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Our dear readers,

We are happy to publish the third issue of our journal for 2022 with 40 articles. In this period when the COVID-19 pandemic has lost its power, we agree that we should devote a significant part of our strength to scientific articles. As we mentioned before, we want to contribute to international literature at an increasing level and to increase the success bar of our journal by entering valuable international indexes. I would like to thank all authors for submitting articles contributing to both domestic and international literature with their comprehensive scientific content for publication in our journal. We hope that this issue will be useful to our readers.

Sincerely yours

Assoc. Prof. Alpaslan TANOĞLU, MD
Editor-in-Chief

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Original Article

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MR imaging characteristics of Morel-Lavallee lesions in pediatric patients

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ABSTRACT

Aim: Morel-Lavallée syndrome (MLS) is a serious posttraumatic soft tissue injury in which the subcutaneous tissues are separated from the underlying fascia by glove-finger (closed type) peeling and replaced by a cavity filled with hematoma and fat. It is most commonly seen in the trochanter major, but it can also be found in the flank, hip, and lumbodorsal regions. The goal of this study is to define the typical findings of MLS in order to avoid misdiagnosis and delay in patient treatment.

Material and Method: This retrospective study was approved by the Institutional Review Board. Informed consent was waived due to the retrospective nature of the study. Between 2015 and 2021, MR images and clinical follow-ups of 22 pediatric patients with clinical and radiological Morel-Lavallée lesions (MLL) were reviewed retrospectively. All patients were evaluated using 1.5T or 3T power MR devices (Siemens Healthineers, Erlangen, Germany).

Results: Of 22 patients diagnosed with MLS, 77% (n=17) were male and 23% (n=5) were female. Patients ranged in age from 7 to 18 years, with a mean of 13.2 years (+/-2,3). The locations of MLL were knee (77%, n=17, 15 patients had anterior knee and 2 patients had posterior knee involvement), hip (14%, n=3) and thigh (9%, n=2) in order of frequency. These lesions all had a similar ovoid shape. The majority of patients (18/22) received solely conservative management but three patients underwent percutaneous drainage.

Conclusion: In our study, the importance of differential diagnosis of MLL from traumatic collections and the importance of MRI findings in diagnosis and treatment were discussed. Accurate diagnosis and treatment of MLL are critical, as the lesion's size may increase as a result of delayed treatment, causing skin necrosis and denervation due to the mass effect.

Keywords: Morel-lavallée, MRI, traumatic collection

INTRODUCTION

Morel-Lavallée syndrome (MLS) is a serious posttraumatic soft tissue injury in which the subcutaneous tissues are separated from the underlying fascia by glove-finger (closed type) peeling and replaced by a cavity filled with hematoma and fat. It is most commonly seen in the trochanter major, but it can also be found in the flank, hip, and lumbodorsal regions (1).

MLS is a trochanter major lesion first described in 1863 by Victor-Auguste-François Morel-Lavellée (2). It is the preferred term for all degloving injuries, including many bone spurs (3-5). Pathological examination of MLS reveals fat lobules within the lesion, which aids in distinguishing MLS from traumatic pathologies such as hematoma and bursitis (3,6).

MLS reduces lymphatic circulation and causes blood vessel damage. The formed cavity collects blood and lymphatic fluid. These fluids' metabolic and inflammatory content increases cellular permeability. As a result, the leakage from the vessels and lymphatics into the formed space becomes more pronounced. This cycle is thought to be responsible for the lesions' continuous growth and development. Because MLS lesions are rare and their diagnosis may be delayed due to trauma history, approximately half of the patients' diagnoses are missed (6,7).

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The goal of this study is to define the typical findings of MLS in order to avoid misdiagnosis and delay in patient treatment.

MATERIAL AND METHOD

This retrospective study was approved by the Erzincan Binali Yıldırım University Clinical Researches Ethics Committee (Date: 25.01.2021, Decision No: 02/21). Informed consent was waived due to the retrospective nature of the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Between 2015 and 2021, magnetic resonance imaging (MRI) and clinical follow-ups of 22 pediatric patients with clinical and radiological Morel-lavallée lesions(MLL) were reviewed retrospectively.

All patients were evaluated using 1.5T or 3T power MR devices (Siemens Healthineers, Erlangen, Germany). Although the modalities used in each patient differed, all patients received fat-suppressed and non-fat-suppressed sequences. Fat-suppressed axial proton density, non-fat-suppressed coronal T1 spin echo (SE), fat-suppressed proton density (PD) turbo spin echo (TSE), fat-suppressed sagittal T2 TSE and PD sequences are available in the standard knee imaging protocol.

A 5-year-experienced pediatric radiologist and a 3-year-experienced radiologist examined subcutaneous collections for location, shape, and signal properties such as the presence of fat, blood products, septations, and thick capsules.

Internal fat droplet presence in the collection was used as a diagnostic criterion for MLL. Furthermore, it was determined which patients required drainage treatment and which MRI findings aided in determining the treatment plan.

Statistical analysis: The data was analyzed using the SPSS package for social sciences (version 20) for Windows (IBM SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to determine whether the data had a normal distribution. Numerical variables with a normal distribution are reported as mean±standard deviation, while variables with a non-normal distribution are reported as medians with minimum and maximum values. Numbers and percentages are used to report categorical variables. The difference of the variables between conservative and percutaneous treatment subgroups was defined via Chi-square test.

A two-tailed P-value of <0.05 was considered significant.

RESULTS

Of 22 patients diagnosed with MLL, 77% (n=17) were male and 23% (n=5) were female. Patients ranged in age from 7 to 18 years, with a mean of 13.2 years (+/-2,3).

The locations of MLL were knee (77%, n=17, 15 patients had anterior knee and 2 patients had posterior knee involvement)(**Figure 1**), hip (14%, n=3) and thigh (**Figure 2**) (9%, n=2) in order of frequency. These lesions all had a similar ovoid shape. Twenteen (55%) had septation, 8 of them had one and 4 of them had multiple septations. Six (27%) had internal blood products and percutaneous drainage was performed in 3 of these patients. 2 (9%) had thick capsule (>2mm) both of them was treated by percutaneous drainage.

The majority of patients (18/22) received solely conservative management but three patients underwent percutaneous drainage. All of the patients who received treatment were over 11 years old.

Older age (over 11 years), the presence of thick capsule and hemorrhagic content of subcutaneous collection were risk factors for treatment and were statistically significant (p<0.05).

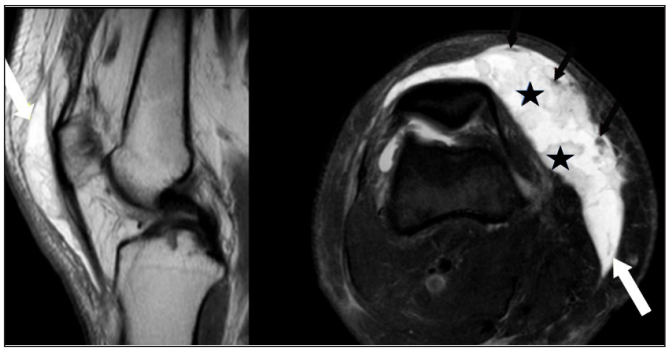

Figure 1. 15 year old boy had post traumatic pain and swelling at the knee. Sagittal proton density without fat saturation demonstrate a fluid containing lesion over the medial retinaculum, with a thin tail extending superficially over the patella (white arrows) with hemorrhagic content (asterix) and fat droplets (black arrows)

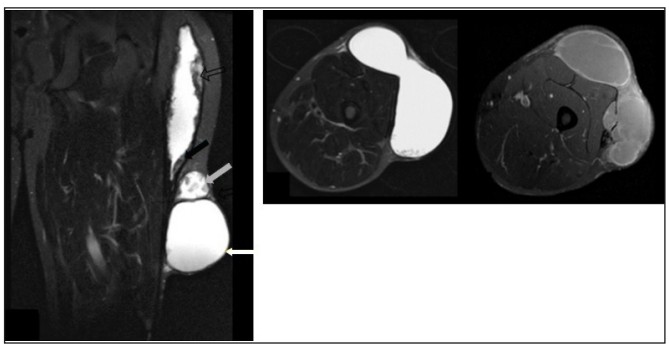

Figure 2. 13-year-old boy with swelling and pain at the level of the thigh. Coronal and axial T2 with fat saturation and contrast enhanced T1 with fat saturation demonstrate a large fluid collection containing a fluid-fluid level (white arrow), dark T2 fat droplet (grey arrow) and septations (open arrows) with a thicker capsule (black arrow).

DISCUSSION

Morel-Lavallée is a closed degloving injury, which results from shearing injury that separates the subcutaneous layers from the fascia and it represents a potential space. Then, hemolymphatic collection fills the potential space. Although it is mainly defined in adults when located at the hip in the literature, a limited number of cases in the pediatric age group have been reported. Unlike adults, Morel-Lavallée lesions most commonly affect the knee and especially the anterior knee in children. Lesions are usually ovoid shape and may contain septa, internal blood products and thick capsule. The presence of the internal fat droplet in the collection is an important criteria for the diagnosis of the Morel-Lavallée and it is required for differential diagnosis from other post-traumatic pathologies such as hematoma and prepatellar bursitis (7,8).

Early detection of Morel-lavallée lesions prevents delays in treatment and shortens recovery time. If patients do not comply with treatments such as compression cuffs and do not avoid doing sports, the probability of recurrence and worsening of the lesions increases. The continued growth of the lesion may result in denervation by causing fat and/or skin necrosis as a result of the mass effect (6,9). It is critical to better understand the imaging findings of these traumatic degloving injuries because approximately half (44%) of MLL are misdiagnosed during the initial examination (7).

The majority of patients receive conservative management but older age (>11 years old), the presence of the hemorrhagic content and thick capsule in the collection are MRI findings that can guide the clinician in determining the need for percutaneous drainage. MLL can be misdiagnosed by clinicians-radiologists since they are not very common in the pediatric age group, so Morel-Lavallée should definitely be considered in the differential diagnosis, especially in the presence of a subcutaneous collection in the anterior knee(7,8).

Although there is no definitively accepted treatment for MLL, compression bandages, percutaneous aspiration, drainage, and open debridement have been reported (10-14) In small and acute MLL lesions without fracture, compression bandaging, NSAID, bed rest, and physical therapy can be used as first-line treatments (10-13). When a compression bandage is insufficient and the lesion is large, percutaneous drainage can be used. Sclerotherapy, in addition to percutaneous drainage, may be used if the lesions are chronic (15,16). In patients with long-standing MLL with pseudocapsules that do not respond to percutaneous drainage and may cause recurrence, surgical intervention is indicated (6,17).

Our study's limitations are that it is primarily a retrospective study with a small sample size. Only the patients' MRI findings were presented in our study, and there is a need for large studies in which the patients are evaluated alongside other findings. Children with MLS have a very low cure rate. Our findings on this subject should be validated by increasing the number of children who are eligible for treatment.

CONCLUSION

The importance of differential diagnosis of MLL from trauma collections was determined in our study, and MRI findings aided in treatment planning. Accurate diagnosis and treatment of MLL are critical, as the lesion's size may increase as a result of delayed treatment, causing skin necrosis and denervation due to the mass effect.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Erzincan Binali Yıldırım University Clinical Researches Ethics Committee (Date: 25.01.2021, Decision No: 02/21).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Original Article

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The effect of *Lycium barbarum* on reproductive system and the expression of Crisp-1 protein in experimentally diabetic male rats

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ABSTRACT

Aim: The main purpose of this study is to investigate the effects of *Lycium barbarum* polysaccharides (LBP) on the male reproductive system and Crisp-1 protein expression in experimentally diabetic Wistar Albino male rats.

Material and Method: In this study Wistar Albino male rats (3-4 months old) were randomly selected and divided into four groups; Control, LBP control (only LBP), Diabetic and Treatment (diabetic + LBP) group. For the experimental diabetes model, a single dose of 55 mg/kg STZ was injected intraperitoneally. In the treatment group, the diabetic rats were administered with 200 mg/kg of LBP by gastric gavage for 15 consecutive days.

Results: In histological examinations, increased intertubular connective tissue, congestion, vacuolization and edema were observed in testicular tissues of the diabetic group. The histopathological changes were improved after LBP treatment. Also, the number of total sperm count and sperm motility were significantly increased in the treatment group. Our biochemical analysis results showed that the serum testosterone level were significantly increased and serum MDA level were significantly decreased after treatment with LBP. Compared to the diabetic group, the apoptotic cells were decreased in the treatment group. Crisp-1 protein expression was increased in the treatment group, Crisp-1 possitive vesicle-like structures and apical blebs were also examined in the epididymal tissues.

Conclusion: It is concluded that *Lycium barbarum* polysaccharides have a therapeutic effect on the male reproductive damages of diabetes and also enhances Crisp-1 protein expression in the epididymis.

Keywords: Antioxidant, crisp-1 protein, cysteine-rich secretory protein, diabetes mellitus, infertility, *Lycium barbarum*

INTRODUCTION

Diabetes mellitus (DM) is a major metabolic disorder worldwide. It is characterized by chronic hyperglycemia and imbalances in carbohydrate, fat and protein metabolism that develops as a result of failures related to insulin production (1). Hyperglycemia caused by DM causes tissue damage and disruption of the balance between free radical production and antioxidant defence by triggering oxidative stress (2,3). Oxidative stress which occurs as a result of high production of reactive oxygen species (ROS) is responsible for important complications related to diabetes (4). One of these important complications is a malfunction in the male reproductive system and infertility (5-7).

In fertilization process, important events such as sperm binding and penetration of the zona pellucida and merging with the egg plasma membrane take place through cell connections and are regulated by specific molecules found in both gametes. One of these molecules is the rat epididymal Crisp-1 protein, which is the first member of the Cysteine-Rich Secretory Protein (CRISP) family (8). Crisp-1 is expressed from the epididymal epithelium and released into the lumen where it is associated with the surface of the maturing sperm (9-11). Crisp proteins accompany sperm through the passage of both male and female reproductive systems and have many important functions in the fertilization (12). Crisp-1 protein plays important roles in sperm capacitation, sperm-zona pellucida (ZP) binding, and sperm-egg fusion (13-16). As a result of in vitro fertilization studies, it has been shown that sperm lacking Crisp-1 has a disadvantage in its communication with ZP and its ability to fuse with the egg (17).

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In recent years, natural herbal solutions have been sought in the field of treatment and these studies have attracted attention. *Lycium barbarum*, also known as Goji berry, has been used in traditional Chinese medicine for centuries in herbal treatments and stands out with its antioxidant properties that suppress oxidative stress. Studies have shown many important properties of *Lycium barbarum* polysaccharides (LBP) such as immune system regulator, antitumor, antioxidant, and anti-radiation. For this reason, *Lycium barbarum* stands out as an effective natural material in the treatment of diabetes and male fertility (18-24).

MATERIAL AND METHOD

Experimental Animals and Ethics

The study was carried out with the approval of Abant Izzet Baysal University Clinical Researches Ethics Committee (Date: 28.01.2016, Decision No: 2016/04). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The experimental animals were obtained from Abant İzzet Baysal University Experimental Animal Application and Research Center. The animals were kept in a 25±3 °C environment suitable for 12 hours light/dark cycle during the study and were fed with ad libitum food and water. The study was started with 40 healthy male Wistar Albino rats (3-4 months old) but was completed with 31 rats due to deathly complications of diabetes.

Study Design and Groups

Schematic summary of the experimental study design is shown in **Figure 1**. Rats were randomly selected and divided into four groups:

Group 1: Control group (n=6),

Group 2: LBP control group (only LBP administration) (n=6),

Group 3: Diabetic group (STZ) (n=9) and

Group 4: Treatment group (STZ+LBP) (n=10).

