efficient and pretty safe in pediatric NPSLE patients (29). There is no sufficient number of studies about rituximab until this date. The death of the only male patient with NPSLE diagnosis suggested that the course of central involvement may be more severe in men compared to female gender. Other patients were clinically stable, and no new neurological attack was observed in their follow-up.

5. Conclusions

Symptoms such as headache and mild mood disorder are common in SLE. In order to avoid missing NPSLE diagnosis in patients with these symptoms who underwent cranial MRI, the use of imaging methods such as PET and SPECT may be important in early diagnosis and treatment since they are more efficient in showing microvascular involvement. It should be considered that patients may rarely present with stroke secondary to RVCS and PRES, and differential diagnosis is important due to the difference in their treatment. Since there is no standard optimization in treatment and no gold standard method for diagnosis yet, it is important for patients to be evaluated by an experienced clinician. There is a need for new studies to be performed with large groups in order to determine a standard in therapy.

Conflict of Interests
All authors declared that there is no conflict of interest
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Author Contributions
FŞ contributed to the conception
and design of the study. FŞ and MC contributed to the collection of the data, statistical analysis, evaluation of the results, and writing of the manuscript. FŞ contributed to revising the work and final approval of the version.
Ethical Approval
Ethics committee approval was taken for the study (06/17/26.09.2019).
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Comparative Evaluation of Tocilizumab Versus High Dose Methylprednisolone Therapy in Mild Acute Respiratory Distress Syndrome Related to Covid-19 Pneumonia: A Retrospective Cohort Study

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Abstract: Coronavirus disease 2019 (COVID-19) is a respiratory infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) . The underlying causes of severe COVID-19 are related to systemic inflammatory responses that can lead to lung damage. Tocilizumab and high-dose glucocorticoids are practically used in ARDS cases associated with COVID-19. In this study, we wanted to compare the beneficial effects of tocilizumab and high-dose methylprednisolone therapy in mild acute respiratory distress syndrome (ARDS) caused by COVID-19. The study included 152 patients who received two doses of tocilizumab 400 mg or pulsed methylprednisolone therapy (500 mg/day for three days) due to mild ARDS related to COVID-19 pneumonia. The two groups were compared in terms of age, gender, comorbid diseases, hospital stay, admission to intensive care unit, length of stay in the intensive care unit, intubation status, mortality, C-reactive protein (CRP) level, white blood cell (WBC) count, platelet, neutrophil, lymphocyte, ferritin and D-dimer levels. There was no statistically significant difference between the groups in gender, comorbid diseases, need for intubation, mortality and need for intensive care. There was no statistically significant difference between the groups inage, total length of hospital stay, length of stay in intensive care, CRP, WBC, platelet, neutrophil, lymphocyte counts, ferritin and D-dimer values. The present study found that treatment with pulsed methylprednisolone which is cheap and easy to access can be a good alternative to tocilizumab therapy in mild ARDS related to COVID-19 pneumonia.
Keywords: COVID-19; Tocilizumab; methylprednisolone.

1. Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a newly emerging virus that was first detected in Wuhan/China (1). Although the disease is mild to moderate, almost one third of patients are at risk of developing a more serious illness due to acute respiratory distress syndrome (ARDS). Mechanical ventilation (MV) and the need to stay in the intensive care unit develop in almost one third of the patients. These patients with poor prognosis have an increased risk of developing acute respiratory distress syndrome (ARDS), which can lead to death. The

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mechanisms underlying severe COVID-19 are related to systemic inflammatory responses that can lead to lung injury and multisystem organ dysfunction (2, 3).
Understanding the pathophysiology of cytokine storm in the treatment of COVID-19 pneumonia is important. Various immune cells such as T cells, B cells, dendritic cells (DCs) or macrophages are involved in this mechanism. Similarly, various inflammatory cytokines such as tumor necrosis factor (TNF)-α, type I and II interferons (IFNs), interleukin (IL)-1, IL-6, CCL2, or monocyte chemotactic protein-1 (MCP-1) or immunosuppressive cytokines such as chemokines IL-10 or transforming growth factor-are included. Among these, attention has been paid to the activation of macrophages, particularly due to macrophage activation syndrome (MAS) (4).
Based on this assumption, systemic anti-inflammatory drugs have been proposed as an alternative treatment tool to avoid the SARS-CoV-2-induced inflammatory state and to reduce mortality in these patients (3, 5-7).
Tocilizumab is a monoclonal antibody for IL-6 receptor and is now widely used in hospitals to treat COVID-19 (8, 9). The average price of a 400mg vial of tocilizumab is $250-500, with an overall cost of $500-1000 for two doses. Thus, it is expensive and inaccessible.
There are many studies supporting steroid treatment in COVID-19 and showing reduced mortality, ICU admission and the need for mechanical ventilation. Steroids are recommended for severe cases in China [10]. In addition, high dose glucocorticoids are used in practice in patients with ARDS associated with COVID-19 (11) A 500 mg vial of methylprednisolone costs approximately $10.
Corticosteroids are inexpensive and easily available drugs compared to tocilizumab. There is no study comparing these two drugs, which are becoming widely used in treatment-unresponsive COVID-19 pneumonia. The present study aimed to compare the benefits of tocilizumab and pulsed methylprednisolone which are among the treatment options in mild ARDS related to COVID-19 pneumonia. The mortality, length of hospital stay, need for intensive care, time spent in intensive care unit and need for intubation were compared in patients with mild ARDS who were treated with pulsed methylprednisolone (500 mg/day for 3 days) vs. tocilizumab.

2. Material and Methods

During the pandemic, the first-line treatment in our hospital was administered as standard favipiravir, heparin, protein pump inhibitor and antibiotherapy. In our hospital, high-dose methylprednisolone or tocilizumab have been used in second-line treatment to patients who developed acute respiratory dyspnea syndrome due to COVID-19 and did not benefit from first-line treatment. The choice was entirely at the discretion of the doctor because there was no standard practice in this regard. The study started with the approval of a university's ethics committee. In this study, we have compared second-line treatments in COVID-19 pneumonia. The study included 152 patients who received two doses of tocilizumab 400mg or pulsed methylprednisolone therapy (500 mg methylprednisolone/day for three days) due to mild ARDS related to COVID-19 pneumonia between 01.05.2020 and 01.12.2020. Patients with mild acute respiratory dyspnea syndrome due to COVID-19 pneumonia were selected retrospectively. The patients were divided into two groups; those who received tocilizumab 800 mg and those who receivedpulsed methylprednisolone (500 mg methylprednisolone/day for three days) therapy. The two groups were compared in terms of age, gender, comorbid diseases, hospital stay, admission to intensive care unit, length of stay in the intensive care unit, intubation status, mortality, C-reactive protein (CRP) level, white blood cell (WBC) count, platelet, neutrophil, lymphocyte, ferritin and D-dimer levels.
The inclusion criteria were as follows**:**

1. PCR positivity or thorax CT consistent with COVID-19 (Figure 1).
2. Patients who received first step (favipiravir, heparin, protein pump inhibitor and antibiotherapy) therapy for at least 5 days but were unresponsive to treatment and whose COVID-19 pneumonia progressed.
3. Patients with mild ARDS manifestations related to COVID-19 (200 mmHg<PaO_2/FiO_2<300 mmHg+ PEEP or CPAP≥5 cm H_2O) (Table 1).

Table 1: Berlin Criteria for the Diagnosis of ARDS.

Timing	➢ New or worsening respiratory distress occurring within 1 week
Chest X-ray	➢ Bilateral opacities that cannot be explained with effusion, collapse or nodule
Source of edema	➢ Showing that respiratory distress is not due to heart failure or hypervolemia using objective criteria such as ECHO
Oxygenation	
• Mild	➢ 200mmHg<PaO2/FiO2<300mmHg + PEEP or CPAP≥5cmH2O
• Moderate	➢ 100mmHg<PaO2/FiO2<200mmHg + PEEP≥5cm H2O
• Severe	➢ PaO2/FiO2≤100mmHg + PEEP≥5cm H2O

Abbreviations: PaO2: Arterial partial oxygen pressure, FiO2: Fractioned O2 in inspired air CPAP: Continuous positive airway pressure PEEP: Positive end-expiratory pressure.

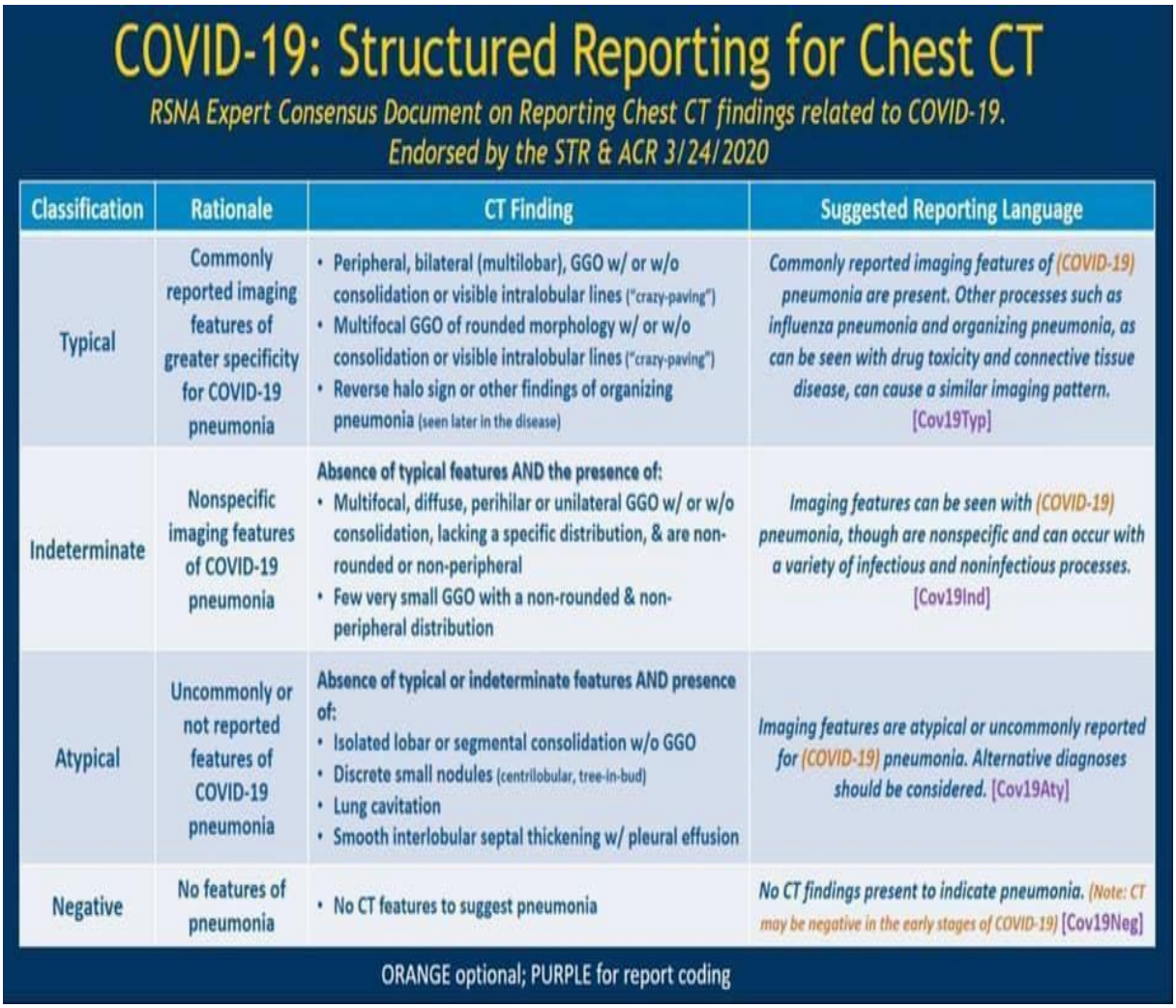

Figure 1: Consensus Statements on Chest Findings of COVID19.

The exclusion criteria were follows:

1. Receiving a single dose of tocilizumab, different dose methylprednisolone treatment other than 500 mg/day for 3 days, immune plasma therapy
2. Those who receive treatment other than favipiravir, heparin, protein pump inhibitor and antibiotherapy in initial treatment.
3. Age under 18 years.

2.1. Statistical Analyses

Statistical analysis was performed using the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The assumption of normal distribution of data was tested by Kolmogorov-Smirnov test. Descriptive statistics of continuous variables were shown with mean and standard deviation (SD) values. The Chi-square test was used to compare nominal variables while Student's t test was used to compare the means values of scalar data between the two groups. The hypotheses are two-sided and $p \leq 0.05$ was considered statistically significant at 95% confidence interval.

3. Results

Of the 152 patients retrospectively reviewed between 01.05.2020 and 01.12.2020, 70 were treated with pulsed methylprednisolone and 82 were treated with tocilizumab. Comparisons of the parameters of the groups treated with pulsed methylprednisolone or tocilizumab are provided in Tables 2 and 3.

Table 2: Comparison of the parameters between the two groups.