Before the experimental study, blood-glucose levels were measured and the rats with normal values (<250 mg/dl) were included in the study. Streptozotocin (STZ) (Sigma Aldrich, CAS no: 18883-66-4) was used to induce experimental diabetes. Rats were fasted 12 hours before STZ injection. Just before the injection was performed, rats were weighed and fasting blood-glucose values were measured using the Accu-Check Go device from the blood obtained by cutting the tail tip. Besides, the amount of STZ solution was calculated according to the body weight of the rats. A single dose of 55 mg/kg STZ dissolved in citrate buffer (0.1 M pH: 4.5) was administered intraperitoneally. In the 24 hours after injection, 30% dextrose solution was added to the water containers of the rats to prevent deaths due to hypoglycemic shock. Diabetes was determined by measuring blood-glucose values from the blood taken from the tail end of the rats 48 hours after STZ injection. Rats with a blood-glucose level of 250 mg/dl and above were considered as diabetic as previously stated (24).

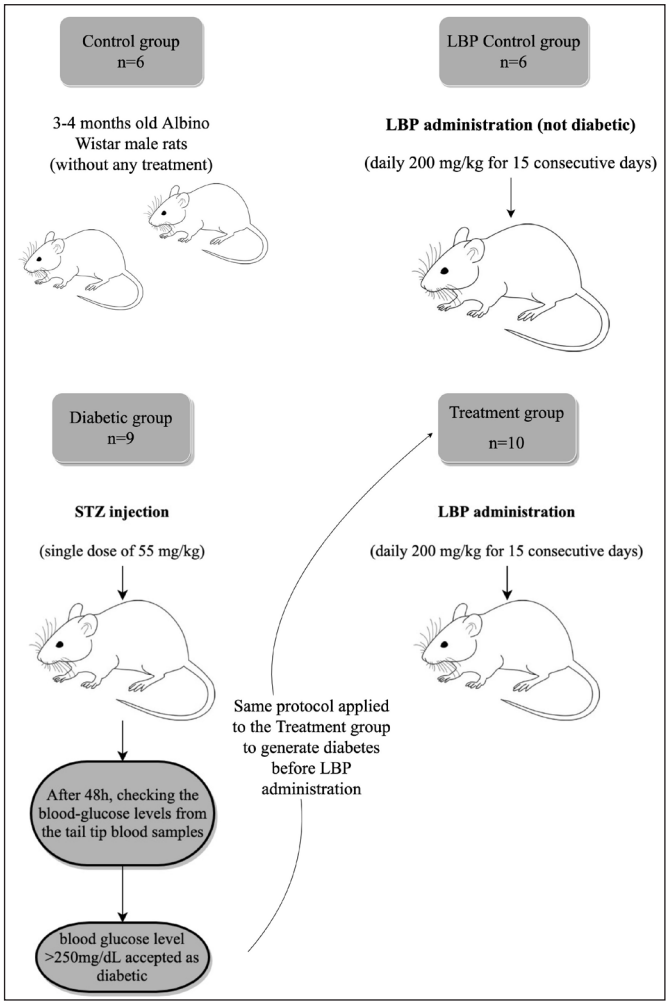

Figure 1. Schematic summary of the study design and experimental groups

After the rats were accepted as diabetic, *Lycium barbarum* polysaccharides (LBP) were administered by gavage of 200 mg/kg once a day for 15 consecutive days in the LBP treatment group. At the end of 15 days, animals were sacrificed after being treated with anaesthetic agents administered at the indicated doses. All tissues were fixed by 10% formaldehyde. Epididymal tissues were used for both histological examination and sperm smear analysis.

Preparation of *Lycium barbarum* Polysaccharides

LBP was purchased commercially and prepared based on previous methods (25). Briefly, dried fruit was pulverized with a blender. To separate the fatty parts; chloroform + methanol (2:1) was used and then refluxed with 80% ethanol at 80°C to separate oligosaccharides. The residue was added four times hot water and extracted four times. Then, the filtrate was combined and concentrated in

rotary at 60°C, and precipitation was performed with 95% ethanol, 100% ethanol and acetone, respectively. The precipitate was collected and dried in vacuum. The extract was freshly prepared and used.

Histological and Immunohistochemical Examination

After the fixation with 10% formaldehyde, the testicular tissues were embedded in paraffin and sectioned into 4-µm thick slices with microtome (Leica). Hematoxylin & Eosin, Masson's Trichrome stainings were used for histological examinations. Caspase-3 primary antibody (Invitrogen, Cat no: PA5-16335) was used for examining apoptotic cells in testicular tissue sections. Also, Crisp-1 primary antibody (Mybiosource, Cat no: MBS2032852) was performed for epidydimal tissue sections to examine Crisp-1 protein expression.

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labelling (TUNEL) Assay

TUNEL assay was used to evaluate apoptosis in testicular tissues. The 4-µm thick slices firstly deparafinized and then all the protocol were assessed according to the ApopTag® Peroxidase In Situ Apoptosis Detection Kit manual (cat no: S7100).

Biochemical Analysis

Blood samples were taken from the inferior vena cava and heart from anaesthetized rats for biochemical analysis. Then, sacrification was performed by cutting the aorta. The blood samples were placed in yellow-capped 5 ml gel vacuum serum separator tubes and centrifuged at 4000 rpm for 10 minutes. The serum parts obtained were taken into eppendorf tubes and stored at -80 ° C until the analysis. Testosterone (T) (Elabscience, Catalog no: E-EL-0072), follicle stimulating hormone (FSH) (Elabscience, Catalog no: E-EL-R0391) and oxidative stress parameter malondialdehyde (MDA) (Elabscience, Catalog no: BC0025) levels were examined. In the analysis of these parameters, "Enzyme-Linked Immunosorbent Analysis" (ELISA) method and thiobarbituric acid (TBA) method were used. The kits used in the analyzes were the original Elabscience (Elabscience Biotechnology Co. Ltd; Wuhan; P.R.C.) kits and were used by following the procedure specified by the manufacturer.

Assessment of Sperm Number, Motility and Aniline Blue Staining

For semen analysis, sperms revealed by linting method from cauda epididymis in PBS were counted at 20x magnification under light microscope using Makler sperm counting camera (Makler counting chamber sefi-medical instruments). In semen analysis, number, motility and morphology parameters were examined according to the criteria of the World Health Organization (WHO). Acidic aniline blue staining is used to evaluate sperm chromatin condensation. The sperm smear preparations fixed with 3% glutaraldehyde for 30 minutes and then stained with aniline blue (pH:3.5) for 10 minutes. Washed twice in PBS and left to dry. The preparations were examined under a light microscope at 20x and 100x magnification.

Statistical Analysis

The obtained data were analyzed using SPSS statistical program (IBM Statistics for Mac Os, Version 21.0). Mann Whitney-U test, Kruskal Wallis and One-Way ANOVA tests were used to compare data. Results were evaluated with the significance level as $p \leq 0.05$.

RESULTS

The body weights of the rats weighed before and after the experiment. It was observed that the rats in the treatment group lost weight at the end of the experiment, but there was no significant difference. Also, polyuria was observed in diabetic rats.

STZ-Induced Diabetes Leads to Histopathological Changes and LBP Has Therapeutic Effects on Testicular Tissues

In the testicular tissues of the control group (**Figure 2. A**), there is no structural abnormality; seminiferous tubules and spermatogenic cells preserved their structural integrity. In the testicular tissues of the diabetic group, edema in the intertubular areas (**Figure 2. B**) and cell debris in the lumen of the seminiferous tubule (**Figure 2. C**) were observed. In addition, vacuole formation within the seminiferous tubules, increased connective tissue in the intertubular area and congestion in the vessels were observed (**Figure 2. D,E,F,G**). In the testicular tissues of the treatment group, similar to the control group, general structural integrity and intertubular connective tissue were normal (**Figure 2. H**).

LBP Decreases the Diabetes-Related Apoptosis of Spermatogenic Cells

TUNEL assay method was used to identify apoptosis and the apoptotic cells were counted in five different areas with 20x under the light microscope. A statistically significant difference was observed between the control and the diabetic groups ($p=0.001$, $p \leq 0.05$) and also between the diabetic and treatment groups ($p=0.024$, $p \leq 0.05$) (shown in **Figure 3**).

In the immunohistochemical examinations with light microscopy, increased number of TUNEL positive apoptotic cells in the diabetic group indicates that diabetes leads to apoptosis by damaging spermatogenic cells (shown in **Figure 4**). In the treatment group, decreased number of TUNEL positive cells indicates less spermatogenic cell damage and apoptosis compared to the diabetic group.

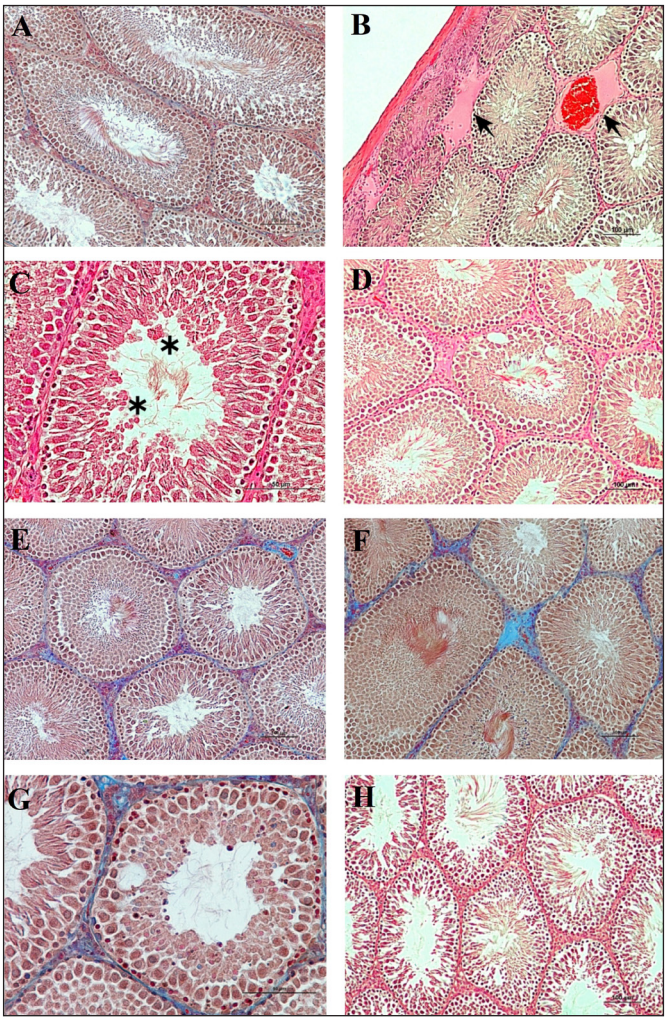

Figure 2. Histological examinations of testicular tissues. A: Control group. Seminiferous tubules and spermatogenic cells preserved their structural integrity. B-C: Diabetic group, edema in the intertubular areas and cell debris in the lumen of the seminiferous tubule were observed. D-G: Diabetic group, vacuole formations within the seminiferous tubules, increased connective tissue in the intertubular area and congestion in the vessels can be seen. H: Treatment group, the seminiferous tubules were similar to the control group. Masson's Trichrome and Haematoxylin & Eosin histological stainings were performed and examined with x200 and x400 magnifications.

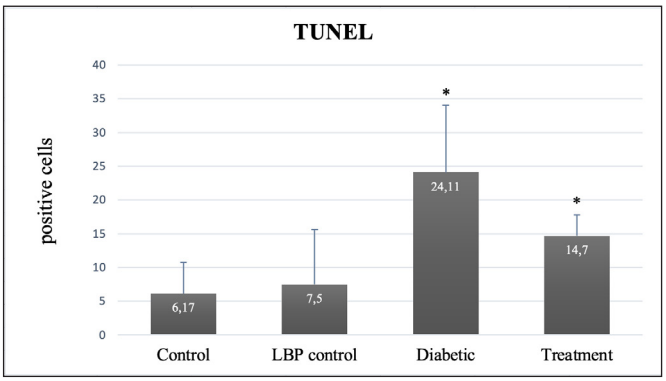

Figure 3. TUNEL immuhistochemical staining. TUNEL positive (apoptotic) cells were counted for each group. A significant difference is found between the diabetic and treatment group (p=0.024, p ≤0.05). Results were evaluated with the significance level as p ≤ 0.05

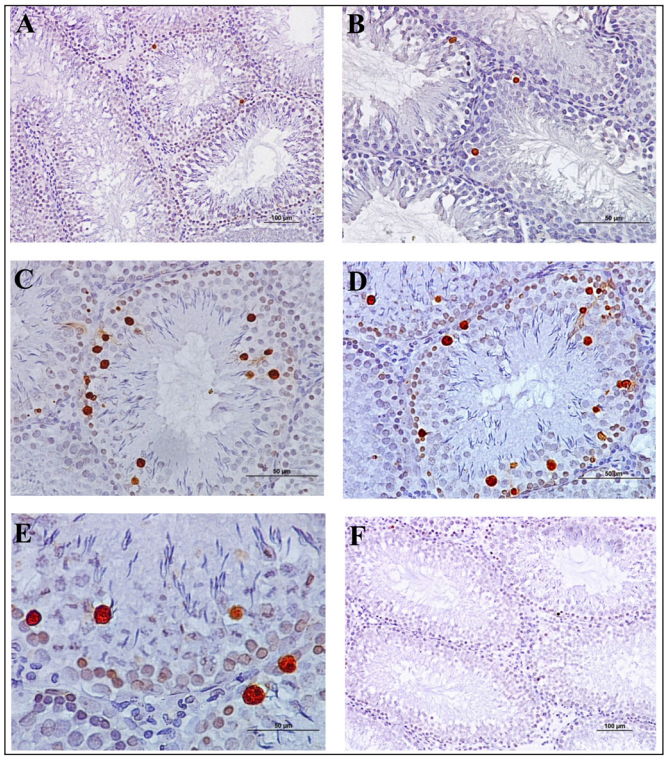

Figure 4. TUNEL apoptotic assay. In the immunohistochemical examinations, increased number of TUNEL positive apoptotic cells in the diabetic group indicates that diabetes lead to apoptosis by damaging spermatogenic cells. A: Control group, B: LBP control group, C-E: Diabetic group, F: Treatment group

Increased Caspase-3 Activation Indicates Apoptotic Cells in Diabetic Testicular Tissue

To determine apoptotic cells in testicular tissues, positively stained cells with Caspase-3 immunolabeling were counted for each group (shown in **Figure 5**). There was a significant difference between the control and diabetes groups (p=0.026, p≤0.05). The increase in the number of caspase-3 positive cells in the diabetes group showed that diabetes-induced apoptosis by increasing caspase-3 activation in testicular tissue (shown in **Figure 6**). The number of positive cells in the treatment group showed a significant decrease compared to the diabetes group (p=0.013, p≤0.05). In addition, there was no significant difference between the control group and the treatment group (p=0.958, p>0.05).

LBP Enhances Fertility-Related Crisp-1 Protein Expression in the Epididymis

In the Crisp-1 immunolabeling, we observed cytoplasmic Crisp-1 staining in epididymal epithelial cells and also stereocilia stained with Crisp-1 antibody (shown in **Figure 7**). Spermatozoa inside the epididymis tubule lumens were also positively stained. In addition, vesicle-like secretory structures protruding towards the lumen stained positively were observed both in the lumen and on the surface of the epithelial cells like apical blebs (shown in **Figure 7D**).