Gender	Pulsed Methylprednisolone n=70	Tocilizumab n=82	p
Female	14(20%)	30 (36.6%)	0.1
Male	56 (80%)	52 (63.4%)	
Hypertension	24 (34.3%)	40 (48.8%)	0.2
Diabetes mellitus	10 (14.3%)	16 (19.5%)	0.5
Chronic Renal Failure	2 (2.9%)	4 (4.9%)	0.6
Heart failure	12 (17.1%)	4 (4.9%)	0.08
Chronic obstructive pulmonary disease	8 (11%)	6 (7%)	0.5
Other chronic diseases	4 (5%)	6 (7%)	0.7
Comorbid diseases			
Yes	38 (54.3%)	50 (60.9%)	0.5
No	32 (45.7%)	32 (39.1%)	
Need for intubation	12 (17.1%)	12 (14.6%)	0.7
Mortality	8 (11.4%)	8 (9.8%)	0.8
Need for intensive care	14 (20%)	18 (22%)	0.8

p≤0.05 was considered statistically significant. There was no statistically significant difference between the groups ingender, comorbid diseases, need for intubation, mortality and need for intensive care (p>0.05).

Table 3: Comparison of the parameters between the two groups.

	Pulsed Methylprednisolone n=70 median (range min-max)	Tocilizumab n=82 median (range min-max)	P value
Age	67 (32 to 90)	65 (28 to 88)	0.3
Total length of hospital stay	13 (5 to 47)	13 (6 to 41)	0.7
Length of stay in intensive care	0 (0 to 10)	0 (0 to 24)	0.7
C Reactive Protein (CRP)	70 (11.30 to 254)	72.5 (4 to 306)	0.3
White Blood Cell (WBC)	8.1 (4,74 to 24,60)	9.56 (1.50 to 91)	0.5
Platelets	199 (112 to 486)	208 (88 to 373)	0.5
Neutrophils	6.80 (2.30 to 21.49)	7.94 (0.27 to 21.00)	0.4
Lymphocytes	0.88(0.21 to 2.73)	0.90 (0.23 to 5.90)	0.5
Ferritin	700 (82 to 1777)	807 (71.10 to 3669)	0.2
D-dimer	860 (315 to 7570)	785 (188 to 4420)	0.4

p≤0.05 was considered statistically significant. There was no statistically significant difference between the groups inage, total length of hospital stay, length of stay in intensive care, CRP, WBC, platelet, neutrophil, lymphocyte counts, ferritin and D-dimer values (p>0.05).

4. Discussion

In this study, no statistically significant difference has been found between the tocilizumab and pulsed methylprednisolone in patients developing mild ARDS related to COVID-19 pneumonia. A comparison between the two patient groups showed that there was no statistically significant difference in the length of hospital stay, the rate of need for intensive care, the length of stay in intensive care, and the rates of intubation and mortality (p<0.05). Similarly, no statistically significant difference was found between the two groups inage, comorbid diseases, ferritin levels, neutrophil values, lymphocyte values, platelet values, CRP and WBC levels which are other variables that may affect mortality, length of stay in the hospital and in the intensive care unit and status of intubation (p>0.05).

There are many controversial therapies in COVID-19 as it is a new disease. Tocilizumab therapy has shown promising outcomes in COVID-19 pneumonia in subjects that were not responsive to treatment. Malgie *et al. (*12) published that tocilizumab treatment have

reduced mortality by 12% in COVID-19 patients. Xiaoling *et al.* (9) also stated that tocilizumab was an effective treatment in COVID-19 pneumonia and reduced mortality.

Similarly, in another study, 29 patients receiving tocilizumab treatment were compared with 58 patients receiving only routine care, and patients treated with tocilizumab have required less ventilation andextubation was performed at an earlier stage in more patients. Both the length of stay in the intensive care unit and the length of stay in the hospital were significantly shorter in patients treated with toxilizumab (13).

The relative benefits of tocilizumab compared to other immune modulator drugs have not yet been reported. In the literature, there are reportson high-dose glucocorticoids in COVID-19 pneumonia. In an intensive care study by Ramin Hamidi Farahani et al including 29 patients, significantly higher systolic (P=0.018) and diastolic (P=0.001) blood pressures were detected in patients with high-dose methylprednisolone. Patients who were given high-dose methylprednisolone therapy had significantly (P<0.001) higher Glasgow coma scale (GCS) in the methylprednisolone group and with improvement in SpO_2 in the methylprednisolone group, none of the patients required mechanical ventilation (11).

Guillermo Ruiz-Irastorza *et al*. stated that patients with respiratory distress that deepened with respiratory failure and who showed inflammatory activity might benefit from high-dose glucocorticoids. They pointed out that this group should be identified in the early period with good observation (14).

Wu et al. also showed that treatment with methylprednisolone in COVID-19 patients who developed ARDS was associated with a reduced risk of mortality (risk ratio: 0.38; 95% CI: 0.20-0.72) [15]. In another study with high-dose methylprednisolone and dexamethasone (1000 mg methylprednisolone for 3 days plus 8 mg dexamethasone for another 3-5 days), the treatment showed a rapid anti-inflammatory effect, but it was also observed that it increased the risk of thromboembolism. Neutrofyl/Lymphocyte ratio and D-dimer level (16). On the other hand, there are also some studies that do not recommend treatment with high-dose glucocorticoids and tocilizumab (17-19).

The limitation of our study can be elaborated as the beneficial effects of these two treatment modalities seemed similar, we did not have enough data about their short-term and long-term adverse effects and we did not have a placebo group and the absence of a control group that did not receive both treatments.

5. Conclusions

The average cost of tocilizumab therapy is $500-1000, while it is $30 in pulsed methylprednisolone treatment. The present study found that treatment with pulsed methylprednisolone was cheap and easy to access could be evaluated as an alternative to tocilizumab therapy in mild ARDS related to COVID-19 pneumonia. Future studies with control group are required to obtain more data on this topic.

Conflict of Interests

We do not have any conflicts of interest

Financial Support

We do not have financial resources to declare

Author Contributions

Barış Çil was responsible for the organization and coordination of the study. Barış Çil was the chief investigator and responsible for the data analysis. Barış Çil and Mehmet Kabak developed the study design. All authors contributed to the writing of the final manuscript. All members of the Team contributed to the management or administration of the study.

Ethical Approval

Ethical approval was obtained from the ethics committee of Mardin Artuklu University Ethics committee number: 79906804-050.06.04.

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Biochemical and Histopathological Evaluation of the Protective Efficacy of Thymoquinone in Experimentally Ischemia Reperfusion Induced Rat Ovaries

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Abstract: In our study, oxidant-antioxidant and inflammation markers of ovarian tissue were evaluated in order to determine whether thymoquinone has a protective effect against experimentally induced ischemia/reperfusion in rat ovaries. Oxidant-antioxidant and inflammation markers in ovarian tissue were evaluated in order to determine whether thymoquinone has a protective effect against damage in rat ovaries in which ischemia/reperfusion was created. The rats, which randomly divided into 5 groups and were experimentally induced ischemia/reperfusion in their ovaries, were administered thymoquinone at the determined doses. The markers such as TAS, TOS and MDA from ovarian tissues were determined as spectrophotometrically obtained by experimental procedure. Tissues examined histopathologically were evaluated for immunopositivity after immunohistochemical staining with NF-kβ antibodies. While MDA, TOS, OSI levels in tissue were significantly higher in ischemia/reperfusion groups and TQ, MDA, TOS, OSI levels were significantly lower rather than the control group ($p<0.05$). TAS levels in tissue were significantly lower in the I/R group but after treatment they increased significantly ($p<0.05$). NFKβ1 immunopositivity could not be detected in Sham and experimental groups ($p> 0.05$). It was concluded that thymoquinone protected the tissues from ischemic damage by causing significant increase in TAS levels that were decreased by I/R injury and significant decrease in elevated levels of MDA and TOS that were increased by I/R injury.
Keywords: Ischemia/Reperfusion; Oxidative Stress; Thymoquinone.

1. Introduction

Ovarian torsion in women is a gynecological syndrome that results in oophorectomy (removal of the ovaries) by blocking blood circulation in the ovaries both in childhood and adolescence (1). In case of early detection of torsion, infarction departure can be prevented. In this case, ovarian detorsion is performed to restore blood flow (2,3). Although blood flow has been fixed, ischemia/reperfusion (I/R) injury may occur in ovarian torsion. In this case, various problems may arise in the organism (4). However the mechanism causing the damage has not been fully elucidated, studies have shown that reactive oxygen

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species (ROS) produced by polymorph core leukocytes (PMNL), cytokines and complement activation have impact on I/R damage (5, 6). Anti-inflammatory and antioxidant defense systems generally play an active role in preventing I/R damage in tissues (7).

'Nigella sativa (NS)', in other words black cumin, is an important plant species that has been used for the treatment of many diseases in the Middle and Near East countries for over 2000 years. Thymoquinone (TQ), one of the main components of its seeds of NS, is a monoterpene. Many studies have shown the antibacterial, antitumor, antioxidant, antineoplastic, antifungal, anti-inflammatory, reactive oxygen positive effects of TQ on the central nervous system (CNS). In addition to all these, it is known that TQ has important effects on cell cycle, immune system and apoptosis (8, 9).

Oxidative stress results from the disruption of the balance between the formation of ROS and antioxidant defense capacity. ROS cause serious damage to macromolecules such as cellular membrane lipids, proteins and DNA and lead to more toxic products such as MDA (10).

Antioxidant means that any substance prevents or delays oxidative damage on the target molecule. There are many substances that act as antioxidants. Although the basic system of protection from ROS damage is enzymatic systems that prevent oxidation, there are also non-enzymatic antioxidant compounds. ROS can cause many diseases such as cataract, skin aging, Alzheimer's, asthma, Parkinson's, and it is the first among the causes of I/R damage (11).

No studies have been found on whether TQ, known for its anti-inflammatory and antioxidant properties, has an effect on ovarian I/R damage or not. According to the this litarature findings, in this study we aimed to investigate that whether there is an effectiveness of TQ in the prevention of experimentally I/R-induced damage to rat ovaries or not. For this reason, oxidative stress such as malonyldialdehyde (MDA), total oxidant status (TAS), total antioxidant status (TOS) in ovarian tissue samples after ovarian I/R parameters were measured.

After these measurements, the oxidative stress index (OSI), which is expressed as a percentage of the ratio of TOS levels to TAS levels, was calculated. In addition to this, it was evaluated histopathologically whether TQ had an effect on post-I/R inflammatory activity in ovarian tissue samples immunohistochemically stained with NFKβ1 antibodies for Nuclear Factor Kappa B (NFKβ1), which is known to have a role in events such as cell proliferation, cell differentiation, apoptosis and inflammation.

2. Material and Methods

2.1. Study Animals

'Wistar albino' female rats (12-16 weeks, with an average weight of 200 to 250 grams), which were obtained from Atatürk University Medical Experimental Application and Research Center (ATADEM), were included in our study which was approved by Atatürk University Rectorate, Animal Experiments Local Ethics Committee with the decision dated 23.02.2018 and numbered 2018-29.

2.2. Chemicals

TOS and TAS of tissue homogenates were determined using kits (Reel Assay Diagnostics, Turkey). Sodium Dodecyl Sulfate (SDS) was obtained from Merck, Acetic acid (CH_3COOH) from Sigma®Thiobarbuturic acid (TBA) from Sigma® Tetraethoxypropane ($C_{11}H_{24}O_4$) from Sigma® for the measurement of malondialdehyde. Anti-NF-kβ 1 antibody Abcam (Cat. No. ab7971) kit was used for immunochemical analysis NF-kB.

2.3. Experimental Procedure

Wistar albino rats were fasted the night before for ischemia and reperfusion experiments and were provided free access to water. Rats were randomly separated into 5 groups with 8 animals in each group. Rats were anesthetized with intraperitoneal ketamine (75 mg/kg) and Xylazine (8 mg/kg). Thymoquinone was applied to the rats with ischemia/reperfusion at determined doses. Animals have been underwent the following experimental procedures after 8 days.

Group 1 (Sham Control). Peritoneum were reached by making 1-2 cm incision in the lower abdomen of the experimental animals in this group and closed back without any other procedure.

Group 2 (3 Hours Ovarian Ischemia). Peritoneum was reached by making 1-2 cm incision in the lower abdomen of the experimental animals. The ovarian tissue was exposed to ischemia with the help of a clamp for 3 hours, and at the end of ischemia, the clamp was removed and reperfusion was started. Meanwhile, the incision was closed with a 3-0 surgical silk suture and 3 hours of reperfusion was achieved. After the reperfusion was completed, the ovarian tissues of the rats were removed.

Group 3 (3 Hour Ovarian Ischemia+Thymoquinone 4 mg/kg (intraperitoneal)). Experimental procedures in Group 2 were performed. 4 mg/kg thymoquinone was applied intraperitoneally 30 minutes before reperfusion.

Group 4 (3 Hour Ovarian Ischemia+Thymoquinone 8 mg/kg (intraperitoneal)). Experimental procedures in Group 2 were performed. 8 mg/kg thymoquinone was applied intraperitoneally 30 minutes before reperfusion.