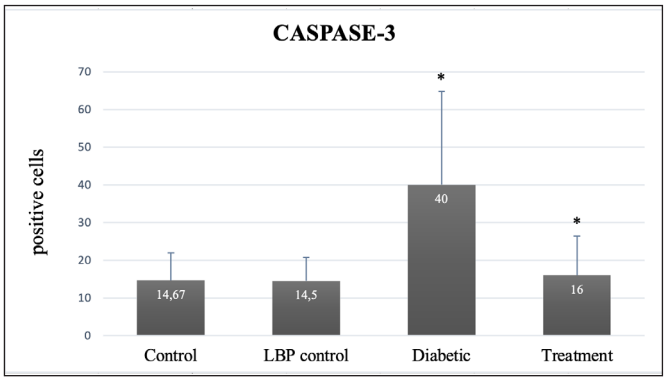

Figure 5. Caspase-3 immunohistochemical staining. Caspase-3 positive cells were counted for each group. A significant difference is found between the diabetic and treatment group (p=0.013, p≤0.05). Results were evaluated with the significance level as p ≤ 0.05

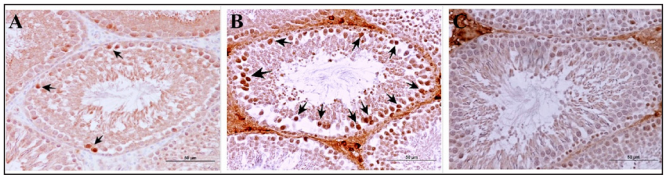

Figure 6. Caspase-3 immunohistochemical staining for apoptotic cells. A: Control group, B: Diabetic group, C: Teratment group (x200).

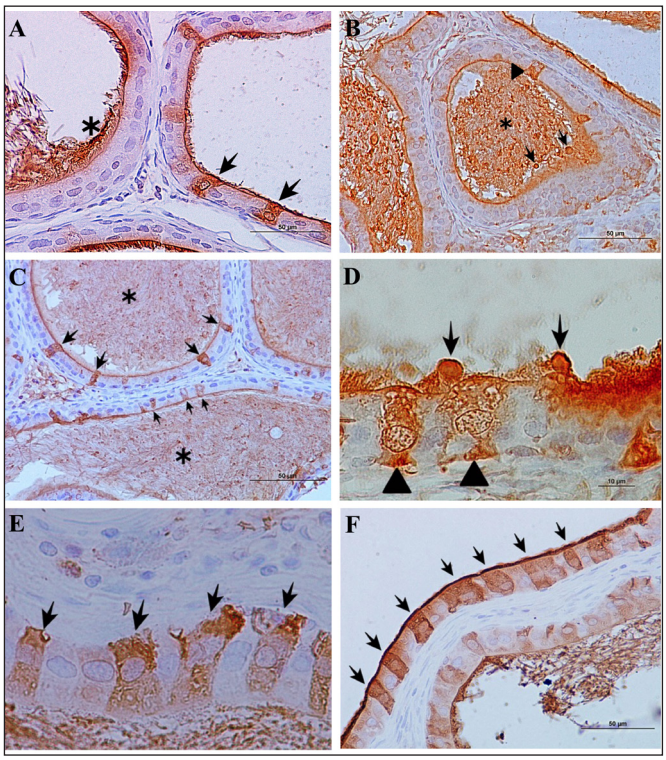

Figure 7. Examination of the Crisp-1 protein expression in the epididymal tissues. A,E,F: The cytoplasmic Crisp-1 staining in epididymal epithelial cells and also stereocilia stained with Crisp-1 antibody. B,C: Spermatozoa inside the epididymis tubule lumens were also positively stained. D: Vesicle-like secretory structures protruding towards the lumen and staining positively were observed both in the lumen and on the surface of the epithelial cells like apical blebs

Crisp-1 positive cell counting was performed for each group (shown in **Figure 8**). There was no significant difference between the control and diabetes groups (p=0.346, p>0.05). There was a statistically significant difference between diabetes and the treatment group (p=0.001, p≤0.05). There was also a statistically significant difference between the control and treatment groups (p=0.013, p≤0.05).

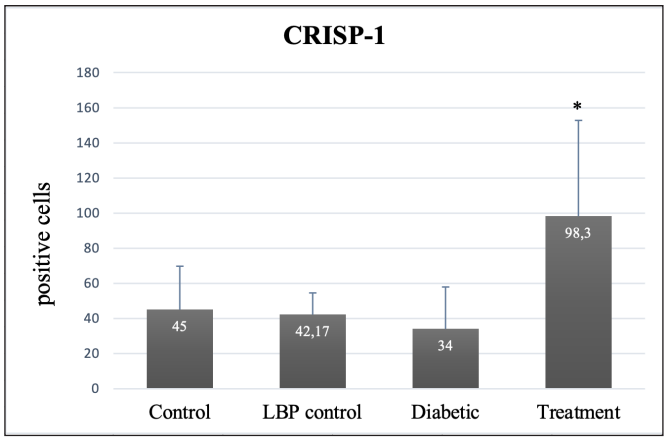

Figure 8. Crisp-1 immunohistochemical staining. Crisp-1 positive cells were counted for each group. A significant increase is found in the treatment group (p=0.001, p≤0.05). Results were evaluated with the significance level as p≤0.05

It was determined that there was a decrease in the number of Crisp-1 positive cells in the epididymis tissue of the diabetes group, but there was no statistically significant difference when compared with the control group. In the treatment group, there was a significant increase in the number of Crisp-1 positive cells.

LBP Treatment Reduces Diabetes-Related Lipid Peroxidation

To examine oxidative stress, we analyzed the MDA (Malondialdehyde) levels among all groups. A statistically significant difference was observed between the diabetes and treatment groups (p=0.050, p≤0.05) (shown in **Figure 9**). In the diabetic group, there was a slight increase in MDA values, as expected, indicating lipid membrane damage. It was observed that MDA significantly decreased in the treatment group, that is, lipid peroxidation decreased. The MDA values of the treatment group were found to be close to the control group values.

LBP Treatment Has Enhancer Effects on Decreased Serum Testosterone Level

Testosterone levels were analyzed to examine the effect of diabetes on male stereoid hormones and reproductive ability. Testosterone levels were expected to decrease significantly (p=0.015, p ≤0.05) in the diabetic group (shown in **Figure 10**), indicating that damage to the male reproductive system and spermatogenesis were

negatively affected. It was observed that testosterone increased significantly (p=0.003, p≤0.05) in the treatment group, that is, positively affected spermatogenesis. In addition, testosterone values of the treatment group were determined to be close to each other, not showing a significant difference with the control group.

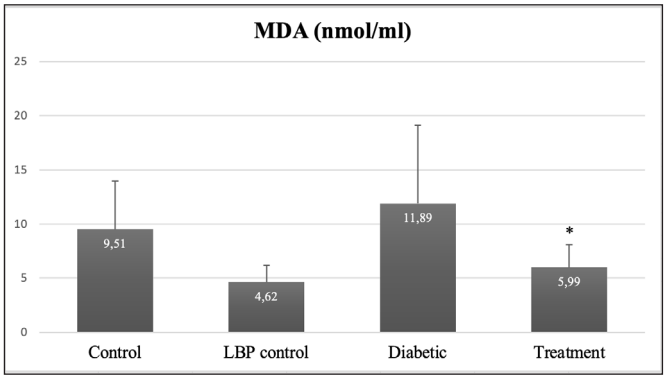

Figure 9. Biochemical analysis of serum MDA levels. A significant difference is found between the diabetic and treatment group (p=0.050, p≤0.05). Results were evaluated with the significance level as p≤0.05

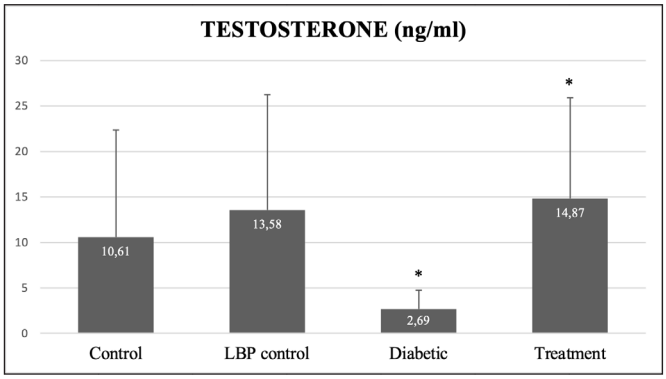

Figure 10. Biochemical analysis of serum Testosterone levels. Testosterone levels decreased significantly (p=0.015, p ≤0.05) in the diabetic group. It was observed that testosterone levels increased significantly (p=0.003, p≤0.05) in the treatment group. Results were evaluated with the significance level as p≤0.05

LBP Treatment Has No Significant Effect on the Serum FSH Level

The obtained serum FSH values of the groups can be seen in the **Figure 11**. Although there was an increased serum FSH level in the LBP control and the LBP treatment groups, compared to the diabetic group, it was not statistically significant (p=0.356, p>0.05).

Total Sperm Count and Motility Increased with LBP Treatment after STZ-Induced Diabetes

The total sperm count and sperm motility values of the groups were performed immediately after sacrification and shown in the **Table**. The values should be multiplied by 105. There was a statistically significant difference for total sperm count between diabetes and treatment groups (p=0.000, p≤0.05). Total sperm count of the diabetic group showed that diabetes caused a decrease in sperm number. Compared to the control and LBP control groups, the sperm motility of the diabetic group was decreased. It was also determined that the sperm motility in the LBP treatment group was increased significantly compared to the diabetic group (p=0.000, p≤0.05). There was no difference between the groups for sperm morphology. In addition, no difference was detected with the Aniline blue staining performed to determine the sperm DNA integrity (shown in **Figure 12**).

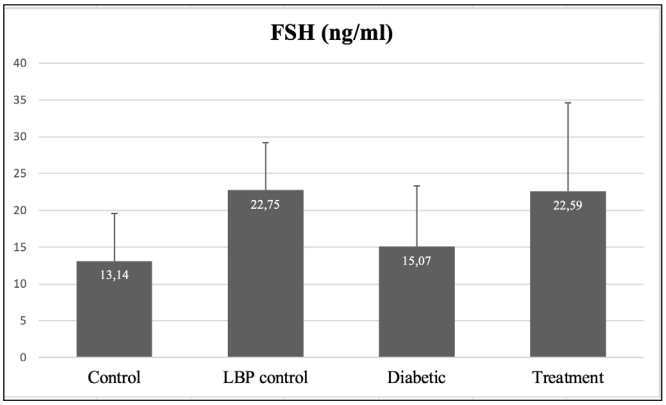

Figure 11. Biochemical analysis of serum FSH levels. There was no statistically significant difference. Results were evaluated with the significance level as p≤0.05

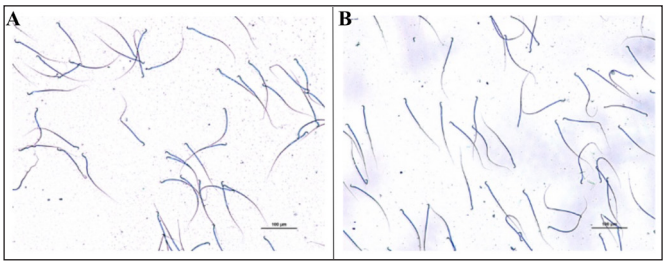

Figure 12. The Aniline blue staining was performed to epididymal sperm smears. A: Control group. B: Diabetic group

Table. Total sperm counts and sperm motility values of the groups. (The values should be multiplied by 105)

Groups	Control	LBP control	Diabetic	Treatment
Total sperm count±SD	106±26.65	165.5±67.21	111.89±50.29	191±103.54
Motile sperm count±SD	18.17±9.15	15.33±19.13	1±1.2	28.20±20.14

DISCUSSION

Diabetes Mellitus (DM) is a very common metabolic disease worldwide. One of its most important complications is spermatogenic dysfunction and infertility (26-28). Oxidative stress increases with diabetes and damages directly testicular and epididymal tissues (29). Crisp-1 protein is one of the molecules that regulates fertilization by playing a role in important events such as capacitation, sperm-egg interaction and gamete fusion. Crisp-1 protein secreted from epididymal epithelial cells and delivered to the lumen binds to the sperm surface during epididymal passage (11,30).

In current study, we demonstrated that the diabetes-related oxidative stress leads to the histopathological and biochemical changes of male reproductive system. In addition, we showed that the LBP, as a natural antioxidant, reduces the harmful effects of diabetes. Also, we showed that the expression of Crisp-1 protein is affected by diabetes-associated oxidative stress, and LBP treatment enhances its expression in the epididymis.

The molecular mechanism of diabetes-related male reproductive system damage and the protective effects of LBP have been elucidated in several studies (19-23). It has been found that LBP had an anti-apoptotic effect by reducing the expression of Caspase-3, which is an indicator of apoptosis, in testicular tissues of diabetic rats (31). Also, it has been reported that LBP could reduce irradiation-induced apoptosis of spermatogenic cells by upregulation of Bcl-2 expression, could increase serum testosterone level and decrease MDA level (22). Consistently, our data showed that the number of apoptotic cells decreased significantly after the LBP treatment. In addition, we showed that the LBP improved serum testosterone level and reduced serum MDA level. The serum FSH level analysis was also performed in our study. Although we showed an increase in the FSH level for the LBP treatment, we could not examine a significant difference between the diabetic and LBP treatment groups. This result could be related with the different mechanisms of hormonal control in the male reproductive system. Treatment of infertility-related hormonal dysfunction requires an understanding of the hormonal basis of spermatogenesis (32). While FSH stimulates spermatogenesis by effecting Sertoli cells, testosterone is mainly secreted from Leydig cells under the control of LH (luteinizing hormone). This can explain why the serum testosterone level was significantly increased but the serum FSH level was not increase at significance level after the LBP treatment. It has been showed that LBP could also improve sperm motility and total density (33). Consistently, our study showed that the total sperm number and sperm motility were improved after LBP treatment. Although we expected a decrease for the sperm motility in STZ-induced diabetic group, it was unexpectedly very low. It might be caused by toxic effect of STZ or a mistake in experimental process during sperm linting etc. Lei et al. (34) reported that the LBP treatment increased the number of HIF-1a positive cells in the testicular tissues of diabetic rats compared with the control group. They also showed that LBP reduce apoptosis, increase sperm motility, and also upregulate PCNA and SIRT-1 expression. Our data was consistent with this study in terms of the histopathological examination, apoptosis and sperm motility. Unlike, we also performed the biochemical analysis for the serum testosterone, MDA and FSH levels. In addition, immunohistochemical examination of the Crisp-1 protein expression in epididymal tissues was performed in our study.

The Crisp-1 protein was first identified in the rat epididymis by Cameo and Blaquier (8). They reported that Crisp-1 is secreted in the epididymis and then it attaches to the sperm surface during the epididymal passage. It has been reported that when Cuasnicu et al. (35) applied polyclonal anti-Crisp-1 to block the Crisp-1 protein, the fusion of sperm with oocyte was inhibited. Busso et al. (36) reported that the epididymal Crisp-1 protein binds to the relevant regions on the egg surface and plays a role in gamete fusion and sperm-ZP interaction. The effect of LBP, which has a known fertility-enhancing effect, on Crisp-1 protein expression was investigated for the first time with our study. We showed that LBP enhances Crisp-1 protein expression in epididymal tissues. Our work is unique in this respect. In our Crisp-1 immunolocalization study, it was observed that stereocilia extending along the epithelium and sperms in the epididymal lumen were stained with Crisp-1 antibody. We also observed that round vesicles that protrude from the epididymal epithelium to the lumen and are free in the epididymal lumen showed Crisp-1 positive staining. Our findings are consistent with the previous studies of Sullivan et al. (37-38). They indicated that there is an apocrine secretion in the apical cytoplasm of principal cells and these apical blebs contain epididymosomes, so we might have observed Crisp-1 protein containing epididymosomes. Epididymosomes were defined as membranous vesicles involved in the transfer of epididymal proteins to sperm and were reported by Sullivan et al. (37-38). Thimon et al. (39) investigated the protein compositions in the epididymosome and determined that it contains many proteins, including the Crisp-1 protein, and reported that the Crisp-1 protein was secreted in the epididymis and then assembled in the epididymosomes.