Group 5 (3 Hour Ovarian Ischemia+Thymoquinone 25 mg/kg (intraperitoneal)). Experimental procedures in Group 2 were performed. 25 mg/kg thymoquinone was applied intraperitoneally 30 minutes before reperfusion. After the reperfusion was completed, the ovarian tissues of the rats were removed.

2.4. Biochemical Analysis

50 Mm, pH: 7.2, KH_2PO_4/ K_2HPO_4 buffer was used for tissue homogenization. Ovarian tissues weighing 0.1 g

were placed in glass tubes and homogenized in ice with 2 mL of phosphate buffer. The homogenized tissues were centrifuged at 5000 rpm for 20 minutes at +4 ºC and the supernatants was carefully separated. The separated supernatants were used for analysis of MDA, TAS, TOS and protein. The protein concentration of the supernatant was measured using the method described by Bradford MM (12). Biochemical results were expressed on the basis of protein for per gram.

2.5. Determination of Malondialdehyde (MDA)

In the measurement principle of MDA formed as a result of lipid peroxidation, the absorbance of the pink colored complex formed as a result of the reaction of MDA and thiobarbutyric acid (TBA) is measured at 532 nm (13). Results are expressed in µmol/L. Tissue MDA concentration was calculated as µmol/g protein using the formula below. Tissue MDA (µmol/g protein) = MDA (µmol/L) /Tissue protein (g/L).

2.6. Determination of TAS and TOS

Rel Assay kit was used for the TAS analysis method. The principle used in the analysis method of this kit is based on the reduction of the colored complex ABTS (Ethylbenzathiazoline Sulfonic Acid) cationic radical by all antioxidant molecules in the samples and the decolorization of the colored radical in proportion to the total concentrations of antioxidant molecules (14).

Results are measured in a spectrophotometer microplate reader at a wavelength of 660 nm and expressed as mmol Trolox Equiv./L. For tissue samples, the result was divided by protein and reported as µmol Trolox Equivalent/mg protein. The measurement principle of the kit (Rel Assay Diagnostics, Turkey) used for TOS analysis is based on the conversion of oxidants in the samples into ferric ion chelator complexes (15).

In an acidic environment, ferric ions form a colored complex with the help of chromogen. The intensity of the color measured spectrophotometrically at 530 nm. wavelength is related to the total amount of oxidant molecules in the samples. Results are expressed in (µmol H_2O_2 equiv/L). For tissue samples, the result was divided by protein and reported as µmol H_2O_2 equiv/g protein. The percentage ratio of TOS to TAS was used as the OSI. OSI was calculated as TOS divided by 10xTAS.

2.7. Histopathological Assessments

The removed ovarian tissues were determined in 10% neutral formalin solution. After washing, the tissues passed through routine alcohol xylol series were placed in paraffin blocks and 5 µm parts were taken. After deparaffinization, the parts were kept in microwave oven for 15 minutes with 400 watts of antigen retrieval solution (pH 6.0). Parts washed with PBS were incubated with NFKβ1 (cat no. ab7971, dilution ratio 1/200, Abcam) antibodies for 15 minutes at room temperature for inflammatory activity. Parts washed with PBS were stained with Expose mouse and rabbit specific HRP/DAB detection IHC kit. 3,3′ diaminobenzidine (DAP) was used as a chromogen. After counterstaining with hematoxylin, parts passed through alcohol xylol series were examined under a light microscope. The immunopositivity was evaluated as no (0), mild (1), moderate (2), and severe (3).

2.8. Statistical analysis

Statistical Package for Social Sciences (SPSS) for Windows 23.0 program was used for statistical analysis. Biochemical results were given as mean±standard deviation (X±SD), and P values below 0.05 were considered statistically significant. The normality test of the data was evaluated with Kolmogorov Smirnov. As the data fit the normal distribution, the groups were compared with the parametric test, one-way ANOVA. The significance of the difference between the groups was determined by the Post Hoc Tests Tukey HSD test. Kruskal Wallis, one of the non-parametric tests, and MannWhitney U test were used to determine the differences between the groups for histopathological data ($p<0.05$).

3. Results

3.1. Biochemical Results

Tissue MDA, TAS and TOS analysis X±SD and p values of all results are shown in Table 1.

When the MDA levels in Table 1 were compared, it was observed that the MDA level in the I/R damaged groups (8.99±1.14) increased significantly compared to the sham group (5.17±1.07) ($p=0.001$). It was observed that MDA levels in the groups treated with TQ approached the sham group after I/R (Figure 1).

When the mean of MDA levels was compared in the study groups, statistically significant difference were found between the groups ($p<0.05$). The amount of MDA in the IR+4 mg/kg TQ ($p=0.000$) and IR+8 mg/kg TQ ($p=0.001$) groups were statistically significantly decreased compared to the IR group. But there is no significant difference compared to the IR+25 mg/kg TQ group ($p=0.114$).

When the mean of OSI levels was compared in the study groups, there was a statistically significant difference among the groups ($p<0.05$). The amount of OSI in the IR+4 mg/kg TQ ($p=0.000$) and IR+8 mg/kg TQ ($p=0.000$)and IR+25 mg/kg TQ ($p=0.001$) groups were statistically and significantly decreased compared to the IR group. (Table 1).

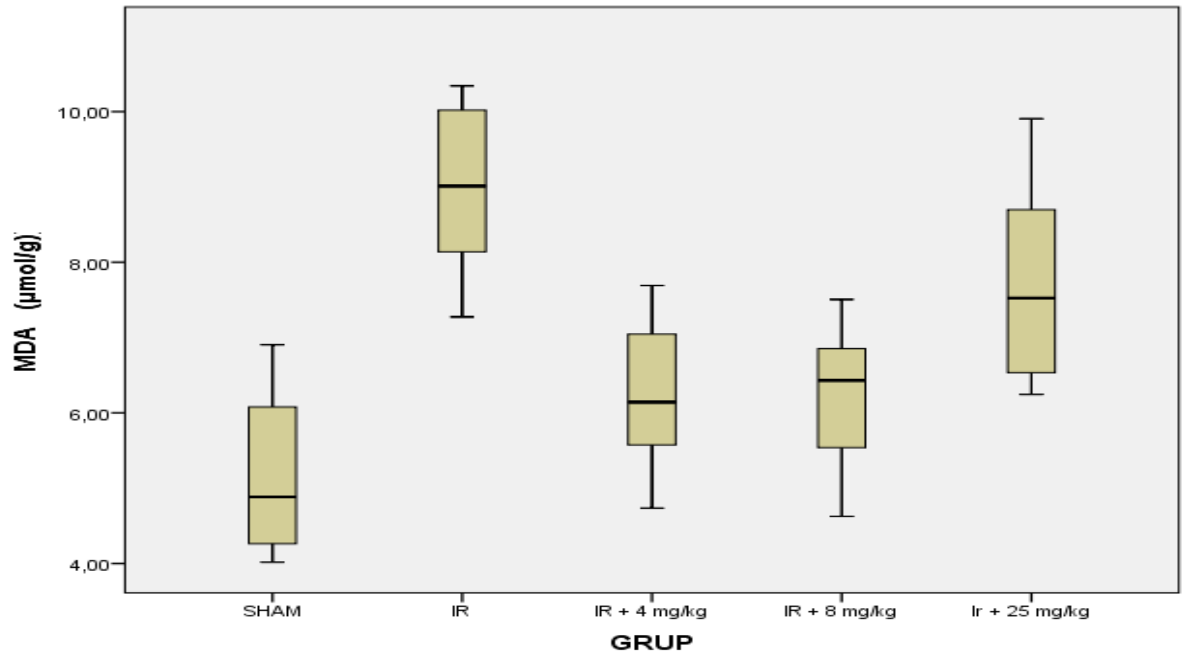

Figure 1: Ovarian Tissue MDA levels in Groups.

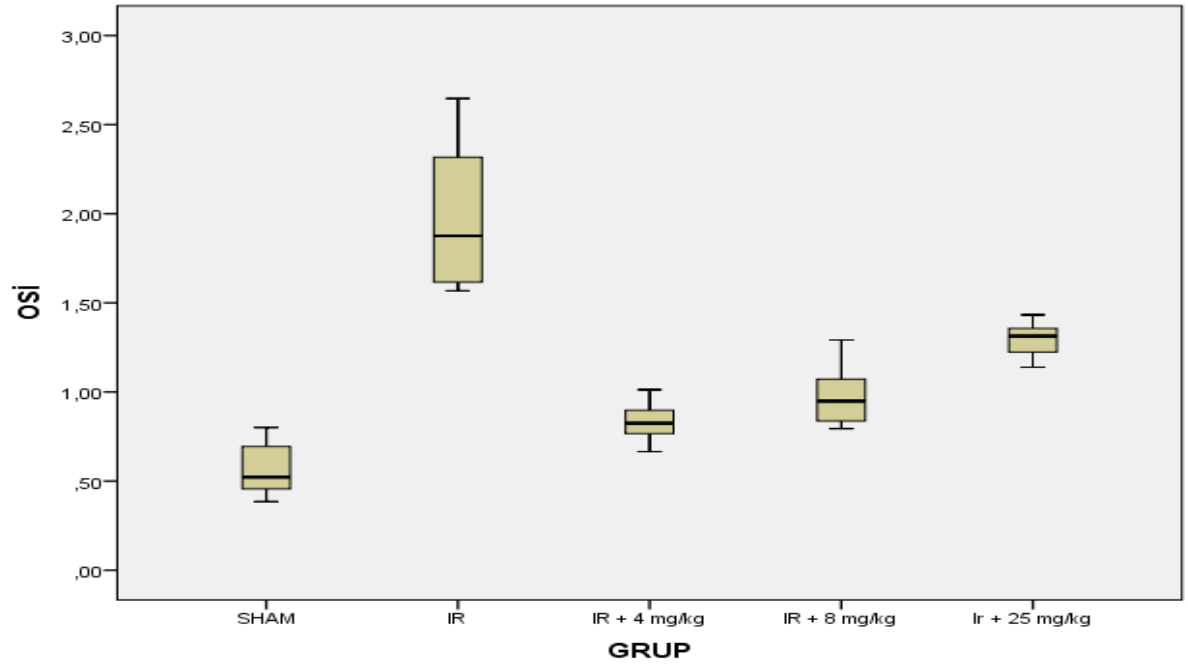

Figure 2. Ovarian Tissue OSI levels in Groups.

In Figure 2, Oxidative Stress Index (OSI) levels, known as the percentile of the ratio of TOS levels to TAS levels, are given. When the OSI levels in Table 1 were compared, it was observed that the OSI level was significantly higher in the groups with I/R injury (1.97±0.41) compared to the sham (0.56±0.14) group (p=0.001). OSI levels were significantly decreased after I/R injury in groups treated with TQ (p=0.001).

3.2. Histopathological Results

NF-Kβ 1 immunopositivity in ovaries could not be detected in Sham and experimental groups (Figure 3, Table 2).

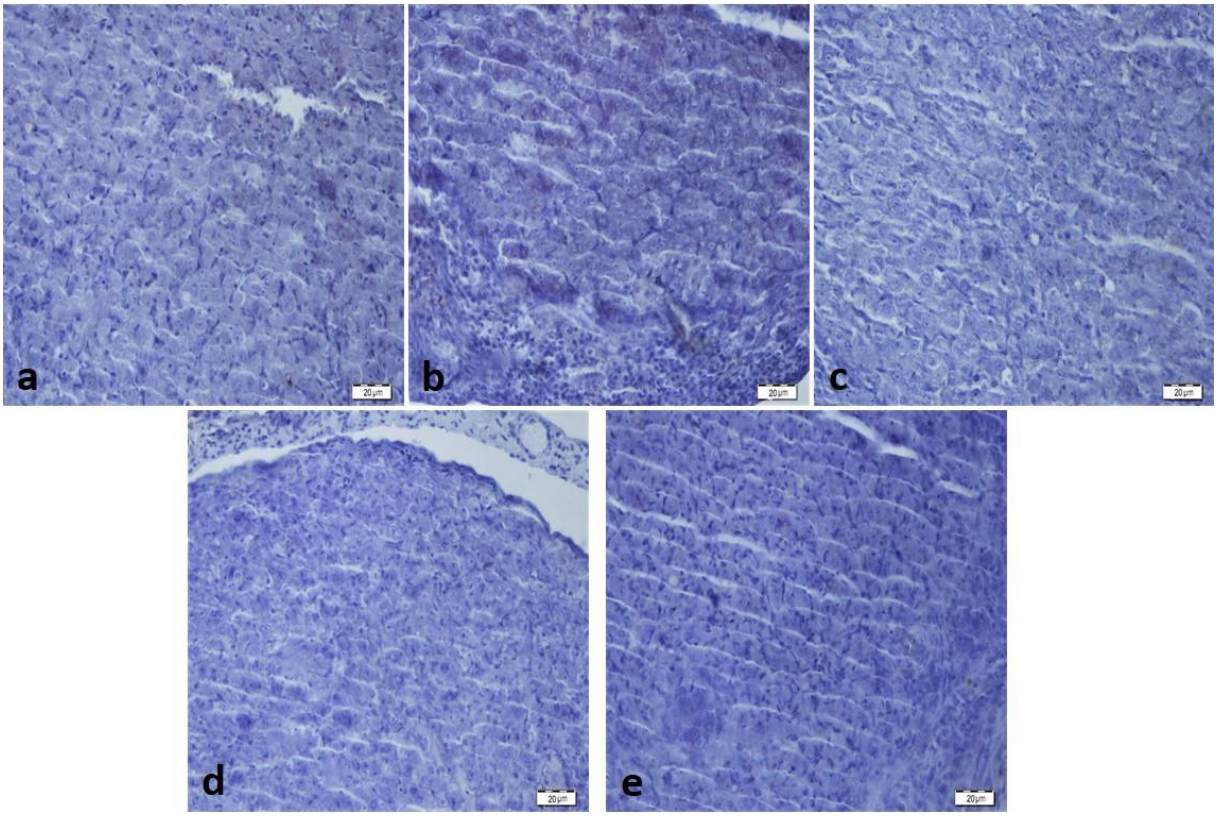

Figure 3: Immunnegativity in NFKB1 staining. (a) sham group, (b) I/R group, (c) I/R+4 mg/kg TQ group, (d) I/R+8 mg/kg TQ group, (e) I/R+25mg/kg TQ.

Table 1: Analyte levels in ovarian tissue.

	SHAM X±SD* (N:8)	**IR** X±SD* (N:8)	**IR+4 mg/kg TQ** X±SD* (N:8)	**IR+8 mg/kg TQ** X±SD* (N:8)	**IR+25 mg/kg TQ** X±SD* (N:8)	**p**
MDA µmol/g protein	5.17±1.07[b]	8.99±1.14[a,b,c,d]	6.24±0.99[b]	6.22±0.97[a,b]	7.70±1.31	0.000**
TAS mmol Trolox Equiv/mg protein	11.92±1.08[a,b]	7.10±1.09[b,c]	10.06±0.94	9.77±1.01	8.92±1.08	0.000**
TOS µmol H_2O_2 Equiv/mg protein	6.66±1.42	13.76±1.9[c]	8.46±1.34[b]	9.52±1.85	12.03±1.26	0.000**
OSI	0.56±0.14[b]	1.97±0.41[a,c,d,e]	0.84±0.13[b]	0.97±0.16[b]	1.35±0.13[b]	0.000**

SHAM: Control, IR: Ischemia-reperfusion, IR+4 mg/kg TQ: Ischemia-reperfusion+4 mg/kg Thymoquinone group. IR+8 mg/kg TQ: Ischemia-reperfusion+8 mg/kg Thymoquinone group. IR+25 mg/kg TQ: Ischemia-reperfusion+25 mg/kg Thymoquinone group. MDA: Malondialdehyde, tGSH: total glutathione, TAS: Total antioxidant status, TOS: Total oxidative status. OSI: oxidative stress index. a: statistically significantly different compared with SHAM, b: statistically significantly different compared with IR, c:statistically significantly different compared with IR+4 mg/kg TQ. d: statistically significantly different compared with IR+8mg/kg TQ. e: statistically significantly different compared with IR+25 mg/kg. *x±SD: Mean±STD. ** p<0.05.

Table 2: Data Obtained from Staining with NF-kB1 Antibodies.

	Sham	I/R	IR+4mg/kg TQ	IR+8mg/kg TQ	IR+25mg/kg TQ
NFKB1	0.25±0.46	0.37±0.51	0.50±0.53	0.12±0.35	0.25±0.46

4. Discussion

Ischemia is defined as the inability to oxygenate and feed the organs or tissues as a result of a clot that occurs in the vessels that provide blood flow or a mechanical factor. Some structural and metabolic changes occur in cells with ischemia (16).

Reperfusion is defined as the increase in the amount of oxygen in the tissue with the mechanisms developed by the tissue after ischemia in order to prevent ischemia and restore blood flow. However, the reperfusion of tissues exposed to ischemia and the initiation of the inflammatory process with activated mechanisms cause more tissue damage than ischemia (17, 18).

Hesperetin (19), aprotinin (20), tacrolimus (21), ursodeoxycholic acid (22), N-acetyl cysteine and resveratrol (23), erythropoietin (24), curcumin (25) and sildenafil (26) were used in studies to reduce reperfusion damage in the ovaries and the studies showed that ovarian damage was reduced to a certain extent.

Studies showed that oxidative stress is primarily responsible for the formation of I/R damage (27). MDA, the end product of lipid peroxidation, is used as an indicator of oxidative stress in tissues (28). Kılıç et al. examined the effects of N-Acetyl Cysteine and Resveratrol on I/R damage in rat ovaries and showed that MDA levels increased significantly in I/R groups compared to the control group (23). On the other hand, Yuan et al. showed that MDA levels increased in the I/R group in their animal experiment studies in which they investigated hepatic I/R damage and MDA levels decreased significantly after treatment (29). In a study conducted by Beheshtian et al. shown that MDA levels increased in the I/R group compared to the control group in ovarian tissue and MDA levels were significantly decreased in the groups treated with sildenafil (30). In another study investigating the protection of atorvastatin in the ovarian I/R model, it was reported that MDA levels increased significantly in the I/R group, whereas MDA levels decreased significantly in the atorvastatin applied groups and approached the control group (31). In our study, as the other studies in the literature, tissue MDA levels were found to be significantly higher in the I/R group than in the sham group. In recent years, it has been determined that MDA levels have decreased significantly in the treatment groups in which TQ has been applied, which has focused on many properties such as antioxidant, antitumoral, antineoplastic, anticarcinogenic, antifungal and anti-inflammatory.

Considering recent studies, TOS parameter is used to analyze lipid peroxidation activities. Protective enzymes such as SOD, GSH, CAT react against damage caused by ROS, and these protective enzymes form TAS (30). Oxidative stress parameters such as TOS and TAS are important markers used to determine the effectiveness of I/R damage and treatment modalities. In studies conducted, TAS levels were found to be significantly lower in I/R injury compared to control groups, while TOS levels were found to be higher (32, 33). Avni et al. while TOS and OSI levels were higher in I/R formed rat ovaries compared to the control group, it was reported that there was a significant decrease in oxidative stress levels after treatment with resveratrol and N-acetyl cysteine (18). In our study, while TOS and OSI levels were increased as markers of damage, TAS levels decreased in groups with I/R in ovarian tissue. While TOS and OSI values decreased significantly in TQ applied groups, it was determined that TAS levels increased and approached the sham group.

The immunopositivity of NF-Kβ1, which is known as one of the important indicators of oxidative stress in histopathological evaluations, could not be detected in the sham and experimental groups in the immunohistochemical sections. When the studies have been examined in the literature, it has been seen that the activation of NF-Kβ increases during ischemia, and it shows excessive reactivity in I/R damage in immunohistochemistry sections (34).

5. Conclusions

In our study, it is aimed to research the effectiveness of TQ, which is known for its anti-inflammatory and antioxidant properties, in the prevention of experimentally I/R-induced damage in rat ovaries, and we showed the protective effect of TQ in line with our findings. TQ showed these effects by reducing the oxidative stress parameters that occur in I/R damage. It was observed that MDA, TOS, OSI levels in particular increased in I/R injury and approached the control group with TQ application. In our current study, in addition to biochemical parameters, NF-Kβ 1 immunopositivity was examined histopathologically, but immunopositivity could not be detected in Sham and experimental groups.

Conflict of Interests

The authors approved that they have no conflict of interest

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Author Contributions

Turkeri ON, Tanyeli A and Bakan N contributed to the constructing the idea for research. Turkeri ON contributed to the planning the design of the work. Turkeri ON, Ekinci Akdemir FN, Tanyeli A, Kurt N and Mokhtare B contributed to the execution of the experiments. Turkeri ON, Tanyeli A and Kurt N

contributed to the analysis and interpretation of data. Turkeri ON contributed to the providing tools and instruments. Turkeri ON, Ekinci Akdemir FN, Kurt N and Mokhtare B contributed to the biological materials. Turkeri ON contributed to the literature review. Turkeri ON and Bakan N contributed to the critical review. Turkeri ON, Kurt N, Tanyeli A, Bakan N, Ekinci Akdemir FN, and Mokhtare B contributed to the final approval of the version to be published.

Ethical Approval

The study was approved by Atatürk University Rectorate Animal Experiments Local Ethics Committee with the decision dated 23.02.2018 and numbered 2018-29.

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How did the Treatment Modalities Effect the Kinesiophobia for the Treatment of Unstable Intertrochanteric Fractures? Retrospective Clinical Trial

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Abstract: Kinesiophobia is the fear of pain and re-injury resulting from movement. It may occure after surgery and affect functional outcomes and patient comfort. In this study, we compared fixation with proximal femoral nail antirotation (PFNA) and hemiarthroplasty, which are two essential methods in treating geriatric unstable intertrochanteric femur fractures, by performing functional evaluation and kinesiophobia measurement Patients who were treated with PFNA or hemiarthroplasty for AO 31A2 hip fracture in our clinic between January 2017 and May 2019 were retrospectively evaluated. A total of 72 patients (age range 60-89, mean age 75.2 ± 7.7 years) with at least 1 year follow-up were included in the study. Functional evaluation of the patients was done with the Harris Hip Score (HHS), pain evaluation was performed with the Numerical Rating Scale (NRS), and the kinesiophobia measurement was carried out with the Tampa Scale (TSK). The mean TSK scores in the PFNA and hemiarthroplasty groups were 47.9±4.9 (95% CI 46.4-49.5) and 51.7±5.7 (95% CI 49.6-53.4), respectively ($p<0.05$). On the other hand, while the mean HHS was 89.1±3.7 (95% CI 87.2-90.3) in the PFNA group, it was 86.2±4.1 (95% CI 84.8-87.6) in the hemiarthroplasty group ($p<0.05$). The NRS score was 2.81±2.62 in the PFNA group and 3.11±2.81 in the hemiarthroplasty group ($p=0.672$). There was no correlation between age and TSK, NRS, or HHS scores ($p=0.316$). However, a significant negative correlation was observed between the HHS and TSK scores ($r=-0.77$, $p<0.01$). Hemiarthroplasty in geriatric unstable intertrochanteric femur fractures is associated with high levels of kinesiophobia. Fixation with PFNA is more advantageous in terms of functional results and kinesiophobia.. © 2021 NTMS.

Keywords: Intertrochanteric Fractures; Hemiarthroplasty; Kinesiophobia and PFNA; Pain..

1. Introduction

The rise in the elderly population increases the rate of intertrochanteric femur fractures (1). which have been reported to have high mortality and morbidity rates, reaching 38% in the first year (2). When these fractures are detected early and treated appropriately, mortality and morbidity can be minimized, and the rapid decrease

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in the quality of life can be prevented (3).
Various methods, such as bipolar hemiarthroplasty, total hip arthroplasty and various fixation methods (PFNA, DHS, Plate Screw Systems) are used to treat intertrochanteric femur fractures. Many factors, such as the patient's general medical status, patient profile, surgeon's experience, and preference, determine the treatment selection (4). Despite several studies, there is no consensus on the optimal treatment option in this patient group (4-7).
On the other hand, kinesiophobia is defined as ‘‘an excessive, irritational, and debilitating fear of physical movement and activity resulting from feeling of vulnerability to a painful injury or re-injury’’ (8). Kinesiophobia is a hot topic still under investigation. It is associated with success following surgery and affects the quality of life (9-17).
Kinesiophobia after intertrochanteric femoral fracture will make this difficult-to-treat condition even more complicated. This study aimed to compare fixation with PFNA and hemiarthroplasty, which are two essential methods used in the treatment of geriatric unstable intertrochanteric femur fractures, by performing functional and kinesiophobia analysis. Our hypothesis was that fixation with PFNA would cause less kinesiophobia compared to that with hemiarthroplasty.

2. Material and Methods

Patients in the 60-89 age range, who were admitted to our clinic between January 2017 and May 2019, operated due to intertrochanteric femur fracture, and underwent fixation with PFNA or hemiarthroplasty, were examined retrospectively in the study. The study was conducted in accordance with the principles of the declaration of Helsinki. Ethics committee approval was obtained from the local ethics committee for this study between 2019-2020 (2017-KAEK-189_2019.11.27_13). Data retrieval was done from the patient files using the records at the one-year follow-up. Written informed consent was obtained from every patient at the time of the operation. The mechanism of injury in all patients was a fall from the same level. The fracture classification was performed according to the AO classification (18). A total of 121 patients with AO 31A2 fractures were operated. Surgical interventions of all patients were performed by the same orthopedic surgeon.
Patients with pre-operative unassisted walking capacity, good nutritional status, BMI between 19-24, no post-operative complications, good cognitive status, no psychiatric disease, no pathological fracture were included.
Forty-nine patients, who had a neurological pathology that caused movement disorder (n=3), had a pathological (metastatic or primary tumoral lesions) fracture (n=25), were mentally disabled (n=9), had some psychiatric disease (n=8), and who had missing data in the patient file (n=2), no informed consent (n=2) were excluded from the study. Fixation with PFNA was performed in 40 patients (PFNA group) (26 patients 31A2-2, and 14 patients 31A2-3), while in 32 patients received hemiarthroplasty (hemiarthroplasty group) (17 patients 31A2-2 and 15 patients 31A2-3) (Figure 1). The patients’ median follow-up duration was 16 months for the PFNA group, and 14 months for the hemiarthroplasty group. All patients were mobilized within 48 hours postoperatively, with loads as they could tolerate.