CONCLUSION

We showed that the Crisp-1 protein is related with the molecular mechanism of diabetes-induced damages of male reproductive system and the protective effects of LBP. However, future comprehensive studies are needed for the Crisp-1 protein to reveal the detailed mechanism of function and the relationship with infertility.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the approval of Abant Izzet Baysal University Clinical Research Ethics Committee (Date: 28.01.2016, Decision No: 2016/04).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Relationship between the amniotic fluid prolactin level at early second trimester and pregnancy outcome

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ABSTRACT

Aim: The aim of this study was to determine whether early second trimester amniotic fluid prolactin level were associated with pregnancy outcome.

Material and Method: This study included 125 women who underwent amniocentesis for variable indications. Healthy subjects with no history of drug use were included in this study. The gestational age was determined by ultrasonic examination before 10 weeks in all cases. Amniotic fluid specimens were taken while performing amniocentesis for other indications. Amniotic fluid was collected by transabdominal amniocentesis. Amniotic fluid prolactin concentrations were utilized to predict pregnancy complication among women who underwent amniocentesis due to the variable indications.

Results: Among all study population, pregnancy was unremarkable in 102 (81.6%) cases, on the other hand, most common fetal abnormality was found to be the fetal hydrops fetalis and the second most commonly encountered fetal anomaly was trisomy, intrauterine growth restriction and intrauterine fetal demise. All study population was divided into two groups as complicated (n=23) and uncomplicated (n=102) pregnancies. Amniotic fluid prolactin concentration did not have any predictive value for complicated pregnancies (AUC=0.479).

Conclusion: Our data showed; there is no relationship between early second trimester amniotic fluid prolactin level and pregnancy outcome.

Keywords: Decidual prolactin, pregnancy outcome, early second trimester

INTRODUCTION

The biological roles of the decidual protein hormones during pregnancy are unclear, countless studies suggest that the hormones may act locally to affect the function of the placenta, decidua, and fetal development. These decidual hormones are released into the circulation and function to regulate uterus, placenta and fetal membrane activity via autocrine/paracrine factors (1). Prolactin which is one of the decidual hormones ,increase during pregnancy and are involved with many aspects of maternal metabolic adaptation to pregnancy. The metabolic roles of prolactin propose an interesting scenario in which the central nervous system and indirectly whole body development (2).

In a previous study, which was conducted to figure out the possible effect of decidual prolactin on materno-fetal physiology,late second trimester amniotic fluid (AF) prolactin levels were shown to be associated with recurrent polyhydramnios (3).

Additionally, previous data indicated significant relationship between AF prolactin and preterm premature rupture of membrane at third trimester , study revealed that increased AF prolactin resulted in impairment of structural integrity of fetal membranes through electrolytes disturbances (4). Furthermore, fetal and maternal tissues were shown to express prolactin receptors in late pregnancy. Increased expression in the chorion, decidua, and placenta was reported during labor and delivery which supported an autocrine/paracrine role for decidual prolactin in the peripartum (5). There is no study in the literature so, we aimed to assess the relationship between early second trimester AF prolactin level and pregnancy outcome.

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MATERIAL AND METHOD

This prospective cohort study was performed at perinatology clinic of Zeynep Kamil Women and Children's Health Training and Research Hospital between January 2017 and April 2018. This study was approved by Zeynep Kamil Women and Children's Health Training and Research Hospital Clinical Researches Ethics Committee (Date: 18.11.2017, Decision No: 144) and informed consent was obtained from each participant. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Sample and study participants: The study group included 125 women who underwent amniocentesis for variable indications.

Healthy subjects with no history of drug use were included in this study. The gestational age was determined by ultrasonic examination before 10 weeks in all cases. AF specimens were taken while performing amniocentesis for other indications. AF was collected by transabdominal amniocentesis.

AF samples were centrifuged at 400 rpm for 10 minutes. The amniotic supernatant and the serum were separated and stored at 20"C until assayed. Total prolactin in AF samples was measured by the Tandem-E PRL immunoenzymetric assay.

Intrauterine growth retardation was diagnosed in cases with a fetal weight that is below the 10th percentile for gestational age as determined through an ultrasound (6).

Cases were accepted to have spina bifida defect if the spinal cord and its meninges are exposed through a gap in the backbone (7).

Hydrops fetalis was defined as abnormal accumulation of fluid in two or more fetal compartments, including ascites, pleural effusion, pericardial effusion, and skin edema (8).

Pregnancies with high blood pressure (140/90 mmHg) that develops during pregnancy or during the postpartum period that is associated with overt protein in the urine or the new development of decreased blood platelets, with the kidney or liver function tests, fluid in the lungs, or signs of neurological symptoms such as seizures and/or visual disturbances were diagnosed to be complicated with preeclampsia (9).

In all cases, amniocentesis was indicated based on the results of screening tests such as ultrasound or biochemical markers. AF prolactin concentrations were utilized to predict any pregnancy complication among women who underwent amniocentesis due to the variable indications.

Data Analysis: SPSS version 15 (Chicago, USA, 2006) was used for statistical analysis. Student-t test was used to compare continuous variables while categorical variables were compared by Chi-square test. $P<0.05$ was accepted to be statistically significant.

RESULTS

Summary characteristics of some demographic and clinical characteristics of whole study population were shown in **Table 1**. Indications for amniocentesis were shown in **Table 2**, which shows that the most frequent indication as high risk for trisomy detected by first or second trimester screening tests. Fetal sex distributions were shown in **Table 3**. Among all study population, pregnancy was uneventful in 102 (81.6%) cases, on the other hand, most common fetal abnormality was found to be the fetal hydrops fetalis and the second most commonly encountered fetal anomaly was trisomies, intrauterine growth restriction and intrauterine fetal demise (2.4%, **Table 4**). All study population was divided into two groups as complicated (n=23) and uncomplicated (n=102) pregnancies. AF prolactin concentration did not have any predictive value for complicated pregnancies (AUC=0.479, **Figure 1**).

Table 1. Summary characteristics of some demographic and clinical characteristics of whole study population

	Minimum	Maximum	Mean	Std. Deviation
Age (Years)	19	45	33.4	5.9
Gestational age (weeks)	15	22	18.1	1.5
AF prolactin concentration (mcg/L)	28.9	31.412	5972.7	4774.4
Gestational age at delivery (weeks)	17	42	36.5	5.7

Table 2. Indications for amniocentesis

	Frequency	Percent
Cystic Hygroma	1	.8
Double Bouble	3	2.4
High risk for trisomy in first trimester screening test	59	47.2
High risk for trisomy in second trimester screening test	41	32.8
Advanced maternal age	6	4.8
High NT thickness	5	4.0
Maternal anxiety	6	4.8
High risk for trisomy in quadriple test	3	2.4
Fetal Hydocephaly	1	.8
Total	125	100.0

Table 3. Fetal sex distributions

	Frequency	Percent
Undetermined sex	15	12.0
Male	55	44.0
Female	55	44.0
Total	125	100.0

Table 4. Pregnancy outcome distributions		
	Frequency	Percent
Normal fetus	102	81.6
Undetermined	1	0.8
Di-George	1	0.8
Hidrops Fetalis	4	3.2
Hipoplastic left heart	1	0.8
Skelatal Dysplasia	2	1.6
IUGR	3	2.4
Intrauterine Fetal Demise	3	2.4
Preeclampsia	1	0.8
Spina Bifida	2	1.6
Termination for variable reasons	2	1.6
Trisomy 13-18	1	0.8
Trisomy 21	2	1.6
Total	125	100.0

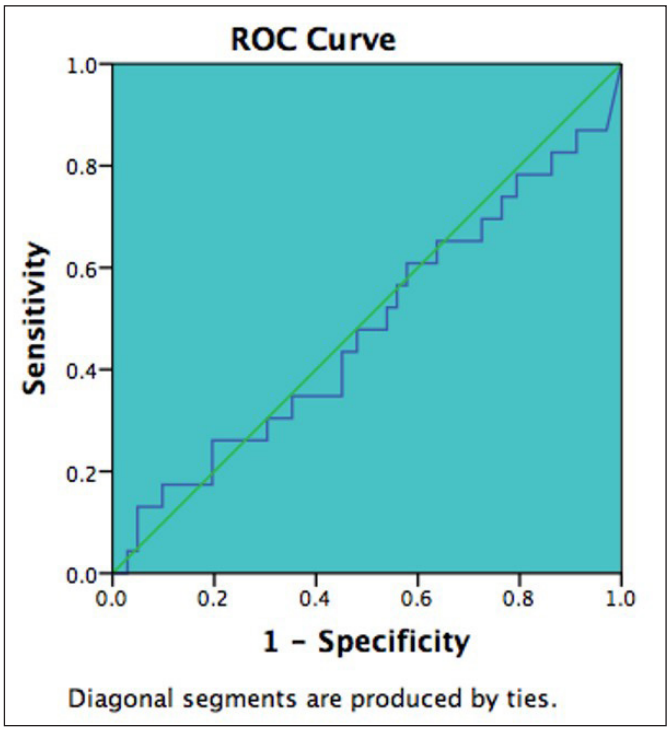

Figure 1. ROC cur of AFprolactin to predict poor pregnancy outcome

DISCUSSION

Measuring prolactin level at different trimester of pregnancies inside decidual cells and amniotic fluid indicated that changes in decidual cells are consistent with the changes of the concentration of amniotic fluid prolactin (10) Prolactin was found to be increased by the fetal pituitary gland and was immunologically determined by 10th week and biologically determined at the 18th week. Fetal plasma prolactin levels were low until 30 weeks of gestation, then a striking increase occured between 30 weeks and term (11)

Tyson et al. suggested that AF decidual prolactin may have an inhibitory influence on prostaglandin (PGE2) synthetic pathways (12). When the amniotic membrane disrupts, this inhibitory function of AF prolactin disappears, additionally increasing surface area of distrupted membrane result in higher production of prostaglandin (PGE2). All this data indicate the critical role of AF prolactin on active labor (13,14). This hypothesis led us to consider that, AF prolactin values in postterm pregnancies may be higher than in term pregnants. No significant differences were shown between the amniotic fluid, maternal serum and cord blood prolactin values in term and post-term pregnancies. Thus, the hypothesis has not been supported by the results of the study by Demir et al.(15).

Amniotic fluid, maternal serum and cord blood prolactin values of normal term pregnancy group in a study by Demir et al. were in agreement with those in the literature (13-16). On the other hand, other regulatory factors such as platelet activating factor and calcium in conjunction with estrogens, progesterone and cortisol were shown to have significant impact on the release of prolactin from the decidua (18). Amniotic transport occurs through intercellular channels between amniotic epithelial cells and can be modulated by AF prolactin levels (19). Based on these information some studies have been conducted to assess relationship between prolactin and some pregnancy complications, one of these studies assess prolactin concentrations in women with preeclampsia. Preeclampsia was shown to be characterized by increased excretion of urinary prolactin. Prolactin concentrations and isoforms were suggested to be appropriate markers for assessing the severity of preeclampsia and the occurrence of adverse outcomes. Therefore, it was suggested that prolactin and /or isoforms may play a role in the pathophysiology of preeclampsia (20). Furthermore, it was suggested that hormonal changes may play a role in AF volume arrangement. Prolactin receptors, on both fetal and maternal tissues increase while the pregnancy progresses. ıt is a deciduous prolactin and it was shown to affect amniotic permeability (2).

In a previous study, prolactin concentrations secreted by decidua were compared among groups of women with different pregnancy complications including induced abortions, and diabetes mellitus, preeclampsia, chronic hypertension, and polyhydramnios and normal term pregnancies. Study revealed lower prolactin levels in amniotic fluid, decidual prolactin content and production were in pregnancies complicated by either hypertension or polyhydramnios (21).

Some physiologic functions of prolactin have been defined, for example prolactin has been found to inhibit the secretion of different proinflammatory chemokines by human fetal membranes (22). Additionally, amniotic prolactin was shown to play a role in the pathogenesis of polyhydramnios due to osmoregulatory effect (23).

Maternal serum prolactin levels in normal pregnancies was not found to differ from serum levels in complicated pregnancies, on the other hand significantly lower levels of prolactin in AF of pregnancies complicated by hypertension or polyhydramnios were determined in a previous study, authors suggested that this difference was probably due to adverse effects of these conditions on the synthesis and release of prolactin by decidua (24). Previously published studies showed that amniotic prolactin concentrations in normal human pregnancy decrease from the second trimester of pregnancy and a plateau of low levels were observed in the late third trimester (25, 26). This data consistently support the possible effect of prolactin on labor.

First report of acute recurrent polyhydramnios with high levels of AF prolactin levels was published by De Santis et al. (2). In that study, AF prolactin levels was within normal limits in cases with acute recurrent polyhydramnios but close up to the upper limits of levels in normal pregnancies (24,25). De Santis et al. put forward hypothesis of acute recurrence of polyhydramnios may be linked to the dysfunctional chorionic receptors for prolactin. Previous study assessed AF prolactin levels three times during the pregnancy, study revealed no change with advancing gestational age (27). No change was determined in this study, however intervals that the samples were obtained too short to detect decreasing concentrations with advancing gestational age.

In normal pregnancies, a significant correlation between the production of AF and the amniotic prolactin level, which was shown to peak at 23-25 gestational weeks and reaches a plateau after 34 gestational weeks (26). Peak levels were obtained at 23-25 weeks of gestation in their study. Consistently, decreasing amniotic prolactin levels in the third trimester of normal pregnancies was shown to be parallel to the decreasing amniotic fluid production (28), study speculated that amniotic prolactin concentration measurements may be utilized to determine whether the AF production in polyhydramnios begins to normalize or not.

Further data indicated that, prolactin may have a role in fetal growth process and maturation of the gut mucosa (29).

Decidual cells synthesize endometrial prolactin and some variations in prolactin gene expression were shown within each area of decidua. Study showed periodic differences in prolactin gene expression in the decidual cells during pregnancy (30).

Plasma and AF prolactin concentrations were compared between preterm and term pregnancies. Study included 20 patients with preterm labor and 20 patients with preterm labor who responded to tocolysis and delivered at term. Prolactin concentrations were generally found to be significantly higher in preterm than at term (31).

Our data showed that, AF prolactin concentration determined between 16 to 20 gestational ages was not a significant predictor for any pregnancy complication.

All these aforementioned studies suggest that there is still contraversies on the physiological function of decidual prolactin during pregnancy and its relationship between the pregnancy complications.

CONCLUSION

No significant relationship was determined between early first trimester prolactin level and any of the pregnancy complication.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Zeynep Kamil Women and Children's Health Training and Research Hospital Clinical Researches Ethics Committee (Date: 18.11.2017, Decision No: 144)

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Hyperlipasemia is a poor prognostic factor in patients with COVID-19

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ABSTRACT

Introduction: COVID-19 disease may pose a considerable health threat to healthy individuals and individuals with comorbidity. The SARS-CoV-2 virus affects the respiratory tract and may cause damage to the pancreas by binding to the ACE-2 receptor in the pancreas. In our study, we investigated the effects of hyperlipasemia on morbidity and mortality in patients diagnosed with COVID-19.

Material and Method: In this study, 2350 patients diagnosed with COVID-19 between November 2020 and December 2020 were retrospectively reviewed. Other possible causes of hyperlipasemia were excluded. Hyperlipasemia secondary to COVID-19 was detected in 338 patients. These patients were divided into two groups based on their lipase elevation rates.

Results: Hyperlipasemia was detected in 14.4% of the patients diagnosed with COVID-19, and severe hyperlipasemia (>3x) was detected in 2.3%. The mean age of the patients was 64±13.8 (18-92), of which 59.5% (201) were male. In our study, 24 patients (1%) were diagnosed with acute pancreatitis. When compared according to lipase level, a significant difference was found between the groups regarding the history of HT, CCI score, development of ARF at follow-up, development of ARDS, need for ICU hospitalization, need for intubation, length of stay in ICU, and death rates. A weak correlation was found in the correlation analysis between hyperlipasemia and ARDS development and mortality.