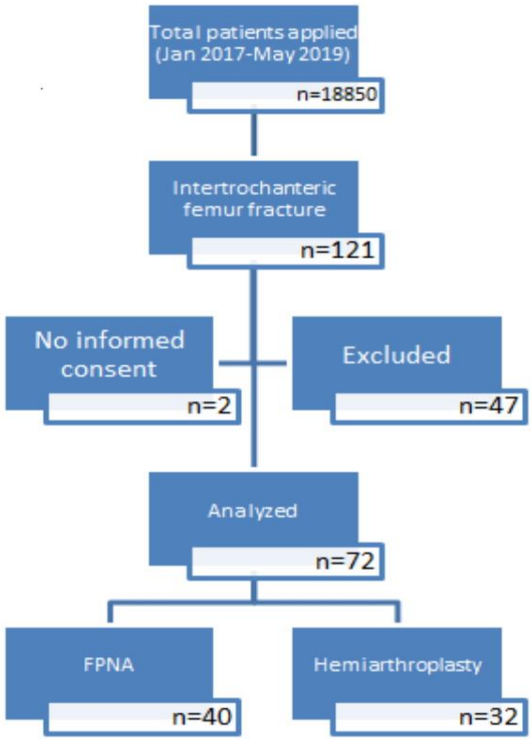

Figure 1: Study flow diagram.

Using the applet developed by RussLenth (http://homepage.divms.uiowa.edu/~rlenth/Power/), a post-hoc sample size calculation was done based on the primary outcome variable TSK. Given a common standard deviation of five, a two-tailed alpha error of 0.05, and a true difference of means of four (effect size=0.8), a sample size of 64 participants (32 PFNA + 32 hemiarthroplasty) would achieve a power of 88.3% to compare the two groups concerning mean TSK scores with the independent samples t-test.

2.1. Measurement Scales

Functional evaluation: The groups were assessed with the Harris Hip Score (HHS) regarding functional outcomes. HHS is a commonly used score for evaluating patients after surgery. The maximum score that can be received from the scale is 100. Less than 70 points are reported as poor results. The instrument asks questions about pain, function, deformity, and range of motion (19). Functional evaluation was performed in all patients in the first postoperative year.

Pain evaluation: Pain was assessed with the Numerical Rating Scale (NRS). NRS is a scale classified as zero (no pain at all) and ten (worst imaginable pain). Patients answer the tool based on the subjective pain they feel by choosing a score between zero and ten) (20).
Kinesiophobia evaluation: Patients were evaluated with the Tampa Scale (TSK) concerning kinesiophobia. TSK is a scale consisting of 17 self-response items. Each item is numbered from one to four. The minimum and maximum obtainable scores from the instrument are 17 and 68, respectively (8).

2.2. Surgical Technique

Regional anesthesia was used in all patients. In the PFNA group, the procedure was performed in the supine position on the fracture table. The fracture was reduced carefully, With an incision of approximately 5 cm from the superior of the trochanter major, the appropriate entry point was determined under the scope and nails were (Dyna Locking Trochanteric nail System, Uijeongbu Gyeonggi-do COREA) applied conventionally.
In the hemiarthroplasty group, a posterior intervention was performed by using an approximately 10-12 cm incision. the external rotator muscles were marked and the capsule was incised in a t-shape. TIPSAN (TIPSAN Co, Izmir, Turkey) prosthesis was used, and at the end of the operation, capsule and external rotator muscles were routinely sutured. An Hemovac drain was placed at the end of the procedure.

2.3. Statistical Analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) Version 22.0 (SPSS, Chicago, IL) statistical analysis software. The normal distribution of the numerical variables was assessed by the Kolmogorov–Smirnov test. Postoperative comparisons were performed by the Student t-test. A two-tailed p-value of <0.05 was considered significant. The Pearson correlation analysis was used to check for correlations between the NRS, HHS, and TSK scores.

3. Results

Data of 72 patients operated due to intertrochanteric femur fracture were analyzed. Fixation with PFNA was done in 40 patients (55.6%), while hemiarthroplasty was performed in 32 patients (44.4%). Forty patients were female (55.6%), and 32 were male (44.4%). There was no difference in terms of gender distribution between the groups. The patients' mean age was 75.2±7.7 years (range, 60-89) (95% CI 73.4-77.1). The mean age in the PFN group was 73.9 ±8.1. The mean age in the hemiarthroplasty group was 77.4±6.9. There was no significant difference between the groups in terms of ages (p=0.382). The median follow-up duration of the patients was 16 months (13-28 months) in the PFNA and 14 months (12-24 months) in the hemiarthroplasty group (p=0.621).
The mean TSK score was 49.6 ±5.5 (95% CI 48.3-50.9). The mean HHS was 87.8±4.1 (95% CI 86.9-88.8). While there was a significant difference concerning HHS and TSK scores, age and NRS scores were not significantly different between the groups (Table 1).
There was no correlation between age and TSK, NRS, or HHS scores (p=0.316). However, a significant negative correlation was observed between the HHS and TSK scores (r=-0.77, p<0.01) (Figure 2).

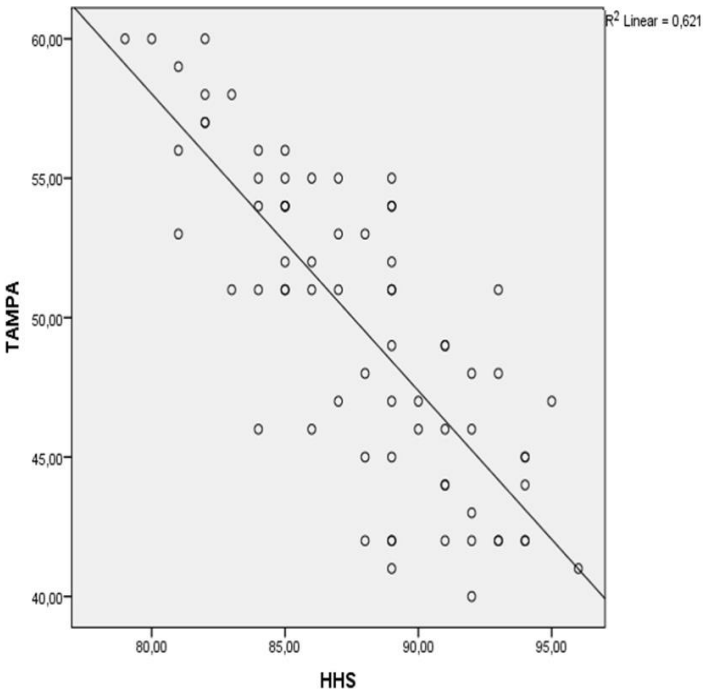

Figure 2: Correlations between HHS and TSK scores.

4. Discussion

This study confirmed that comparing PFNA and hemiarthroplasty in the treatment of unstable intertrochanteric fractures in elderly people, PFNA caused less kinesiophobia.
Fixation with PFNA and hemiarthroplasty are among the commonly used methods in treating geriatric unstable intertrochanteric femur fractures. Many studies compared these two methods from various aspects (21-26). In many studies, it has been stated that fixation with PFNA is superior regarding short operation durations, a little bleeding, and low surgical complications, while hemiarthroplasty is more advantageous in certain aspects such as early mobilization and being a safer method in unstable and osteoporotic fractures (5, 7).

Table 1: Demographic data of the patients.

	PFNA group (n=40)	95% CI	Hemiarthroplasty group (n=32)	95% CI	p*
Age (years)	73.9 ±8.1	71.8-75.6	77.4 ± 6.9.	74.8-80.3	0.382
HHS (score)	89.1 ±3.7	87.2-90.3	86.2 ±4.1	84.8-87.6	0.002
TSK (score)	47.9 ±4.9	46.4-49.5	51.7 ±5.7	49.6-53.4	0.004
NRS (score)	2.81 ±2.62	2.1-3.4	3.11 ±2.81	2.7-4.2	0.673

NRS: numerical rating scale; TSK: Tampa Scale; HHS: Harris Hip Score.

Many studies revealed that both approaches are beneficial treatment options in geriatric intertrochanteric femur fractures. Besides, there are no significant differences between them in terms of functional scores (24-26).

Kinesiophobia is a problem that generates fear of re-injury during an activity preventing movement (8). In geriatric unstable intertrochanteric femur fractures, early mobilization after treatment is vital in reducing mortality and morbidity. From this perspective, it is seen that kinesiophobia has a critical potential to affect the success of rehabilitation, and thus, morbidity and mortality in geriatric intertrochanteric femur fractures.

Using TSK scoring, Şengül et al. found that the pain scores and kinesiophobia were significantly higher in the osteoarthritis group compared to the hip-fracture group in patients receiving a total hip replacement. However, they also stated that they detected high kinesiophobia scores in the hip fracture group (27). As far as we know, there are no studies in the medical literature evaluating these two different surgical methods concerning kinesiophobia in geriatric unstable intertrochanteric femur fractures. In this context, we think that our research will shed light on the rehabilitation process and success of PFNA and hemiarthroplasty treatments applied in geriatric unstable intertrochanteric femur fractures.

Kristin et al. stated that 63% of the patients had moderate pain in the first year after major traumas (28). Pain control is an important factor that affects rehabilitation and treatment success. In our study, we did not determine a significant difference between the groups regarding pain scores. However, we discovered that the PFNA group was significantly superior concerning kinesiophobia. In this context, we can state that the significant difference in the TSK scores does not arise from pain.

When we examine the literature, there are many studies reporting that hemiarthroplasty is better concerning early movement and rehabilitation (21-22). In some studies, it was determined that HHS scores were substantially higher regarding hemiarthroplasty in the first 6 months in the PFNA and hemiarthroplasty groups. However, after the 6th month, no significant difference was observed between the two groups (5,7,22). Ozkayın et al. compared proximal femoral nails with hemiarthroplasty and showed higher functional outcomes in the PFNA group after 12 years of follow-up (29). When we looked at the functional outcomes in our study, we found significantly higher results in the PFNA group in mean HHS. We also found that the PFNA group was superior in TSK scores. In this context, it can be stated that PFNA is an enhanced treatment method in terms of patient satisfaction.

Even if fixation with PFNA does not provide a full load to the patient in the early period, kinesiophobia is less common in these patients because of less harm. This may explain that the HHS values in our study were higher in the PFNA group after a follow up of more than one year. For this reason, in our research, we found a negative correlation between HHS and TSK values. Also, although hemiarthroplasty allows for an early full load, possible blood loss, surgical fatigue, and muscle damage are higher in these patients (29-32).

In their study, Kristin et al. stated that kinesiophobia can cause psychosocial problems and depression (28). We can state that, kinesiophobia may cause psychological and social problems and affect the quality of life, a problem that should not be neglected. It can be predicted that having a higher level of kinesiophobia and fear of injury in the hemiarthroplasty group can cause psychological issues, lifestyle changes, and restrictions in the patients.

Although we collected our data prospectively, the long duration required to accumulate sufficient patients necessitated a retrospective file analysis, which can be mentioned as a limitation of the study. Besides, other variables such as the amount of blood loss and information about the follow-up status are missing. Hence, this study should be interpreted in light of these limitations. On the other side, it worth mentioning that this research is important in terms of being the first publication comparing these two common surgical methods in respect of kinesiophobia..

5. Conclusions

Based on our findings, it can be stated that fixation with PFNA and hemiarthroplasty are successful treatment methods in geriatric unstable intertrochanteric femur fractures. However, when evaluated regarding kinesiophobia, fixation with PFNA proves superior compared to a hemiarthroplasty. Future studies should investigate long-term outcomes of PNFA, including patient satisfaction in a larger series of cases.