Conclusion: Elevated lipase levels were associated with poor prognosis and mortality in patients with COVID-19 infection.

Keywords: SARS-CoV-2, hyperlipasemia, COVID-19, pancreatitis, viral pancreatitis

INTRODUCTION

The SARS-COV-2 virus has been spreading rapidly worldwide and has threatened all humanity (1). The course of the disease may range from subclinical infection to hospitalization and death (2). As of August 22, 2021, the cumulative number of cases reported globally in the COVID-19 pandemic is roughly 210 million, and the cumulative number of deaths is just over 4.4 million (3). The SARS-CoV-2 virus mostly affects the respiratory tract (2). However, symptoms, such as nausea, vomiting, abdominal pain, and diarrhea, in some patients suggest that the virus also affects the gastrointestinal system (GIS) (4).

Acute pancreatitis (AP) is an inflammatory disease of the pancreas. The clinical spectrum of AP ranges from mild edematous pancreatitis to severe necrotizing pancreatitis (5). There are various causes of acute pancreatitis in adults. Gallstones are the most common cause of acute pancreatitis. Other causes of pancreatitis include alcohol use, some metabolic disorders, autoimmune diseases, viral infections, exposure to drugs and toxins (5-6). Many viral, bacterial and parasitic infectious agents may cause AP (5). Diagnosis of infection-induced AP requires the absence of other potential causes of AP with evidence of active infection. It has been revealed that viruses, such as coxsackievirus, mumps, cytomegalovirus, hepatitis A, B, C, and Epstein-Barr virus, play a role in the etiology of AP (5). In studies conducted in patients with SARS-CoV-2 infection, ACE-2 expression was slightly higher in pancreatic tissue than in the lungs (7). Moreover, it has been reported that the SARS-CoV-2 virus may lead to pancreatic damage by binding to the ACE-2 receptor in the pancreas (7).

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In our study, we analyzed the correlation of hyperlipasemia with morbidity and mortality in patients diagnosed with COVID-19.

MATERIAL AND METHOD

Our hospital is a tertiary center for the diagnosis and treatment of patients with COVID-19 during the pandemic. This study was designed retrospectively. The diagnosis of COVID-19 was made by polymerase chain reaction (PCR) of nasopharyngeal swab samples. All patients over 18 who were positive for SARS-CoV-2 were included in the present study. A total of 2350 patients who underwent COVID-19 PCR testing and were diagnosed with COVID-19 between November 2020 and December 2020 were reviewed. Patients with known chronic renal failure and other causes of acute pancreatitis (biliary pancreatitis, hypertriglyceridemia, alcoholic pancreatitis, malignancy, hypercalcemia) that may be the cause of hyperlipasemia were excluded from this study. Three hundred thirty-eight patients with serum lipase levels above the upper limit of normal reference values were included in this study. One of the revised Atlanta diagnostic criteria is serum amylase and lipase levels >3 times the upper limit of normal. Hence, in our study, we divided the patients into two groups, considering the lipase elevation level as <3-fold (mild elevation) and >3-fold (severe elevation) (8). According to the revised Atlanta criteria, patients with at least two of the three criteria (abdominal pain consistent with pancreatitis, more than 3-fold increase in serum amylase-lipase levels, and appearance compatible with acute pancreatitis on radiological imaging) were diagnosed with AP. Our study was approved by the Amasya University Non-Interventional Clinical Researches Ethics Committee (Date: 18.02.2021, Decision No: E.5718) and was conducted in accordance with the Declaration of Helsinki.

Statistical analysis

The software of Statistical Package for Social Sciences (SPSS) (IBM SPSS Inc., Chicago, IL) for Windows 20 was used for our statistical analysis. Normality analysis of the data was evaluated via Kolmogorov-Smirnov and Shapiro-Wilk tests. When analyzing the study data, continuous variables with normal distribution were expressed as mean±standard deviation (SD), while continuous variables without normal distribution were expressed as median (min: max). In group comparisons, a parametric test (Student's t-test) was used for normally distributed continuous variables, whereas a non-parametric test (Mann-Whitney U test) was used for non-normally distributed variables. Fisher's exact test and Pearson's chi-square test were used for discrete variables. Spearman correlation coefficient was used to compute the correlation analysis. Statistical significance was considered $p \leq 0.05$ with a confidence interval (CI) of 95%.

RESULTS

Elevated lipase levels were detected in 14.4% of patients diagnosed with COVID-19, while severe lipase levels were detected in 2.3%. The ages of the patients ranged from 18 to 92, with a mean of 64±13.8% and 59.5% (201) male. All of the patients were hospitalized and followed up and treated. Demographic data of the cases are presented in **Table 1**. In the group with lipase >3 times higher, 22 patients had abdominal pain and one patient had pancreatitis on CT. The patient, whose CT was compatible with pancreatitis, did not have accompanying abdominal pain. In the group with lipase <3 times higher, 82 patients had abdominal pain and three patients had an appearance compatible with pancreatitis on CT. However, only one of the three patients whose CT was compatible with pancreatitis had abdominal pain. A total of 24 patients (n:2350, 1%) met the diagnostic criteria for acute pancreatitis according to the Revised Atlanta criteria.

Table 1. Demographic data of patients with elevated lipase

		Lipase Level Group (n:338)
Age		64±13.8 (22:92)
Gender (F/M)		137/201
BMI*		29.7±3.8
CCI		4.4±2.8 (0:12)
History of CAD [n/(%)]		118 (34.9)
History of DM [n/(%)]		127 (37.6)
History of HT [n/(%)]		229 (67.8)
History of KLD		1 (0.3)
Dyspnea [n/(%)]		322 (95.3)
Anorexia [n/(%)]		256 (75.7)
Nausea [n/(%)]		151 (44.7)
Diarrhea [n/(%)]		23 (6.8)
Abdominal pain [n/(%)]		104 (30.8)
Thorax CT severity (n:66/n:270)	<18 ≥18	236 (70.2) 100 (29.9)
Abdominal CT sign of pancreatitis		4 (1.1)
ARF		86 (25.4)
Lipase rising time		11.6 (1:42)
Length of ICU stay		2.4±5.5 (0:40)
Length of hospital stay		11.6±8.1 (0:50)
ARDS		98 (29)
Intubation		91 (26.9)
Requirement for ICU [n/(%)]		89 (26.3)
Mortality [n/(%)]		91 (26.9)

BMI : Body mass index, CCI: Charlson Comorbidity Index, CAD: coronary artery disease, DM : Diabetes mellitus, HT : Hypertension, CLD:Chronic liver disease, ICU: Intensive care unit, ARDS: Acute respiratory distress syndrome, ARF: Acute renal failure

Parameters, such as age, BMI, symptoms (dyspnea, anorexia, nausea, abdominal pain), thorax CT severity index and CORADS classification, appearance compatible with pancreatitis on abdominal CT, lipase elevation time, and duration of hospitalization were

similar in both groups (p> 0.05)). However, previous history of hypertension (HT), Charlson comorbidity index (CCI) score, development of acute renal failure (ARF) in follow-up, development of acute respiratory distress syndrome (ARDS), need for intubation, need for follow-up in the intensive care unit (ICU), duration of ICU stay and mortality rates were similar in both groups (**Table 2**).

When the groups were evaluated regarding laboratory parameters, urea, creatinine, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), direct bilirubin (DB), amylase, uric acid, ferritin, D-Dimer, protein, albumin, leukocytes, hemoglobin, hematocrit, platelets, and C-reactive protein (CRP) levels were significantly different in both groups (**Table 3**).

Table 2. Some clinical and demographic features in groups with high and low lipase levels

	High Lipase Level Group (n:68)	Low Lipase Level Group (n:270)	P
Age	67.4 (37:87)	62.6 (22:92)	0.03
Gender (F/M)	27/41	110/160	0.9
BMI	28.9±3.8	29.9±3.8	0.08
CCI	5.5 (0:12)	4.17 (0:12)	0.001
History of CAD [n/(%)]	29 (42.6)	89 (33)	0.17
History of DM [n/(%)]	26 (38.2)	101 (37.4)	0.9
History of HT [n/(%)]	54 (79.4)	175 (64.8)	0.03
History of KLD	1 (1.5)	0	0.2
Dyspnea [n/(%)]	65 (95.6)	257 (95.2)	1
Anorexia [n/(%)]	54 (79.4)	202 (74.8)	0.52
Nausea [n/(%)]	37 (54.4)	114 (42.2)	0.07
Diarrhea [n/(%)]	8 (11.8)	15 (5.6)	0.1
Abdominal pain [n/(%)]	22 (32.4)	82 (30.4)	0.86
Thorax CT severity (n:66/n:270) <18 ≥18	47 (70.1) 20 (29.9)	189 (70.3) 80 (29.7)	1
Thorax CT-CORAD classification low risk high risk	15 (22.4) 52 (77.6	55 (20.4) 214 (79.6)	0.7
Abdominal CT sign of pancreatitis	1(1.5)	3 (1.1)	1
ARF	29 (42.6)	57 (21.1)	0.001
Lipase rising time	11 (1:29)	11.47 (1:42)	0.6
Length of ICU stay	3.2 (0:24)	2.2 (0:40)	0.006
Length of hospital stay	11.9 (0:50)	11.9 (1:41)	0.6
ARDS	32 (47.1)	66 (24.4)	0.001
Intubation	29 (42.6)	62 (23)	0.002
Requirement for ICU [n/(%)]	27 (39.7)	62 (23)	0.008
Mortality [n/(%)]	31 (45.6)	60 (22.2)	0.001
Steroid usage requirement	65 (95.6)	265 (98.1)	0.2
NSAID use	6 (8.8)	17 (6.3)	0.6

BMI : Body mass index, CCI: Charlson Comorbidity Index, CAD: coronary artery disease, DM : Diabetes mellitus, HT : Hypertension, CLD:Chronic liver disease, ICU : Intensive care unit, ARDS: Acute respiratory distress syndrome, ARF: Acute renal failure

A strong positive correlation was found between lipase elevation and amylase elevation. Furthermore, a weak positive correlation was found between hyperlipasemia and urea, creatinine, uric acid, d-dimer levels, ARDS development, and mortality, whereas a weak negative correlation was found between hyperlipasemia and albumin.

In the correlation analysis with mortality, a very strong positive correlation was found with ARDS, the need for intubation, and ICU admission A strong positive correlation was found between the duration of ICU stay and mortality. A moderate positive correlation was found with CCI score, development of ARF, urea, creatinine, LDH, CRP, and D dimer levels. A weak positive correlation was found with a previous history of HT, uric acid, DB, and ferritin levels. Moreover, a weak negative correlation was found with protein, albumin, and platelet levels (**Table 4**).

Table 3. Laboratory characteristics of groups with high and low lipase levels

	High Lipase Level Group (n:68)	Low Lipase Level Group (n:270)	P
Glucose	231.1 (60:701)	214.1 (41:1043)	0.4
Urea	110.4 (25:277)	63.4 (17:361)	0.001
Creatinin	1.99 (0.2:14)	1.13 (0.3:10.3)	0.001
ALT (0-55 U/L)	48.7 (9:448)	71.1 (3:1302)	0.15
AST (0-55 U/L)	37.5 (7:182)	52.8 (5:1080)	0.4
GGT (9-36 IU/L)	72.9 (11:297)	72.7 (5:861)	0.9
Alkaline phosphatase	83.7 (44:185)	80.3 (20:622)	0.02
LDH	476 (141:1359)	389.2 (57:1426)	0.001
Total Bilirubin	0.63 (0.19:6)	0.48 (0.11:2)	0.2
Direct Bilirubin	0.36 (0.03:4.6)	0.21 (0.01:0.9)	0.007
Amilaz	463.7 (205:1442)	100.4 (61:192)	0.001
Lipaz	463.7 (205:1442)	100.3 (61:178)	0.001
Uric acid	6 (1.2:19.5)	4.4 (1.1:13.4)	0.001
Triglyceride	170.3 (56:769)	201.3 (56:769)	0.12
Leukocyte	13408 (1630:41350)	11039 (2500:36030)	0.06
Hemoglobin	11.8± 2.1	12.8±1.8	0.002
Hematocrit	35±5.6	38.1±5.1	0.04
Platelet	230704 (66000:513000)	270306 (82000:601000)	0.01
CRP	64.4 (1.34:436)	35.6 (0.17:223)	0.01
Sedimentation	53.5 (9:144)	48.3 (4:135)	0.4
Fibrinogen	516.4 (185:1170)	486.9 (40-1200)	0.2
Ferritin	725.36 (49:3439)	651.8 (7:7591)	0.06
D-Dimer	1.92 (0.05:7.86)	1.5 (0.01:22.3)	0.001
Protein	5.84 (4.1:7.8)	6.1 (3.9:7.8)	0.03
Albumin	3.15 ±0.58	3.45±0.48	0.001

ALT : Alanine aminotranspherase, AST : Aspartate aminotranspherase, GGT : Gamma glutamil transpherase, LDH : lactate dehydrogenase. CRP : C-reactive proteine.

Table 4. Correlation analysis of lipase elevation and mortality and other parameters

	Lipase elevation amount		Mortality	
	r	P	r	p
CCI	0.188	0.001	0.523	0.001
HT	0.125	0.02	0.248	0.001
ARF	0.198	0.001	0.595	0.001
ARDS	0.200	0.001	0.920	0.001
Intubation	0.178	0.001	0.925	0.001
Requirement for ICU	0.152	0.005	0.818	0.001
Length of ICU stay	0.149	0.006	0.786	0.001
Mortality	0.211	0.001	1	
Urea	0.271	0.001	0.565	0.001
Creatinin	0.272	0.001	0.525	0.001
Uric acid	0.226	0.001	0.336	0.001
Protein	-0.132	0.03	-0.239	0.001
Albumin	-0.211	0.001	-0.36	0.001
Amylase	0.694	0.001	0.193	0.001
Lipase	0.694	0.001	0.193	0.001
Alkaline phosphatase	0.140	0.02	0.186	0.002
LDH	0.158	0.004	0.515	0.001
Direct bilirubin	0.148	0.006	0.322	0.001
Hemoglobin	-0.170	0.002	-0.165	0.002
Hematocrit	-0.156	0.004	-0.084	0.12
Platelet	-0.134	0.01	-0.375	0.001
CRP	0.135	0.01	0.490	0.001
Ferritin	0.100	0.06	0.271	0.001
D-Dimer	0.250	0.001	0.432	0.001

r=Spearman's Correlation Coefficient
CCI: Charlson Comorbidity Index, HT : Hypertension, ARF: Acute renal failure, ARDS: Acute respiratory distress syndrome, ICU : Intensive care unit, LDH : lactate dehydrogenase. CRP : C-reactive proteine.

DISCUSSION

Elevations in pancreatic enzymes have been reported in patients diagnosed with COVID-19 (9,10). However, lipase elevation is not unique to pancreatitis (11) and can be released from organs other than the pancreas (12). In renal failure, serum amylase and lipase levels increase due to decreased renal clearance (13,14). In addition to that, increased serum amylase and lipase levels have been reported after trauma, burns, diabetes mellitus, severe gastroenteritis, and cardiovascular surgery (13,14). Based on the Revised Atlanta Criteria, at least two of the three criteria (typical abdominal pain, elevated serum amylase and/or lipase values, and characteristic findings on abdominal imaging) are required to diagnose acute pancreatitis (8). According to the Revised Atlanta Criteria, pancreatitis is a well-known cause of organ failure (such as renal, respiratory and cardiovascular) (8,15). Many viruses may cause pancreatitis (16). However, it has not been fully elucidated whether SARS-CoV-2 directly impacts the pancreas or causes hyperlipasemia secondary to multi-organ failure (MOF) (17,18). Thus, in COVID-19, hyperlipasemia may be secondary to pancreatitis, MOF, or both (19). Studies have demonstrated that ACE-2 expression is higher in pancreatic tissue than in lungs in patients with SARS-CoV-2 infection. This may be a cause of pancreatic injury (7,20).-Pathophysiology of acute pancreatitis activated pancreatic enzymes and pancreatic ischemia secondary to microcirculation disorder of the pancreas play a key role (21). Case-based reports in the literature have revealed cases of acute pancreatitis secondary to COVID-19 (22,23,24). Besides, in the studies, the incidence of pancreatitis secondary to COVID-19 was 0.16%, 7.46%, and 17%, respectively (25,7,9). In our study, the incidence of pancreatitis was 1% (24/2350).