Conflict of Interests
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Author Contributions
Öner K contributed to the conception and design of the study. Paksoy AE contributed to the collection of the data and statistical analysis and evulation of the results. Öner K and Paksoy AE created the manuscript. Öner K contributed to revising the work and final approval of the version.
Ethical Approval
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Il-6 Levels in Induced Sputum and Serum in Acute Attack and Stable Period of Chronic Obstructive Pulmonary Disease

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Abstract: COPD is a disease characterized by chronic inflammation in the airways and increased systemic inflammation. Interleukin-6 is a major immune and inflammatory mediator. To examine the level of IL-6, an inflammatory mediator, in induced sputum and serum of patients with COPD in the acute attack and stable period. Thirty consecutive patients diagnosed with COPD were included in the study. In the attack and stable period, IL-6 levels were studied in the induced sputum and blood taken simultaneously. Twenty-one patients who did not have any pathological findings in terms of chest diseases were selected as the control group. ELISA kit (Bender MedSystems, Vienna, Austria) was used for IL-6 measurements and values were measured as pg/ml. Mann-Whitney U test was used for intergroup comparisons and Wilcoxon Signed Ranks test was used for intragroup comparisons. The serum IL-6 level in the patient group was 6.66±7.49 pg/ml in the acute attack, while it was 3.08±4.07 pg/ml in the stable period. This decrease was found to be statistically significant ($p<0.005$). Sputum IL-6 level did not show a statistically significant change in acute attack (3.54±2.75 pg/ml) and stable period (3.44±6.03 pg/ml). In our study, there was a negative correlation between sputum IL-6 level in acute attack and COPD year (p: 0.02, r: -0.42). A positive correlation was found between acute attack serum IL-6 level (p:0.007,r:0.479) and stable period sputum IL-6 levels (p:0.017, r:0.429) and the number of acute attacks. The high serum IL-6 level in COPD attack shows that the immune response is not only in the respiratory system, but also systemically, and supports that COPD is a systemic inflammatory disease. In our study, we could not detect any contribution of IL-6 level in induced sputum. Studies involving larger numbers of cases and evaluating multiple markers are needed.
Keywords: Chronic Obstructive Pulmonary Disease; Induced Sputum; IL-6.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is an irreversible progressive lung disease and is among the leading causes of death and disability worldwide (1-3). COPD is a disease characterized by chronic inflammation in the airways and increased systemic inflammation. Although the cause of inflammation is not known exactly, it is thought that the toxic effects of smoking and inhalation of other harmful particles and

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gases and autoimmunity play a role in the development of inflammation (4-8). It has been reported that the number of exacerbations is high, especially in patients with moderate to severe COPD (9). The reason why exacerbations are more common in some patients with COPD is not yet known. The diagnosis of acute exacerbation in chronic obstructive pulmonary disease is made when clinically specific symptoms of acute attack occur and lung functions decrease (9,10). Several studies have shown that cytokines (such as IL-1, IL-6, γ-interferon) that control the acute phase response are elevated in serum and sputum during an acute attack of COPD (11,12). There are many studies showing that IL-6 is a major immune and inflammatory mediator (13-15).Induced sputum is a simple and non-invasive method that allows the material obtained from the lower respiratory tract to be examined for various purposes. Since Gibson et al. demonstrated that sputum induction is a reliable and valid method in 1989, it has been used to demonstrate inflammation in asthma and COPD (16). The aim of our study is to examine the level of IL-6, a major immune and inflammatory mediator, in induced sputum and serum of patients with COPD during acute attacks and in the stable period.

2. Material and Methods

2.1. Case selection

Thirty patients were selected consecutively from the outpatient or hospitalized patients who were diagnosed with COPD acute attack and started treatment at Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital between December 2003 and March 2004.Patients with COPD who recently had at least two of the complaints of increased shortness of breath, change in the amount and color of sputum, exacerbation of cough, and high fever were evaluated as COPD acute attack. The time elapsed sınce being diagnosed with COPD was defined as years of COPD. The PA chest radiographs of the patients included in the study did not have any radiological appearance suggestive of pneumonia and/or another disease. The patients were not given any antibiotic and steroid (inhaled or systemic) treatment until sputum induction was performed. After sputum induction, acute attack treatments were arranged according to the COPD stage of the patients. The patients were called again 6-8 weeks after the acute attack to collect stable period sputum; It was evaluated by physical examination, anamnesis and pulmonary function test. Sputum induction was performed again in patients who were accepted to be in a stable period and their acute attacks had passed. Twenty-one patients who did not have any pathological findings in terms of chest diseases were selected as the control group.

2.2. Sputum Induction

After measuring the initial FEV1 value of the cases, 3% hypertonic saline was inhaled with an ultrasonic nebulizer for 7 minutes. HicoUltrasonat 806 E (Germany) with an output of 0.5 ml/min and a particle size of 5 micrometers was used as ultrasonic nebulizer. Afterwards, the FEV1 value was measured again. The nose was closed, the mouth was rinsed with water, and sterile coarse sputum was extracted. FEV1 value; The process was repeated with an interval of 7 minutes until it fell more than 20%. Sputum obtained in this way was kept in the refrigerator for half an hour or for a maximum of two hours and examined in the microbiology laboratory of our hospital (17). The sputum was separated from the salivary part as macroscopically as possible and 0.1% Dithiothreitol equal to the measured amount was added to it (opens the disulfide bonds that bind the glycoprotein fibers and provide the gel form of the sputum, and mucolysis. Thus, homogenization of the sputum sample for cellular analysis) was added. It was incubated at 37 °C for 20 minutes and vortexed every 5 minutes. Then it was centrifuged for 10 minutes at 2000 rpm (ring perminute). A 3 cc sample was taken from the supernatant for biochemical analysis. After two preparations of the sediment were prepared for evaluation in the pathology laboratory, the remaining sediment was mixed with Dulbecco's phosphate buffersalin solution (D-PBS). Total cells were counted by Neubauerhemocytometry. Viability was evaluated by the trypanblueexclusion (0.4%) method. Blue-stained cells were considered dead, and unstained cells were considered live. If cell viability was less than 50% and squamous (epithelial) contamination was more than 20%, sputum was not included in the examination. If not; The cell suspension is prepared as 1x106/ml, 75 ml of cell suspension is placed in the centrifuge dish and left for 6 minutes. After centrifugation at 450 rpm, two smears were prepared. It was air-dried and stained with Wright's dye. Cell distribution was evaluated (400 cells) (18, 19). Cell counting, viability evaluation and evaluation of cell distribution were performed by a microbiologist. The supernatant portion of the sputum was stored at -70 °C for IL-6 to be studied until measurement

2.3. Study of IL-6 in Serum

Simultaneously with sputum induction, IL-6 in 5 cc blood serum taken from patients and control group was stored at -70 °C until measurement was made. ELISA kit (Bender MedSystems, Vienna, Austria) was used for IL-6 measurements and values were measured as pg/ml.

2.4. Statistical Analysis

SPSS 9.0 for Windows package program was used for statistical analysis. Mann-Whitney U test was used for intergroup comparisons and WilcoxonSignedRanks test was used for intragroup comparisons. Pearson correlation analysis was used for correlations. A P value of <0.005 was considered significant.

3. Results

Twelve (40%) of the 30 COPD patients included in the study were female, 18 (60%) were male, and of the 21

subjects in the control group, 10 (47.6%) were female and 11 (52.4%) were male. Biomass exposure was the only factor that varied between the patient and control groups (<0.005). No statistically significant difference was found between the patient and control groups in other parameters. The general characteristics of the groups are shown in Table 1.The mean duration of COPD of the patients was 8.83 ± 5.62 years. The mean annual number of acute attacks in the patient group was 3.53±1.61. When the patient group was compared within the acute and stable periods, it was found that the sedimentation rate was significantly higher in the acute attack. Apart from that, FEV1 in liter and percentage, total cell number in sputum, neutrophil and macrophage percentage were found to be statistically significantly higher in acute attack. In the patient group, serum IL-6 level was 6.66±7.49 pg/ml in acute attack and 3.08±4.07 pg/ml in stable period. This decrease was found to be statistically significant (p<0.005). Sputum IL-6 level did not show a statistically significant change in acute attack (3.54±2.75 pg/ml) and stable period (3.44±6.03 pg/ml) (Table3).When the patient and control groups are compared; induced sputum IL-6 levels in acute attack and stable period and serum IL-6 level in stable period did not show a significant difference with the control group. The only parameter that was significant for IL-6 level was that it was measured as high in the serum in acute attack.

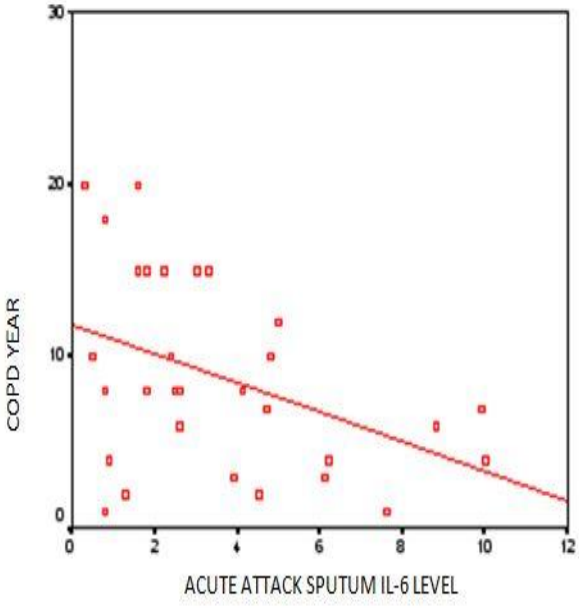

Figure 1: Relationship between acute attack sputum IL-6 level and COPD year.

These values are shown in Table 3.When the patients who had 2 or less attacks per year (10 cases-group1) and those who had 3 or more attacks (20 cases-group2) in the patient group, interestingly, the amount of sputum during the acute attack was found to be statistically significantly higher in group1 compared to group 2. Sputum IL-6 levels were found to be statistically significantly higher in group 2 compared to group 1. These values are shown in Table 4.

In our study, A positive correlation was observed between acute attack serum IL-6 level and number of attacks (p=0.007, r = 0.479). Similarly, a positive correlation was found between stable period sputum IL-6 level and number of attacks (p=0.017, r =0.429). İnterestingly, a negative correlation was observed between acute attack sputum IL-6 level and the year of COPD (p= 0.02, r= -0.42) (Figure 1-2).

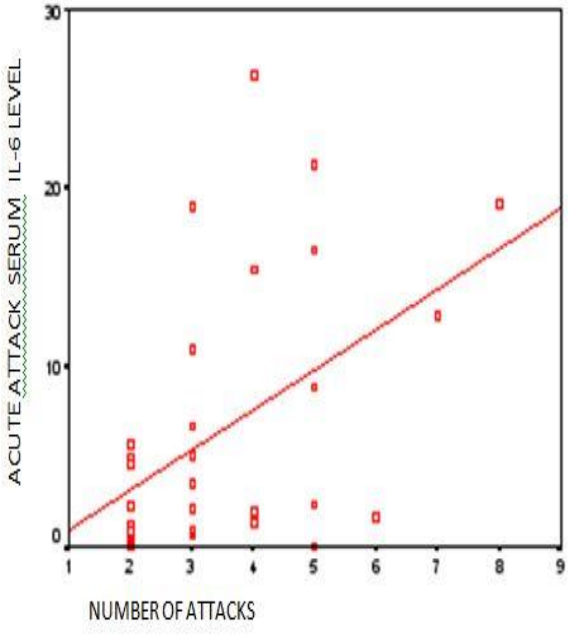

Figure 2: The relationship of serum IL-6 level with the number of attacks in acute attack.

4. Discussion

Induced sputum is a method used to show inflammation in the airways in COPD patients, allowing the study of mediators involved in the pathogenesis of COPD acute attack (20). In our study, induced sputum IL-6 level did not show a significant change in COPD attack and

stable period, while serum IL-6 level was found to be statistically significantly higher in acute attack compared to stable period. The attack serum IL-6 level and the sputum IL-6 level in the stable period were found to be statistically significantly higher.

Table 1: General characteristics of groups.

	Patient (N=30)	Control (N=21)	p
Female/male	12/18	10/11	>0.005
Mean age	61.13 ± 8.15	58.66 ± 10.01	>0.005
Smoker/Non-smoker	18/12	12/9	>0.005
Smoking (pack/year)	19.2 ± 19.92	15.47± 16.57	>0.005

Table 2: Sedimentation rate, FEV1, induced sputum cell count and neutrophil, macrophage percentages in the acute and stable period in the patient group.

	Attack	Stable Period	P
Sedimentation rate (mm/h)	35.4±24.71	16.83±16.16	<0.005
FEV_1 (lt)	1.33±0.28	1.56±0.32	<0.005
% FEV_1	52.9±11.24	61.21±9.33	<0.005
Induced sputum cell count (ml)	$30x10^6$±$35x10^6$	$13x10^6$±$25x10^6$	<0.005
Neutrophil(%)	84.53±16.60	70.47±25.71	<0.005
Macrophage (%)	15.47±16.60	29.53±25.71	<0.005

Table 3: Comparison of serum and induced sputum IL-6 levels of COPD (attack, stable period) cases and control group.

	Attack	Stable Period	Control Group	P (ANOVA)
Serum IL-6 (pg/ml)	6.66±7.49*&	3.08±4.07	1.24±1.41	< 0.005
Induced sputum IL-6 (pg/ml)	3.54±2.75	3.44±6.03	3.61±3.44	>0.005

*p< 0.005 Attack vs Stable, & p< 0.005Attack vs control.

Table 4: Comparison of induced sputum amount and IL-6 (serum/sputum) level according to the number of acute attacks.

	Group1 (n=10)	Group2 (n=20)	p
Sputum amount (cc) (during attack)	6.3±2.21	1.43±0.33	<0.005
Serum IL-6 level (pg/ml) (during attack)	2.2±2.18	7.97±10.14	<0.005
Sputum IL-6 level (pg/ml) (in stable period)	0.87±1.73	3.35±4.12	<0.005

In the study of Pizzichi et al. (21) on the total cell number and distribution in induced sputum in healthy adults, the total cell number in sputum was measured as 3.1x106/ml, and in the cell distribution, 62.9% macrophages, 24.1% neutrophils, 1.3% lymphocytes, 0.5% eosinophils were found. In the study of Peleman et al. (22) including 16 healthy adults and 21 patients with COPD, the total number of cells in induced sputum in patients with COPD was found to be statistically significantly higher than in the healthy adult group. Cell distribution in induced sputum in COPD was 74.9% neutrophils, 20.9% macrophages, 2.4% eosinophils, 0.6% lymphocytes, while in the control group it was measured as 22.5% neutrophils

and 74.0% macrophages. There was no significant change in the percentages of lymphocytes, eosinophils and epithelial cells in either group. As a result, they reported that there was a high number of neutrophils in the sputum of COPD patients, and macrophage dominance in the sputum in healthy adults. In the study of Bhowmik et al. (11) on the relationship between inflammatory markers in sputum in sputum in COPD acute attack and changes in lung functions, the total number of cells in the sputum of patients with COPD in acute attack was 2.86x106 cells/ml, while the cell distribution was 84% neutrophils, 14% macrophages, and 1% eosinophil was determined as 2.8% lymphocyte. In the stable periods of the same patients, the cell number decreased to 1.99x106 cells/ml, and the cell distributions were 81% neutrophils, 13% macrophages, 1% eosinophils, and 2% lymphocytes. According to this result, they reported that the total number of cells in the sputum was high in acute attack and that neutrophils were dominant in the sputum. In our study, when acute attack and stable periods were compared in COPD cases, the number of cells in induced sputum was 30×106±35×106 in the attack period, while it was 13×106±25×106 in the stable period, and the difference was statistically significant ($p<0.005$). When the acute attack and the stable period were compared, there was a statistically significant increase in the percentage of neutrophils in the attack (84.53±16.60% in the attack, 70.47±25.71% in the stable period) and a statistically significant decrease in the percentage of macrophages (15.47±16.60% in the attack, 29.53%±25.71% in the stable period) detected (Table 2).

In addition to cytokines such as TNF-α, IL-8 and LTB4 responsible for the pathogenesis of COPD, IL-6 is also an inflammatory mediator. Interleukin-6 (IL-6); It is a glycoprotein cytokine consisting of 184 amino acids located on the 7th chromosome. IL-6; It affects B and T cells monocytes, hematopoietic system stem cells and hepatocytes. As a result, induction, differentiation and immunoglobulin production in B cells and T lymphocytes occurs, macrophages are activated, hematopoiesis and thrombopoiesis are induced, regulation of the acute phase response is achieved. Increased airway obstruction during acute exacerbations of the disease is held responsible for submucosal edema and mucus production (13-15, 23). In the study of Bhowmik et al. (11) in which they investigated the relationship between IL-6 and IL-8 levels in induced sputum in the acute and stable period and the frequency of attacks in moderate and severe COPD cases; IL-6 level in induced sputum was found to be significantly higher in acute attack (122.7pg/ml) compared to stable period (64.0pg/ml; $p<0.05$). In the same study, induced sputum IL-6 level was 22pg/ml in those who had 2 attacks per year, while this level was 101pg/ml in those who had 3 or more attacks per year.

In our study, however, no significant difference was found in the induced sputum IL-6 level in patients with COPD during acute attack and stable periods. When the cases were re-evaluated according to the annual number of attacks, no statistically significant difference was found in sputum IL-6 levels between the groups. However, the amount of sputum and serum IL-6 level during the attack were found to be statistically significantly higher in patients who had 3 or more attacks compared to the group that had 2 attacks per year. In our study, we may attribute the lack of difference in sputum IL-6 levels according to the number of attacks, due to the low number of cases, and the lack of equal distribution of patients between the groups. Another hypothesis is that the high annual number of acute attacks of the patients in our study may explain the high level of induced sputum IL-6 in the stable period as well as in the acute attack. This shows us that IL-6 level is increased in the airways of those who have frequent acute attacks and that this increase in cytokine level also plays a role in the continuation of inflammation. Wang et al. (24) found no statistical difference in induced sputum IL-6 levels of all three groups in their study on smokers and nonsmokers with healthy adults and patients with stable COPD. In our study, no statistical difference was found when the induced sputum IL-6 levels of the control group were compared with the induced sputum IL-6 levels of the patients with COPD in the acute attack and stable period (Table 3).

In the study of Wedzicha et al. (25), a total of 120 attacks were detected in 93 patients with COPD who were followed up for 1 year. While the acute attack serum IL-6 levels of the cases were 4.3pg/ml, it was measured as 1.10pg/ml in the stable period ($p=0.008$). In the study, it was found that the increased serum IL-6 level in acute attack also caused an increase in fibrinogen; It has been shown that this increase in fibrinogen level increases the tendency to cardiovascular diseases and cerebrovascular events. Similarly, in our study, while serum IL-6 level was 6.69±7.49 pg/ml in acute attack, it showed a statistically significant decrease in stable period and was measured as 3.08±4.07 pg/ml. We can attribute this result to the fact that IL-6 is a major immunomodulator that rapidly rises in response to infection and initiates the acute phase response. Acute attack serum IL-6 level was also found to be significantly higher when compared to the control group (Table 3). The cause of acute exacerbation in chronic obstructive pulmonary disease is usually intercurrent infections. In our study, we found a statistically significant positive correlation (r= r:0.479) between the number of acute attacks and the serum IL-6 level of attacks in patients with COPD. In the UPLIFT study, it was shown that the clinical course of the disease worsened in patients who had frequent attacks. According to this result, the clinical

course of the patients is adversely affected because the inflammation is continuous in those who have frequent attacks (26).

5. Conclusions

The high serum IL-6 level in COPD attack shows that the immune response is not only in the respiratory system, but also systemically, and supports that COPD is a systemic inflammatory disease. In our study, we could not detect any contribution of IL-6 level in induced sputum. Studies involving larger numbers of cases and evaluating multiple markers are needed.

Conflict of Interests

No potential conflicts of interest relevant to this article were reported.

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Author Contributions

Hypothesis for research; FU, Planning the design, patient follow up: MÖA, Analysisof data, literature revewand final approval of the version; FU and MÖA

Ethical Approval

Informed consent form was obtained from the participants.

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The Role of Trace Elements in Thyroid Cancers

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Abstract: Thyroid cancer is one of the most common endocrine cancers. It is known that excessive or insufficient intake of trace elements causes many diseases, including various types of cancer. This study evaluates the element concentration in thyroid tissue, nodule and serum of thyroid cancer patients. The study was conducted on 60 participants, 21 malignant and 39 benign. In thyroid tissue, nodule and serum samples obtained from malignant and benign individuals, copper (Cu), zinc (Zn), aluminum (Al), cobalt (Co), iron (Fe), nickel (Ni), silver (Ag), chromium (Cr), selenium (Se), cadmium (Cd), manganese (Mn), arsenic (As) and lead (Pb) were evaluated using ICP-MS. Nodule Pb level in the malignant group was found to be significantly higher than that of the nodule Pb in the benign group. In addition, as a result of the evaluation between nodule and tissue in the malignant and benign groups, Al and Mn were higher in the malignant group than in the nodule in the thyroid tissue; Ni, Cu and Se were found to be significantly lower. In addition, Al was higher in the benign group than the nodule in the thyroid tissue, while Ni was considerably lower ($p < 0.05$). All these results suggest that trace elements have profound roles in the etiology of thyroid cancer. © 2021 NTMS.

Keywords: Thyroid Cancer; Trace Element; Inductively Coupled Plasma-Mass Spectrometry.

1. Introduction

Although most of them are benign, thyroid gland carcinomas are the most common endocrine system malignancies. The malignancy rate in thyroid nodules is approximately 5% (1-3).

In recent years, trace element analysis has gained importance as the functions of trace elements in different fields have been found out. While the deficiency of essential trace elements causes various

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diseases, the presence of excessive amounts has a toxic effect. Again, the effects of trace elements on the human body and metabolism have determined of trace elements even more critical. For these reasons, trace element analyzes are carried out in many areas, and studies in this area continue.

Although trace elements are very low, they are directly or indirectly involved in many vital functions such as vitamin synthesis, hormone production, and cell respiration (4).

In biological systems, trace elements act as enzyme components or as catalysts in chemical reactions in cells. For this reason, it is known that excessive or insufficient intake of many elements causes many diseases, including various types of cancer. Among these metals, there are studies with the element iron (Fe) in terms of its biological functions in cancerous and normal cells in cancer biochemistry studies. The recent studies are related to the Fe element is carcinogenic due to its catalytic effect on hydroxyl radical formation, suppression of defense cells and triggering of cancer cell proliferation (5). Another study reported that blood selenium (Se) levels in some cancer patients were lower than in healthy individuals (6, 7).

Copper (Cu) and zinc (Zn) play an essential role in various biochemical reactions of the human organism. These metals are cofactors of the superoxide dismutase enzyme and significantly inhibit the initiation and progression of tumors through cell protection against substances that cause the formation of free radicals. In addition, copper takes place in this structure as a cofactor of DNA polymerase and RNA polymerase enzyme. Zinc and copper concentration ratios have been studied many times in different tumor tissues and various body fluids of different patients (8, 9).

All studies show us that element concentration should be evaluated in cancer patients. This study aimed to investigate serum, tissue, and nodule levels of elements such as Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Pb in benign and malignant nodular thyroid diseases.

2. Material and Methods

Sixty patients who applied to Atatürk University Faculty of Medicine Research Hospital with the suspicion of thyroid cancer were included in this study. As a result of the pathological examinations of the patients participating in our study, 21 were found to be malignant and 39 to be benign. Approximately 3 mL of venous blood samples were taken from each patient and transferred to gel biochemistry tubes. The blood samples were waited for 30 minutes at the room temperature and then serum samples were separated by centrifugation at 3,500 rpm for 10 minutes. The thyroid tissues and serum samples taken from the same patients were stored in a deep freezer at -80 °C until the day of elemental analysis.

All tissue and serum samples were properly thawed on the study day. Element levels were pretreated to be measured with an ICP-MS device. The working principle of this device is that the samples in solution are sent to the ionization unit with argon (Ar) gas, and the atoms that ionized at high temperature are separated and detected in the mass spectrometer[10].

For this purpose, the samples were first grinded in the microwave oven (advanced microwave digestion system). For each sample; 0.1 ml of serum samples were taken and 2 ml of 65% HNO_3 was added. Then, 0.25 ml of 30% H_2O_2 was added. For tissue samples; After adding 2 ml of 65% HNO_3 and 0.5 ml of 30% H_2O_2 on 0.1 g tissue, it was waited for 15-20 minutes and then burned in a microwave oven at 180 ^{0}C for 20 minutes.

Standard solutions for the elements to be analyzed (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Pb) were prepared using 2% nitric acid solution at increasing concentrations. Calibration curves were drawn. Indium, Scandium, Germanium and Bismuth were used as internal standards to correct for deviations in the calibration curve during analysis. The milled samples diluted 1/10 times were subjected to elemental analysis in an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7700).

2.1. Statistical analysis

Software SPSS version 21.0 (SPSS for Windows software; SPSS Inc., Chicago, IL, USA) package program was used for statistical analysis in the study. Kolmogorov-Smirnov test was applied to determine the homogeneity of the data. Mann Whitney-U test was performed because the data were not normal. Numerical variables were expressed as Median (Min - Max). The minimum criterion for statistical significance was $p<0.05$ for all comparisons.

3. Results

In table 1, nodule, tissue and serum element levels were compared in malignant and benign groups. According to the data in Table 1, it was observed that the level of nodule Pb in the malignant group was significantly higher than that of the nodule Pb in the benign group ($p<0.05$).

In table 2, the concentrations of all trace elements in the nodules and tissues of all patients were compared and it was determined that the Al element was significantly higher in the tissue compared to the nodule, and the Ni element was significantly lower in the tissue compared to the nodule ($p<0.05$).

Finally, based on the results of the evaluation of nodule and tissue element levels between the malignant and benign groups in Table 3 the Al and Mn element levels in the thyroid tissue in the malignant group were significantly higher than the nodule; Ni, Cu and Se element levels are observed to be significantly lower.

Table 1: Trace element levels of nodule, tissue and serum in malignant and benign groups.