McNabb-Baltar suggested that hyperlipasemia in patients with COVID was not associated with severe illness or poor clinical outcomes. The author stated that hyperlipasemia is not caused by pancreatic injury but might be associated with other gastroenterological manifestations of the virus (11). Liu, on the other hand, argued that in patients with severe COVID-19 infection (even without necrotizing pancreatitis findings), taking pancreatic injury into consideration may impact the prognosis of patients(7). In addition, Lax et al. reported that 36% of patients with COVID-19 without clinical pancreatitis suspected had pancreatic parenchymal necrosis and focal pancreatitis findings (26). Meanwhile, Rasch suggested that pancreatic tissue damage may occur in severe COVID-19 patients, although there are no typical clinical symptoms (10).

In their study, Liu et al. (7) analyzed pancreatic injury due to SARS-CoV-2 infection. They determined elevations in both amylase and lipase levels in 1.85% of mild cases. An increase of 17.9% and 16.4% were detected in amylase and lipase levels of severe COVID-19 patients, respectively. Moreover, McNabb-Baltar detected hyperlipasemia in nine (12.1%) patients (11). In the study of Barlass et al. (19) involving 1003 COVID-19 patients, a >3-fold increase in lipase was found in 14 patients (83% out of 16.8%) . In our study, elevated lipase levels were detected in 14.4% of patients diagnosed with COVID-19, while severe lipase levels were detected in 2.3%.

In the study of Barlass et al. (19), there was a significant predominance of males in the high lipase group (78.6% vs. 38.8%); however, there was no difference regarding other demographic characteristics. Besides, in Rasch's study, it was reported that there was no significant difference between the group with high lipase levels and the control group regarding BMI (11). In our study, no difference was found between the groups regarding sex and BMI. However, patients in the group with severe lipase elevation were older and had a higher CCI score. Moreover, patients with a previous history of HT were more common in the group with severe lipase elevation (p: 0.03).

In the study of Barlas, it was revealed that nausea-vomiting symptoms were more common in the low lipase group (75.4% vs. 42.3%) (19). However, in our study, symptoms, such as shortness of breath, nausea, vomiting, anorexia, and abdominal pain, and the severity of pulmonary involvement secondary to COVID-19 on thoracic CT imaging were similar in both groups.

Liu reported that pancreatic changes were consistent with pancreatitis in five patients (7.46%) with severe disease findings on lung CT, but none of these patients had signs of acute necrosis in the pancreas (7). In the study of Rasch et al., typical pancreatitis imaging findings were not observed in any of the patients (10). In our study, abdominal CT imaging of one patient with severe lipase elevation and three patients with mild lipase elevation revealed enlargement of the pancreatic parenchyma and peripancreatic inflammation ($p>0.05$). On the other hand, in our study, pancreatic necrosis was not detected in any patient's abdominal CT imaging.

Many of the studies in the literature have reported a relationship between high lipase levels and worse clinical outcomes in COVID-19 disease (7,10,19). In the study of Rasch et al., the incidence of ARDS was higher in the group with serum lipase activity >180 U/l (p: 0.003). However, in the same study, it was revealed that there was no difference between the groups regarding ventilation duration and mortality (10). Barlass et al. reported higher ICU admission rates (92.9% vs. 32.8%) and intubation rates (23.5% vs. 78.6%) in the group with high (>3 ULN) lipase levels. In addition, in the study of Barlass, no correlation was found between high lipase levels and the duration of hospitalization (19). In our study, the development of ARF during the follow-up period, the development of ARDS, the need for hospitalization in the ICU, the length of ICU stay, and the mortality rates were higher in the group with severe lipase elevation (**Table 2**). Likewise, in our study, no difference was revealed between the groups regarding the duration of hospitalization. In the correlation analysis with the lipase elevation level, a weak positive correlation was found with amylase, urea, creatinine, uric acid, d-dimer levels, ARDS development, and mortality, whereas a weak negative correlation was found with albumin (**Table 4**).

The limitation of our study was that this study was designed retrospectively. Studies supported by autopsy findings could elucidate the exact cause of hyperlipasemia.

CONCLUSION

Studies support that COVID-19 does not cause a disease that only affects the lungs but a multisystemic disease. In many studies, it has been demonstrated that ARDS, ICU admission, and mortality are higher in patients with hyperlipasemia. Hyperlipasemia could be utilized as a marker for more severe disease and poor prognosis in the monitorization and treatment follow-up of patients with COVID-19 infection. Furthermore, the SARS-CoV-2 virus should be considered in the etiopathogenesis of acute pancreatitis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Amasya University Non-Interventional Clinical Researches Ethics Committee (Date: 18.02.2021; Decision No: E.5718).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Two-year profile of the records of patients referred to Adana city hospital urology clinic due to PSA high in primary care: a retrospective review

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ABSTRACT

Objective: To retrospectively evaluate the two-year records of patients referred to Adana City Training and Research Hospital by family physicians because of high prostate specific antigen (PSA), and to reveal the profile and related outcomes for clinical practices of family physicians about prostate cancer screening.

Material and Method: The files of 102 patients, who were referred to our clinic by their family physicians due to high PSA between April 2019 and May 2021, were retrospectively evaluated. Demographic data of patients, presence of additional disease, family history, control serum PSA value examined in family medicine centers and in our hospital at time of first admission, complete urinalysis (TIT), ultrasonography (USG) and multiparametric magnetic resonance (mpMR) findings, transrectal ultrasonographic biopsy (TRUS-BX) results and biopsy were noted. The treatments administered according to the results (radical prostatectomy, radiotherapy, hormone therapy, chemotherapy) were recorded.

Results: The mean age of the patients was 52.8±8.9 years. The PSA value of the patients at time of admission was 8.0±3.8 ng/ml. The mean PSA values measured at the time of admission to primary care and at the time of admission to Adana clinic after referral were 8.0±3.8 ng/ml and 8.0±3.0 ng/ml, respectively. There was no statistically significant difference between these values (p=0.2). Among all the patients presenting with elevated PSA, 36 (35%) patients underwent TRUS Bx, had prostate cancer as a result of pathology and underwent radical prostatectomy, which was the most common definitive treatment method with statistical significance (p<.001). The sensitivity value obtained from the ROC curve calculated based on the initial PSA value of the patients was 68.09 years and the specificity value was 48.15 years. At the same time, the cut-off PSA value calculated by examining the area under the ROC curve was determined to be <7.5 ng/ml.

Conclusion: An individualized, risk-adjusted strategy for screening should be determined. A shared decision-making process with the patient should be adopted, along with explaining the reasons for and consequences of PSA screening.

Keywords: Family medicine, cancer screening, prostate cancer, prostate specific antigen

INTRODUCTION

Prostate cancer (PCa) is the second most common cancer diagnosed in men worldwide, accounting for 15% of all cancers (1). The prevalence of PCa under the age of 30 years is 5%, while it is 59% over the age of 79 years (2). While digital rectal examination (DRE), serum prostate specific antigen (PSA), transrectal ultrasonography (TRUS) and prostate biopsy (TRUS Bx) are included in the diagnostic evaluation, the introduction of PSA especially has led to serious advances in the diagnosis and treatment of PCa (3). Cancer screening is defined as the systematic examination of asymptomatic people (at risk). Screening for PCa with serum PSA aims to detect PCa at an early and manageable stage, thus directing curative treatment, and ultimately reducing overall and disease-specific mortality (4). Early diagnosis and treatment of PCa was reported to increase cancer-specific survival, and the first widespread screening results, especially in the USA, were associated with a decrease in PCa mortality (5,6). However, clinically insignificant PCa can also be detected as a result of screening, and the number of associated prostate biopsies that may cause sepsis increases in patients who undergo TRUS Bx (7). Therefore, it is useful to develop an individualized, risk-adapted strategy for early detection. People especially at risk including those over the age of

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50 years, with a family history, over the age of 45 years, of African origin, and with familial BRCA1 mutations should be prioritized for early diagnosis (8-10).

As can be seen from the literature above, PCa screening continues to be one of the most controversial issues in urology practice (11). The current situation also affects the decisions of family physicians. However, it is very important for family physicians to inform their patients about cancer screening. In addition, it is important to apply the necessary screening tests or to refer the patients to the relevant branches. Studies in the literature generally analyzed the attitudes of family physicians towards prostate cancer screening by assessing their attitudes towards PCa with questionnaire-based questions (12). In our study, we aimed to reveal the profile and related results about clinical practices of family physicians about prostate cancer screening by retrospectively evaluating the two-year records of patients referred to Adana City Training and Research Hospital (Adana) because of elevated PSA.

MATERIAL AND METHOD

Study Design

Our study retrospectively evaluated the files of 102 patients who were referred to our clinic by primary care family medicine centers between April 2019 and May 2021, after being approved by the Adana City Training and Research Hospital Clinical Researches Ethics Committee (Date: 2021, Decision No: 1595). All procedures adhered to the ethical rules and principles of the Helsinki Declaration. Data recorded included the demographic data of the patients, presence of additional disease, family history, control serum total PSA value examined in family medicine centers and in our hospital at time of first admission, complete urinalysis (TIT), ultrasonography (USG) and multiparametric magnetic resonance (mpMR) findings, transrectal ultrasonographic biopsy (TRUS) -BX) results, the treatments applied (radical prostatectomy, radiotherapy, hormone therapy, chemotherapy) and urinary tract infection according to the biopsy results, and serum total PSA value after antibiotic treatment. Serum total PSA level was measured using an electrochemiluminescence immunoassay (normal range; 0-2.5 ng/ml for age 40-49, 0-3.5 ng/ml for age 50-59, 0-4.5 for age 60-69, 70-79 0-6.5 ng/ml for age) (13).

Data Collection

The principles set out in the Declaration of Helsinki were followed. All methods were carried out in accordance with the relevant guidelines and regulations. Adana electronic hospital information system was used to obtain epidemiological data including demographic, clinical and laboratory findings. The serum total PSA value measured in venous blood samples was recorded. Peripheral venous blood samples were evaluated using standard procedures in the central laboratory of Adana City Training and Research Hospital. Biochemical hormonal parameters were measured with a Siemens ADVIA 1800 automated biochemistry analyzer (Siemens Healthcare Diagnostics Inc, Laboratory Diagnostics, Advia Centaur XPT, manufactured in Erlangen, Germany, Ireland).

Statistical Analysis

SPSS 23.0 package program (IBM, Armonk, NY) was used for statistical analysis of the data. Categorical measurements are summarized as numbers and percentages, and continuous measurements as mean and standard deviation (median and minimum-maximum where necessary). Chi-square and Fisher's exact tests were used to compare categorical variables. Shapiro-Wilk test was used to determine whether the parameters in the study showed normal distribution. In the comparison of continuous measurements between the groups, the distributions were checked and the Mann Whitney test was used for the parameters that did not show normal distribution in the double group analysis, and the Kruskal Wallis test was used for the analysis of more than two groups. In the study, the cut-off value was determined by calculating the sensitivity (sensitivity) and specificity (specificity) values based on the initial PSA value of the patients and examining the area under the ROC curve. Statistical significance level was taken as 0.05 in all tests.

RESULTS

The mean age of the patients was 52.8±8.9 years. Of patients, 8.8% had a family history of prostate cancer. The mean PSA value of the patients at the time of admission was 8.0±3.8 ng/ml. Only 50 patients had TRUS bx (**Table 1**).

The mean PSA values measured at the time of admission to primary care and at the time of admission to the ASEAH clinic after referral were 8.0±3.8 ng/ml and 8.0±3.0 ng/ml, respectively. There was no statistically significant difference between these values (p=0.2) (**Table 2**).

When the data of patients aged 40-45 years, 46-50 years and over 50 years of age were compared, the definitive treatments (RP, RT, HT) and Gleason score values in patients over 50 years old were relatively high, but only the presence of additional disease was statistically significantly higher (p<.001) (**Table 3**).

Among all the patients presenting with elevated PSA, 36 (35%) patients who underwent TRUS Bx, had prostate cancer as a result of pathology and underwent radical prostatectomy, which was the most common definitive treatment method with statistical significance (p<.001). There were 19 (18%) patients who received radiotherapy/hormonotherapy (**Table 4**).

Table 1. Demographic data in the study

	Frequency (n)	Percent (%)
Family history		
No	93	91.2
Yes	9	8.8
Additional illness		
No	50	49.0
Yes	52	51.0
Urinary tract infection		
No	68	66.7
Yes	34	33.3
TRUS Bx		
No	50	49.0
Yes	52	51.0
Radical Prostatectomy		
No	66	64.7
Yes	36	35.3
Radiotherapy / Hormonotherapy		
No	83	81.
Yes	19	18.6
Chemotherapy		
No	100	98.0
Yes	2	2.0
Gleason score		
3+3	24	23.5
3+4	4	3.9
4+3	7	6.9
4+4	9	8.8
4+5	1	1.0
None	57	55.9
	Mean±SD	Med (Min-Max)
Age	52.8±8.9	52 (40-75)
Initial PSA ng/ml	8.0±3.8	7 (3.9-20)
Control PSA ng/ml	8.0±3.0	7.5 (3.8-19)
PSA after antibiotics	4.6±4	3 (1-13)
mpMR PIRADs value	3.25±1.0	3 (1-5)

PSA: Prostate specific antigen, mpMR: Multiparametric magnetic resonance, TRUS-BX: Transrectal ultrasonographic biopsy

Table 2. Comparison of PSA at the time of admission in primary care and the control PSA value measured at first admission to ASEAH

	Mean±SD	Med (Min-Max)	p
Initial PSA ng/ml	8.0±3.8	7 (3.9-20)	0.2
Control PSA ng/ml	8.0±3.0	7.5 (3.8-19)	

PSA: Prostate specific antigen

Table 4. Definitive treatments applied to patients diagnosed with prostate cancer as a result of TRUS Bx

	Radical Prostatectomy		p
	No (n=66)	Yes (n=36)	
Radiotherapy /Hormonotherapy			
No	47 (71.2)	36 (100)	<.001
Yes	19 (28.8)	0 (0)	

TRUS Bx was applied to only 52 (51%) patients with elevated PSA. Gleason score 3+3 pathology was present in 24 (46%) patients, which was statistically significantly higher than other Gleason scores (p<.001) (**Table 5**).

Table 3. Comparison of the data for 40-45 year old, 46-50 year old and over 50 year old patients

	40-45 years	46-50 years	>50 years	p
Family history				
No	24 (88.9)	20 (95.2)	49 (90.7)	0.734
Yes	3 (11.1)	1 (4.8)	5 (9.3)	
Additional illness				
No	21 (77.8)	10 (47.6)	19 (35.2)	0.001
Yes	6 (22.2)	11 (52.4)	35 (64.8)	
Urinary tract infection				
No	20 (74.1)	15 (71.4)	33 (61.1)	0.442
Yes	7 (25.9)	6 (28.6)	21 (38.9)	
TRUS Bx				
No	15 (55.6)	11 (52.4)	24 (44.4)	0.604
Yes	12 (44.4)	10 (47.6)	30 (55.6)	
Radical Prostatectomy				
No	19 (70.4)	13 (61.9)	34 (63)	0.770
Yes	8 (29.6)	8 (38.1)	20 (37)	
Radiotherapy /Hormonotherapy				
No	22 (81.5)	17 (81)	44 (81.5)	0.998
Yes	5 (18.5)	4 (19)	10 (18.5)	
Chemotherapy				
No	26 (96.3)	21 (100)	53 (98.1)	0.654
Yes	1 (3.7)	0 (0)	1 (1.9)	
Gleason score				
3+3	8 (29.6)	5 (23.8)	11 (20.4)	0.753
3+4	0 (0)	0 (0)	4 (7.4)	
4+3	2 (7.4)	2 (9.5)	3 (5.6)	
4+4	1 (3.7)	2 (9.5)	6 (11.1)	
4+5	0 (0)	0 (0)	1 (1.9)	
None	16 (59.3)	12 (57.1)	29 (53.7)	
	40-45 years	46-50 years	Over 50 years	p
Age	6.45 (3.9-15)	6.9 (4-19)	7.25 (3.9-20)	0.214
Initial PSA ng/ml	6 (3.8-16)	8 (3.8-15)	7.9 (3.8-19)	0.119
Control PSA ng/ml	3 (2-13)	3.25 (1-13)	3 (1-13)	0.970
PSA after antibiotics	3 (2-5)	3 (2-5)	4 (1-5)	0.637

PSA: Prostate specific antigen, mpMR: Multiparametric magnetic resonance, TRUS-BX: Transrectal ultrasonographic biopsy

Table 5. Gleason score distribution of patients who underwent TRUS Bx

	TRUS Bx		p
	No (n=50)	Yes (n=52)	
Gleason score			
3+3		24 (46.2)	<.001
3+4		4 (7.7)	
4+3		7 (13.5)	
4+4		9 (17.3)	
4+5		1 (1.9)	
None	50 (100)	7 (13.5)	

TRUS-BX: Transrectal ultrasonographic biopsy

The sensitivity value obtained from the ROC curve calculated based on the initial PSA value of the patients was 68.09 years and the specificity value was 48.15 years. At the same time, the cut-off PSA value calculated by examining the area under the ROC curve was determined to be <7.5 ng/dl (**Figure 1**).

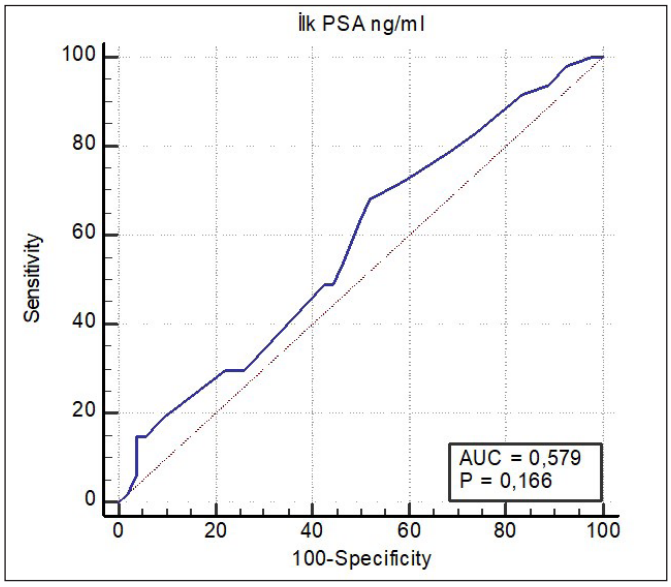

Figure 1. Sensitivity, specificity and cut-off values according to the ROC curve based on the initial serum PSA value

	Initial PSA	95% CI
AUC	0.579	0.476-0.676
Cut-off	<7.5	
Sensitivity	68.09	52.9-80.9
Specificity	48.15	34.3-62.2
+LR	1.31	1.0-1.8
-LR	0.66	0.4-1.1
PPV	53.3	45.3-61.2
NPV	63.4	51.2-74.1
p	0.166	

*p<0.05, **p<0.001, ROC curve

DISCUSSION

Clinically localized PCa is usually asymptomatic. Therefore, PSA and PRM are accepted as diagnostic tests to detect PCa while PCa is confined to the prostate (14). Although the first widespread screening results in the USA were associated with a decrease in mortality (6), in 2012 the US preventive services task force and 2013 AUA (American Urology) guidelines published a recommendation against PSA-based screening, resulting in a reduction in the use of PSA for early detection (15-17). However, Fenton et al. (18) in a systematic review conducted in 2018 and İliç et al. (7) as a result of their systematic review and meta-analysis, concluded that PSA population screening has a long-term benefit in reducing cancer-specific mortality. According to a Cochrane analysis published in 2013 by Ilic et al. (19), the diagnosis of PCa increased with screening and was associated with more localized disease. However, five available randomized controlled trials involving 341,000 men reported no overall survival benefit specific to PCa. Another issue among the questions of whether or not to perform screening is whether only PSA screening is sufficient? In their randomized clinical study investigating the effect of PSA-based screening on prostate cancer mortality in 2018, Martin et al. (20) found that a single PSA screening detected lower-risk PCa, but had no benefit for PCa mortality after 10 years of follow-up. Therefore, for screening and early detection of PCa, men should be offered PSA screening after being given detailed information about the potential risks and benefits and presenting a personalized and risk-adapted strategy to patients with a life expectancy of more than 10 years (EAU 2021).

Early screening for PCa is important for those with family history and inherited germline mutations. Significant cancers were detected at a younger age in male BRCA1 and 2 mutations as a result of PSA screening compared to carriers without mutations (21). Increasing evidence supports the use of genetic counseling and germline testing for early diagnosis and management of PCa. early screening and detection of germline mutations is essential for men with metastatic PCa; men with high-risk PCa and a family member <60 years of age diagnosed with PCa; men with multiple family members diagnosed with PCa at age <60 years, or a family member dying from PCa cancer; with a family history of high-risk germline mutations or a family history of more than one cancer on the same side (22).

PSA is prostate specific but not cancer specific; therefore, it may also increase due to benign prostatic enlargement (BPH), prostate inflammation (prostatitis) and other non-malignant causes. PSA, as an independent variable, predicts PCa better than PRM and TRUS, but serum PSA value is considered as <4 ng/dl despite the lack of a standard PSA value range.3 The European Association of Urology (EAU) prostate guidelines also contains a threshold value. Although not specified, the PSA<2.5-3 ng/dl range is recommended for young men. Broeck et al. reported that those with PSA >1 ng/dl at the age of 40 and PSA >2 ng/dl at the age of 60 are at risk of death due to PCa, and that these patients should be followed up every 2 years. Those who are not in this risk group according to their initial PSA value should be followed up but they suggested that it could be delayed to 8 years (23).

Apart from PSA, DRE is also important for the diagnosis of PCa. PCa develops from the prostate peripheral zone and the tumor volume must be at least >0.2 ml for cancer detection by DRE. At the same time, tumors can be detected in 18% of cases with DRE alone, regardless of PSA level (24). Imaging methods also play an important role in PCa detection. Smeenge et al. (25) reported that standard transrectal USG is not a reliable method for detecting PCa in their study. EAU guidelines suggest multiparametric magnetic resonance imaging (mpMR) for patients undergoing biopsy for the first time or for whom a repeat biopsy is recommended due to elevated

PSA to be performed before biopsy. If suspicious areas are detected in imaging (PI-RADS 3 and above lesion description), targeted and systematic biopsy should be performed by the transrectal route. TRUS Bx recommends taking at least 10-12 core biopsies from each lobe and region (EAU 2021 guideline). The most commonly used histological grading system pathologically after TRUS bx is the Gleason score. The Gleason score ranges from 2 (1+1) to 10 (5+5). It is calculated by adding the most common primary pattern and the second most common secondary pattern forming the tumor in the evaluated tissue and finding a value between 2-10 (International Society of Urological Pathology (ISUP) 2014). Treatment of localized PCa is radical prostatectomy. It is recommended for patients with organ-confined disease and a life expectancy of more than 10 years (23). Radiotherapy is another method that provides biochemical control and survival, similar to radical prostatectomy in the same patient group, and the standard recommended dose is 74-80 Gy (26). Hormone suppression therapy combined with radiotherapy, as well as luteinizing hormone-releasing hormone (LHRH) analogue therapy, are applied for 3 months, between 6 months and 3 years in medium and high risk groups, and its superiority was proven in this risk group compared to radiotherapy treatment along (27-29).

If the results of our study are evaluated in light of the above information, family physicians most frequently refer patients with PSA values of 7.5 ng/dl and below to our urology clinic. At the same time, the most sensitive age group in the patient group referred for PSA elevation was 68 years, while the most specific age group was 48 years. Although only 9 (8.8%) of the referred patients had a family history, these patients were screened for PSA under the age of 45 years in line with the literature. Although the lowest patient age was 40 years and the oldest was 75 years, the median age of the patients was 52 years. In light of this information, PSA screening was performed at around the age of 50 years in accordance with the literature, and the screening reached the target age group. As standard, according to the studies described above and EAU 2021 guideline recommendations, every patient with high PSA level, regardless of the PSA value, had prostate lesion screening with mpMR imaging and PIRADS risk classification before the biopsy. It was observed that the PIRADS risk classification in mpMR is relatively higher in patients over 50 years of age (4 and above), leading to more biopsies and more prostate cancer detection. Another issue that deserves attention is that only 52 (50.9%) of the 102 patients referred to us with PSA elevation were biopsied. Fifty patients who were not biopsied were called for 3-month PSA follow-up because the PSA value fell below 4 after antibiotic treatment, they were PIRADS 3 and below in mpMR images, and the control PSA value checked in our clinic was lower than the PSA value from family medicine center. One of the remarkable issues in our study are the results of the patients who underwent biopsy. Cancer was not detected in only seven (13%) of the patients who underwent biopsy. The most common pathological Gleason score in 45 (87%) patients with cancer was 3+3 (46.2%); in other words, most of the patients were caught at an early stage and this demonstrates the importance of screening. The first definitive treatment choice of patients with PCa as a result of biopsy was radical prostatectomy, with statistical significance, and cancer patients over 50 years of age choose surgery relatively more often. Among the patients with PCa as a result of biopsy, only one patient had metastatic disease (bone metastasis; M1b metastatic group), was referred to oncology and received chemotherapy (CT).

CONCLUSION

The issue of screening in prostate cancer is still controversial. An individualized, risk-adapted strategy for screening should be established. A shared decision-making process with the patient should be adopted, along with detailed information about the reasons for and consequences of PSA screening. At the end of the shared decision-making process between the family physician and the patient, if PSA screening is decided; instead of looking at PSA alone, digital rectal examination together with PSA should definitely be included in the screening. At the same time, PSA elevation does not mean that the patient has prostate cancer; it should be explained that PSA may increase due to many conditions. In addition, patients with PSA elevation were not directly biopsied by urology, control PSA was examined after treatment in patients with urinary tract infection, mpMR was taken before biopsy and biopsy was performed for those with 3 or more lesions according to PIRADS staging, other patients were followed up with 3-month PSA checks, if necessary. It should be noted that new mpMR imaging was performed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Adana City Training and Research Hospital Clinical Researches Ethics Committee (Date: 2021, Decision No: 1595).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have participated in the design, execution, and analysis of the paper, and that they approved the final version.

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Original Article

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Muscle hydatid diseases: percutaneous treatment with Örmeci technique

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ABSTRACT

Aim: To present results of percutaneous treatment with Örmeci technique for muscle hydatid diseases.

Material and Method: Twelve patients (9 male, 3 female) with 16 hydatid cysts (10 CE Type 1, 6 CE Type3B) were treated by percutaneous treatment with Örmeci technique. The percutaneous puncture was performed under sonographic guidance using a 22-gauge Chiba needle as a one-step procedure in CE type 1 and 3A. However, two to six Chiba needles according to size of the cysts were used in different locations at the same time in the cyst of CE type 2, and 3B). For every 1 cm of the long diameter of the cyst lesion, 3cc of fluid from the cysts was aspirated, which was almost the same amount of cc in volume for the CE type 1 and CE type 3A hydatid cysts. A 2cc of pure alcohol (96 %) and 1cc of polidocanol 1% (ethoxysclerol 1%, Kreussler Pharma, Wiesbaden, Germany) were injected into the cysts right after the aspiration of CE type 1 and type 3A, without the aspiration of CE type 2 and 3B, for each centimeter of the long diameter of the cysts. The total amount of pure alcohol and polidocanol were injected equally among the CE type 2 and type 3B cyst's needles. It was waited for five minutes for all scolexes to be killed and the needle/or needles were taken back. The patients with hydatid disease were followed up mean 34.75 ±14.39 (maximum 65-minimum 15) months.

Findings: Fifteen out of 16 hydatid cysts (93.75%) cured. We had two complications of treatment. One patient had an abscess in the cyst after the percutaneous treatment. After the percutaneous drainage, patient was cured well, and he had no symptoms during the follow up. Another patient had torpidity in his leg after the treatment. After three months, he had no symptoms.

In Conclusion, Percutaneous treatment with Örmeci technique is outpatient based, successful, safe, repeatable, cheap and It can be used as an alternative treatment in selected patients.

Keywords: Hydatid cyst, percutaneous treatment, Örmeci technique

INTRODUCTION

Hydatid disease (HD) is still an important health and economic problem especially in endemic areas such as Turkey, Eastern Europe, Eastern Africa, South America, Australia, and New Zealand; in which livestock grows up. The larvae of *Echinococcus granuloses* causes the HD in Human as an intermediate host. Normally, adult parasite resides in the intestine of carnivores. Several thousand eggs of parasite are casted by feces in every day. Human being is accidentally infected by those eggs. When the egg is ingested by the host, larvae of *Echinococcus granuloses* hatches and penetrates intestinal wall and comes to the liver by the portal vein. If it pass through the filters of liver and lungs, it may reaches any organ like muscles in the body. When it resides in any organ, grows 1-3 cm in diameter in a year; the bigger the more complications occur. It compresses the vital organs, fistulize into the spaces like peritoneum, pleura, bile ducts. Besides, the disease gives big economic burden on the endemic countries. It was found that the total cost of a single hospitalization, including hospital stay, surgical intervention, personnel,

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drugs, and administrative costs ranged from €.5,874 to 23,077 (median €.11,033) per patient in Pavia, Italy (1).

Hydatid disease mainly locates in the liver (70%) and the lungs (20 %). Musculo-skeletal system is one of the rare locations of this disease; and it varies 0.5-5 % in the literature (2).

Aim of this study is to present the results of percutaneous treatment with Örmeci technique for 12 patients with 16 muscle HD.

MATERIAL AND METHOD

The study was carried out with the permission of Ankara University Faculty of Medicine Ethics Committee (Date: 26.10.2015, Decision No: 16-689-15). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Nine hundred forty-two patients with 1367 cysts were diagnosed as hydatid disease among the ones who applied to Ankara University Medical School, Department of Gastroenterology. Sixteen patients with 24 cysts out of 1367 (1.36 %) were diagnosed muscle hydatid cyst (**Figure 1**). Three patients were not treated because two of them received Albendazole and one patient had type 4 hydatid cyst which is not necessary for the treatment. Four out of twenty-one hydatid cysts located both in the muscles and liver. One patient was lost to follow up. Twelve patients with 16 hydatid cysts included in the study. Nine out of 12 (75%) were males, three of them were females (25%), mean age was 38.83±12.27 year. Ten cysts (62.5%) were CE Type 1, six out of 16 (37.5%) was CE Type 3. Eight cysts (50%) located at upper part of left leg, while five cysts (31.25%) placed upper part of the right leg. One cyst on the right side, another cyst on the left side located at left lower part, lower extremity. One cyst located at right thoracal paraspinal area. Mean diameter of the cysts was 60.15±37.35 mm. Seventeen out of 21 hydatid cysts were in the muscle (80.95%, primary) and four out of 21 cysts (19.05%, secondary) were in the liver in thirteen patients. Baseline characteristics of the patients with muscle hydatid diseases is seen in **Table 1**.