Variables		Groups		
		MG	BG	P
		Median (Min-Max) (N:21)	Median (Min-Max) (N:39)	value
Al	Nodule(ug/g)	0.34 (0-2.07)	0.27 (0-2.05)	0.128
	Tissue(ug/g)	28.32 (0-96.47)	35.5 (0-92.45)	0.280
	Serum(ug/dL)	4.97 (1.18-7.66)	3.6 (1.48-7.8)	0.333
Cr	Nodule(ug/g)	8.3 (0.1-21.9)	8.3 (0.1-29.52)	0.805
	Tissue(ug/g)	7.1 (0.03-27.02)	7.8 (0.05-29.89)	0.595
	Serum(ug/dL)	0.82 (0.49-2.07)	0.98 (0.59-5.7)	0.056
Mn	Nodule(ug/g)	19.5 (0.05-118.22)	15.99 (0.24-1286.46)	0.467
	Tissue(ug/g)	22.22 (0-80.07)	30.74 (0.10-195.37)	0.140
	Serum(ug/dL)	0.07 (0-0.60)	0.15 (0-1.44)	0.062
Fe	Nodule(ug/g)	7824.26 (251-37188.72)	8345.65 (250-29725.12)	0.245
	Tissue(ug/g)	6269.43 (100.08-21467.45)	10360.03 (200.22-29523.42)	0.156
	Serum(ug/dL)	79.35 (7.98-201.28)	84.68 (17.37-301.95)	0.805
Co	Nodule(ug/g)	1.15 (0-8.4)	1.19 (0-5.68)	0.453
	Tissue(ug/g)	1.26 (0-3.09)	1.47 (0-6.27)	0.142
	Serum(ug/dL)	0 (0-0.33)	0.002 (0-0.112)	0.403
Ni	Nodule(ug/g)	10.73 (1.7-42.02)	10.60 (1.95-33.53)	0.793
	Tissue(ug/g)	7.55 (1.16-19.38)	7.22 (1.53-20.75)	0.934
	Serum(ug/dL)	0 (0-0)	0 (0-0)	1.000
Cu	Nodule(ug/g)	73.84 (0.21-304.03)	76.83 (0.13-259.18)	0.251
	Tissue(ug/g)	61.60 (1.28-222.45)	82.90 (1.49-354.93)	0.227
	Serum(ug/dL)	26.83 (15.32-38.23)	25.08 (11.22-37.86)	0.505
Zn	Nodule(ug/g)	2049.63 (60-15689)	2466.57 (59-13877.86)	0.725
	Tissue(ug/g)	1735.58 (500-6488.35)	2747.26 (353.26-16059.23)	0.096
	Serum(ug/dL)	52.78 (30.08-109.76)	53.25 (20-86.68)	0.680
As	Nodule(ug/g)	0.56 (0-3.54)	0.44 (0-1.34)	0.187
	Tissue(ug/g)	0.56 (0-1.86)	0.59 (0-2.15)	1.000
	Serum(ug/dL)	0.079 (0.056-0.142)	0.106 (0.056-0.128)	0.117
Se	Nodule(ug/g)	63.95 (0.36-453.7)	39.10 (0.29-293.18)	0.233
	Tissue(ug/g)	46.19 (0.27-166.74)	58.48 (5.47-483.95)	0.165
	Serum(ug/dL)	1.57 (0.83-2.42)	1.64 (0.78-2.42)	0.658
Ag	Nodule(ug/g)	0.33 (0.203-0.74)	0.28 (0.66-1.13)	0.258
	Tissue(ug/g)	0.268 (0-0.48)	0.29 (0.03-0.66)	0.680
	Serum(ug/dL)	0.135 (0.081-0.504)	0.129 (0.069-0.721)	0.505
Cd	Nodule(ug/g)	24.20 (0.004-197.34)	17.24 (0.008-161.98)	0.431
	Tissue(ug/g)	25.34 (0.01-107.88)	35.50 (0.08-196.79)	0.277
	Serum(ug/dL)	0.001 (0-0.035)	0.00 (0-0.038)	0.362
Pb	Nodule(ug/g)	5.4 (0-9.4)	4.3 (0-9.44)	0.036*
	Tissue(ug/g)	4.7 (0-7.8)	5.24 (0.02-33)	0.066
	Serum(ug/dL)	0.35 (0-2.81)	0.19 (0-3.76)	0.605

MG: Malign Group, BG: Benign Group. * $p<0.05$(statistically significant).

Table 2: Comparison of trace element levels in all nodules and tissues.

	Groups		
Variables	**Tissue(ug/g)** Median (Min-Max) (N:60)	**Nodule(ug/g)** Median (Min-Max) (N:60)	P value
Al	32.98 (0-96.47)	0.28 (0-2.07)	0.000*
Cr	7.63 (0.03-29.89)	8.39 (0.92-29.53)	0.694
Mn	23.82 (0-195.36)	17.63 (0.06-1286.47)	0.492
Fe	9490.25 (100.08-29523.42)	8047.67 (250-37188.72)	0.741
Co	1.31 (0-6.27)	1.17 (0-8.42)	0.721
Ni	7.54 (1.16-20.74)	10.67 (1.76-42.02)	0.000*
Cu	79.31 (1.28-354.93)	76.25 (0.13-304.03)	0.821
Zn	2068.95 (353.26-16059.23)	2339.82 (59-15689.01)	0.916
As	0.58 (0-2.15)	0.49 (0-3.54)	0.416
Se	48.19 (0.27-483.93)	46.67 (0.29-453.7)	0.777
Ag	0.28 (0-0.65)	0.31 (0.07-1.13)	0.287
Cd	27.29 (0.01-196.79)	19.63 (0.004-197.34)	0.817
Pb	4.98 (0-33)	4.99 (0-9.45)	0.960

* p<0.05 (statistically significant).

Table 3: The results of trace element evaluation of nodule and tissue in malignant and benign groups.

Variables		**Groups**			
		MG Median (Min-Max) (N:21)	P value	**BG** Median (Min-Max) (N:39)	P value
Al	Nodule(ug/g)	0.34 (0-2.07)		0.27 (0-2.05)	
	Tissue(ug/g)	28.32 (0-96.47)	0.00*	35.5 (0-92.45)	0.000*
Cr	Nodule(ug/g)	8.3 (0.1-21.9)		8.3 (0.1-29.52)	
	Tissue(ug/g)	7.1 (0.03-27.02)	0.720	7.8 (0.05-29.89)	0.738
Mn	Nodule(ug/g)	19.5 (0.05-118.22)		15.99 (0.24-1286.46)	
	Tissue(ug/g)	22.22 (0-80.07)	0.001*	30.74 (0.10-195.37)	0.137
Fe	Nodule(ug/g)	7824.26 (251-37188.72)		8345.65 (250-29725.12)	
	Tissue(ug/g)	6269.43 (100.08-21467.45)	0.128	10360.03 (200.22-29523.42)	0.601
Co	Nodule(ug/g)	1.15 (0-8.4)		1.19 (0-5.68)	
	Tissue(ug/g)	1.26 (0-3.09)	0.218	1.47 (0-6.27)	0.499
Ni	Nodule(ug/g)	10.73 (1.7-42.02)		10.60 (1.95-33.53)	
	Tissue(ug/g)	7.55 (1.16-19.38)	0.000*	7.22 (1.53-20.75)	0.008*
Cu	Nodule(ug/g)	73.84 (0.21-304.03)		76.83 (0.13-259.18)	
	Tissue(ug/g)	61.60 (1.28-222.45)	0.015*	82.90 (1.49-354.93)	0.175
Zn	Nodule(ug/g)	2049.63 (60-15689)		2466.57 (59-13877.86)	
	Tissue(ug/g)	1735.58 (500-6488.35)	0.059	2747.26 (353.26-16059.23)	0.171
As	Nodule(ug/g)	0.56 (0-3.54)		0.44 (0-1.34)	
	Tissue(ug/g)	0.56 (0-1.86)	0.177	0.59 (0-2.15)	0.967
Se	Nodule(ug/g)	63.95 (0.36-453.7)		39.10 (0.29-293.18)	
	Tissue(ug/g)	46.19 (0.27-166.74)	0.017*	58.48 (5.47-483.95)	0.171
Ag	Nodule(ug/g)	0.33 (0.203-0.74)		0.28 (0.66-1.13)	
	Tissue(ug/g)	0.268 (0-0.48)	0.165	0.29 (0.03-0.66)	0.801
Cd	Nodule(ug/g)	24.20 (0.004-197.34)		17.24 (0.008-161.98)	
	Tissue(ug/g)	25.34 (0.01-107.88)	0.491	35.50 (0.08-196.79)	0.765
Pb	Nodule(ug/g)	5.4 (0-9.4)		4.3 (0-9.44)	
	Tissue(ug/g)	4.7 (0-7.8)	0.662	55.24 (0.02-33)	0.760

MG: Malign Group, BG: Benign Group.* p<0.05 (statistically significant).

In the benign group, the tissue Al level was higher than the nodule, and the tissue Ni level was significantly lower ($p<0.05$). No significant difference was detected in other elements.

4. Discussion

In our study, the concentrations of elements such as Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Pb in the serum, tissues and nodules of patients with thyroid cancer were determined and the relationships between malignant and benign tumors were investigated.

After understanding the various functions of trace elements in the organism, the deficiencies of these elements may be associated with cardiovascular system diseases, neurodegenerative diseases, carcinogenesis, and even the advisability of determining metals in the diagnosis of these pathological conditions has been confirmed in many studies in recent years (11).

The use of Pb in industry, the exploitation of natural resources and its use as a pesticide in agriculture cause environmental and food pollution. The hematopoietic system is the first to be affected by lead (12). Our study observed that Pb malign nodule levels increased significantly compared to benign nodule levels ($p<0.05$). Again, in the measurement we made in the serum samples of patients with malignant tumors, the Pb level was found to be higher than patients with benign tumors, although it was not statistically significant.

Copper is a trace element that plays a role in the biological oxidation and reduction reaction (13). Recent studies have shown that copper plays a role in both the etiology and growth of the tumor (14). In our study, Cu levels in the malignant patient group's tissues and nodules were lower than in the benign patient group. However, this decrease was not statistically significant ($p>0.05$).

In the studies carried out by Rink and Gabriel in 2000-2001, they stated that Zinc (Zn) is an essential element for normal immune system function and in the absence of Zn, function loss is seen in all immune cell types. Therefore, Zn deficiency has been shown to be effective in cancerization by causing immune dysfunction and increasing copper (Cu) absorption. In studies comparing malignant prostate tissue and normal prostate tissue, it was observed that the Zn level was 60-70% lower in malignant prostate tissue[15]. In our study, Zn levels in the serum, tissue and nodules of the malignant patient group were found to be lower than the benign patient group ($p>0.05$).

Cd activates genotoxic mechanisms such as single-strand DNA breakage and causes DNA repair inhibition (16, 17). In addition, Cd activates the protooncogene and inhibits apoptosis. Thus, it causes cancer (18). Studies on various types of cancer have shown significant relationships between cancer and Cd levels in a human who have been occupationally exposed to Cd (19). In the study of Yaman et al. on tissue samples, the Cd concentration was found to be high in malignant and benign prostate tissue, and no statistical difference and significance could be found between them (20). In our study, malignant Cd nodule was found higher than benign Cd. However, no statistical difference or significance was found between them ($p>0.05$).

The studies related to Se, relationships between cancer and Se were first identified, and it was suggested that high doses of Se are effective in the treatment of hematological tumors. However, in later studies, it was reported that high doses of Se caused cirrhosis and hepatocellular tumors in laboratory animals. The view that there is a relationship between cancer and Se was found to be significant when it was understood that Se protects macromolecules from oxidation stress and is a component of glutathione (21). In our study, Se was found to be higher in malignant nodules compared to benign nodules, but this elevation was not statistically significant. Nodule Se levels were found to be significantly higher than tissue Se levels in our malignant groups ($p<0.05$).

Msteo et al. reported that exposure to Ni might play a role in developing colon cancer by causing changes in the catalase enzyme system and antioxidant mechanisms (22). Our current study found nodule Ni levels to be significantly higher than tissue Ni levels in our malignant groups ($p<0.05$). Serum Ni levels could not be detected.

When the literature was examined, no study was found showing the trace element profile of patients with malignant and benign thyroid tumors. Finally, Al and Mn elements were significantly higher in malignant tissue than in malignant nodules; Ni, Cu and Se element levels are observed to be significantly lower in malignant tissue than in malignant nodules. The level of Al in benign tissue was higher than the benign nodule, and the level of Ni in benign tissue was found to be significantly lower than in the benign nodule.

5. Conclusions

The reported results may highlight the role of trace elements in the unexplained etiology of thyroid diseases. In addition, biochemical changes of trace elements on malignant and benign thyroid tumors can form a basis for diagnosis. We think that the trace elements will give more valuable results for the clinic by including control tissue groups from healthy individuals in the study.

Conflict of Interests

The authors approved that there is no conflict of interest.

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Author Contributions

Turkeri ON, Kurt N, Ozgeris FB and Bakan N contributed to the constructing the idea for research. Turkeri ON, Kurt N, Ozgeris FB and Bakan N

contributed to the planning the design of the work. Turkeri ON, Kurt N, Gul MA, Karadeniz E, and Akcay MN contributed to the execution of the experiments. Turkeri ON, Kurt N, Gul MA contributed to the analysis and interpretation of data. Yeni M, Karadeniz E, and Acay MN contributed to the biological materials. Turkeri ON, Kurt N, Gul MA contributed to the literature review. Turkeri ON, Kurt N, Yeni M, Ozgeris FB, Karadeniz E, and Akcay MN contributed to the critical review. Turkeri ON, Kurt N, Yeni M, Bakan N,, Karadeniz E and Akcay MN and contributed to the final approval of the version to be published.

Ethical Approval

This study was approved by the Atatürk University Faculty of Medicine Clinical Research Ethics Committee. (Dated 04.04.2016 and Number 3, Decision No. 02).

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