The patients were diagnosed hydatid disease by ultrasonographic findings which were described by Gharbi HA and WHO-Informal Working Group on Echinococcosis improved Gharbi's classification for the diagnosis of echinococcosis in 2003 (3,4). Besides that On the MRI examination; occurrence of the hydatid matrix, daughter cysts, floating membranes in the cyst cavity, the wall of hydatid cyst which was shown as characteristically low signal intensity, as "rim" on T2 weighted MR images was accepted as hydatid disease.(5). Similarly, On the CT examination; occurrence of water attenuation cyst with well-defined, all daughter cysts which appear as round, peripherally located cystic lesions in the mother cyst, the high density fluid surrounding the daughter cysts which appears like "spoked wheel pattern", small, round daughter cysts among the solid matrix in the mother cyst, detached germinative membrane from the ectocyst which appear floating, thin and hypodense membrane which is called "water lily pattern" was accepted as hydatid disease (5).

Table 1. Basic characteristics

Parameter	Mean±Std. Dev (max-min)			Number (%)
Age	38.83±12.27 (70-21)			
Follow-up	34.75±14.39 (65-15 months)			
Baseline Diameter	60.15±37.35 (160-25)			
Hydatid Cyst Type 1				10 (62.5%)
Hydatid Cyst Type 3				6 (37.5%)
Gender (Male-Female)				9 (75%) 3 (25%)
Primer-Seconder Cysts				13 (81.3%) 3 (16.8%)
Hydatid Cyst Localization	Lower Extermity	Upper part	Right	5 (31.25%)
			Left	8 (50%)
		Lower part	Right	1 (6.25%)
			Left	1 (6.25%)
	Medulla Spinalis			1 (6.25%)

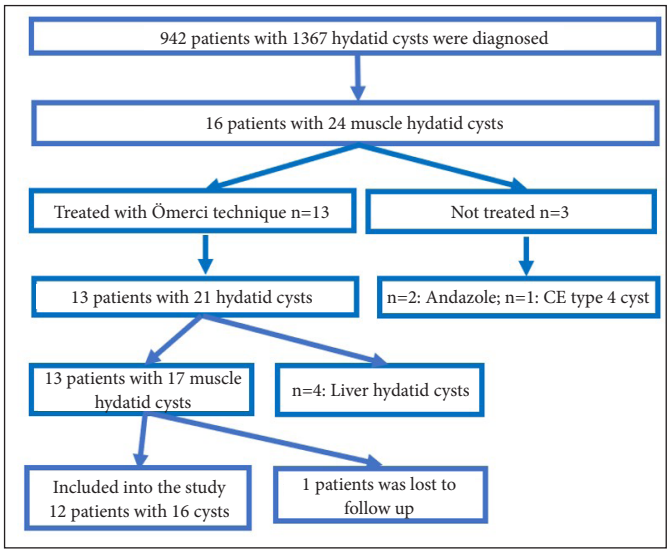


Figure 1. Flowchart of the patients for the study

All alive hydatid cysts were treated by percutaneous way with Örmeci technique. The patients with dead hydatid cyst were excluded from the study. All patients signed an additional informed consent before the enrollment. The study conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013). All procedures were performed under sonographic guidance in the ultrasonography unit of a gastroenterology department that was fully equipped

against an emergency condition. An intravenous line was established. The patients were positioned according to the location of the cysts as face-down or supine position. All patients were given 5mg of meperidine and 40mg methyl prednisolone as sedo-analgesia just before the procedure. The percutaneous puncture was performed under sonographic guidance using a 22-gauge Chiba needle as a one-step procedure in CE type 1 and 3A. However, two to six Chiba needles according to size of the cysts were used in different locations at the same time in the cyst of CE type 2, and 3B. For every 1 cm of the long diameter of the cyst lesion, 3cc of fluid from the cysts was aspirated, which was almost the same amount of cc in volume for the CE type 1 and CE type 3A hydatid cysts. A 2 cc of pure alcohol (96 %) and 1cc of polidocanol 1% (ethoxysclerol 1%, Kreussler Pharma, Wiesbaden, Germany) were injected into the cysts right after the aspiration of CE type 1 and type 3A, without the aspiration of CE type 2 and 3B, for each centimeter of the long diameter of the cysts. The polidocanol (1%), had been used by our group for the first time to close the connection among the cysts and blood vessels, lymphatic vessels and/or biliary ducts since 1991. The total amount of pure alcohol and polidocanol were injected equally among the CE type 2 and type 3B cyst's needles. We waited for five minutes for all scolexes to be killed and the needle/or needles were taken back. This technique was reported before by our group(6). Although CE Type 2 and Type 3B are difficult to treat compared to CE Type 1, we added Albendazole 10 mg/kg/day for 6 months right after the percutaneous treatment. All patients were followed up for the function of vital organs for two to three hours and the patients were sent to their home. The patients were followed up one day, three months and six months after and each year by USG in terms of treatment criteria, blood checking and sometimes CT or MRI.

Every patient was evaluated in terms of treatment criteria as follows:

a. Diameter of the cysts before and after the treatment
b. Detachment of germinative membrane especially in CE Type 1 hydatid cysts
c. Degeneration and pseudo-solidification of the cysts
d. Weather is there any daughter cyst in mother cyst or not?

A statistical analysis was performed using Statistical Package for Social Sciences for Windows version 11.5.(SPSS Inc.; Chicago, IL, USA). Descriptive statistics were summarized as counts and percentages for categorical variables, mean and standard deviations and median (minimum and maximum) for others. The difference between the two dependent groups was evaluated with a Wilcoxon test. A p value less than 0.05 was considered significant.

RESULTS

The patients with hydatid disease were followed up mean 34.75 ±14.39 (maximum 65-minimum 15) months. Diameter of the cysts were decreased after the treatment compared before in 11 out of 16 cysts (P=0.036). Right after injection pure alcohol (96%) and polidocanol 1 % germinative membrane whitened, riddled and detached from ectocyst in 6 (60%) out of 10 Type 1 hydatid cysts. Right after the treatment of type 3 hydatid cyst, daughter cysts distorted, whitened and they showed degenerative changes on the ultrasonographic examinations. (**Figure 2**). Most of the patients showed pseudo-solidification during the follow up. On the MR examinations, it was shown that daughter cysts degenerated, solidified, and decreased the diameter of the mother cysts (**Figure 3A, 3B, 3C, 3D, 3E, 3F, 3G**). Fifteen out of 16 hydatid cysts (93.75%) cured. One out of 16 cysts (6.25%) unchanged (**Table 2**).

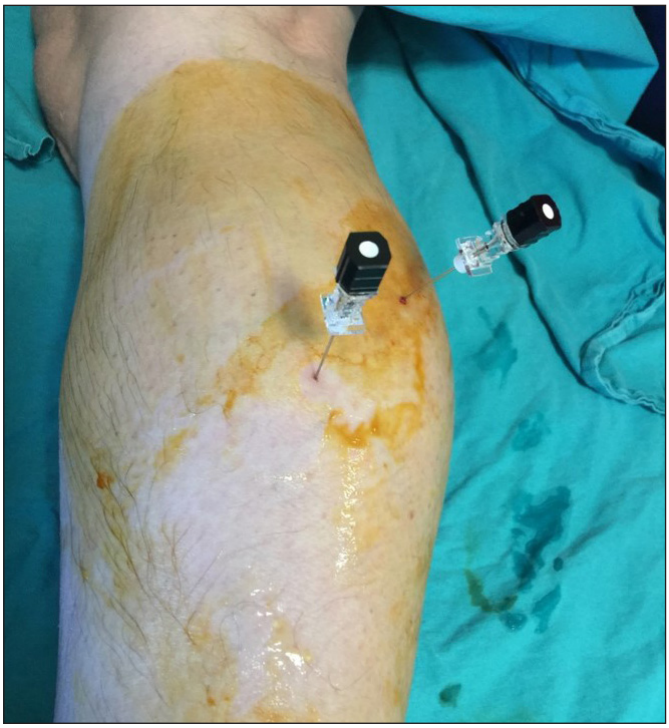


Figure 2. Left leg is swollen because of CE type 3 hydatid cyst in musculus gastrocnemius.Two needles were placed into the cyst at the same session

Table 2. Treatment outcomes of the cysts

	Mean±Std. Dev	Count (Percent)
Previous diameter	60.15±37.35	
Diameter after the treatment	39.75±36	p=0.036
Unchanged		1(6.25%)
Pseudo-solidification 1/3		2(12.5%)
Pseudo-solidification 2/3		1(6.3%)
Pseudo-solidification 3/3		12(75%)
Detachment of cyst germinative membrane		6(37.5%)
Decreasing of the cyst diameter		11(68.8%)

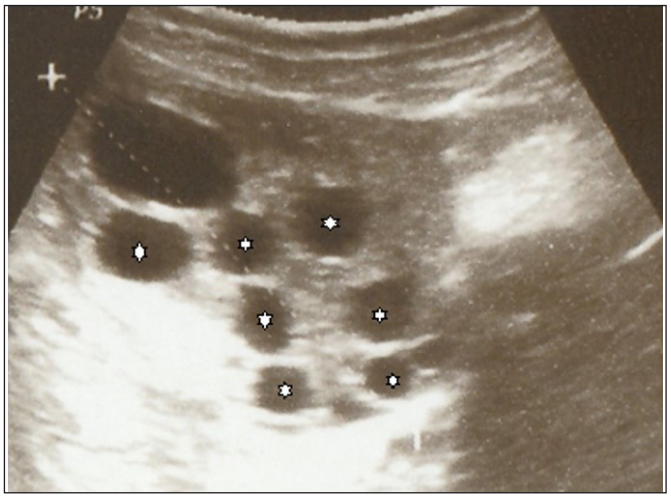


Figure 3A: 44-year-old man with cystic echinococcosis (CE). A well-circumscribed multiple daughter cyst (asterisks) with solid content (CE 3b according to WHO) is observed in the muscle. The cysts appear anechoic with smooth contour and do not contain calcification. The solid matrix appears isoechoic with neighboring muscle structures

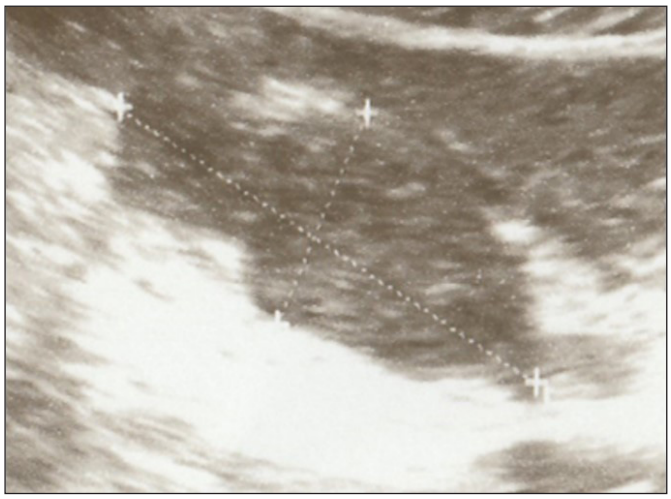


Figure 3B: One-year follow-up ultrasonography after the treatment shows that the cystic lesions are hardly noticeable, and the lesion is almost completely solidified

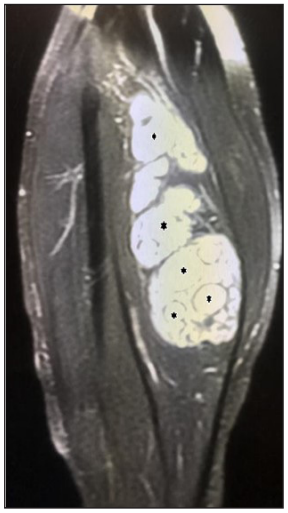
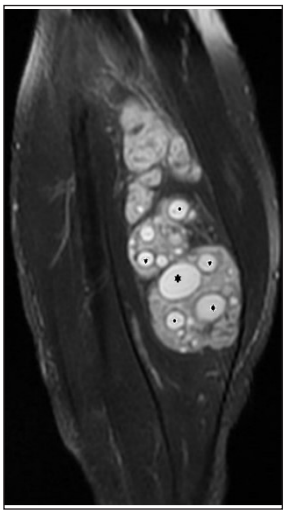


Figure 4A: 37-year-old man with cystic echinococcosis (CE). A large, well-circumscribed semisolid cystic lesion (CE 3b according to WHO) containing a big cyst and multiple daughter cyst (asterisks) with different sizes is observed in the muscle



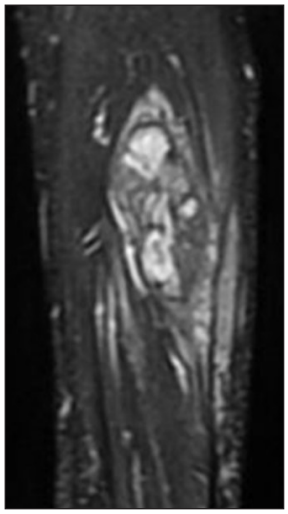


Figure 4B and 4C. Two follow-up ultrasonography's performed one- year- periods after the treatment show that the cystic lesion has solidified

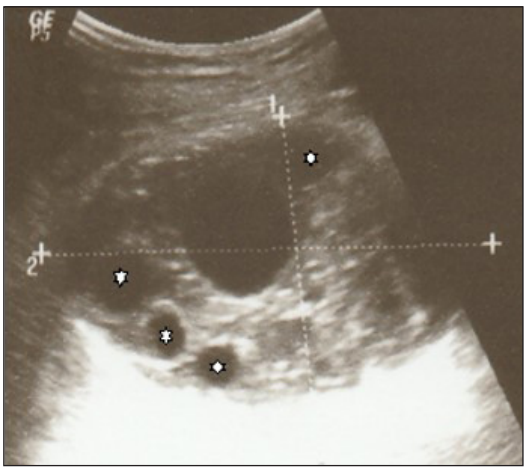


Figure 5A. 44-year-old man with cystic echinococcosis (CE 3b according to WHO). (Same case as Fig.3). A Coronal T2-weighted SPAIR (Spectral Attenuated Inversion Recovery) MR image. A well-circumscribed multiloculated semisolid lesion is observed in the crura region. Multiple, uniformly demarcated, and homogeneous internal structured daughter cysts (asterisks) are present in the lesion. The cysts appear hyperintense on T2 weighted image. The walls of the cysts are seen as hypointense on T2 weighted image (rim sign). Solid matrix of the lesion is seen slightly hyperintense relative to neighboring muscle structures on T2 weighted image. Calcification has not been detected yet

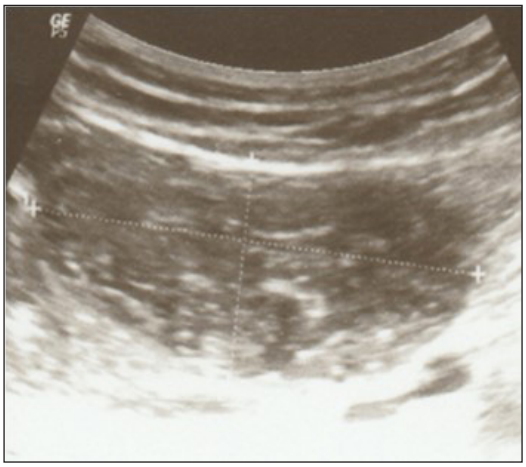


Figure 5B. Coronal T2-weighted SPAIR MR image, after 1 year, overall size of the lesion is constant, but there is minimal reduction in the size of the cysts

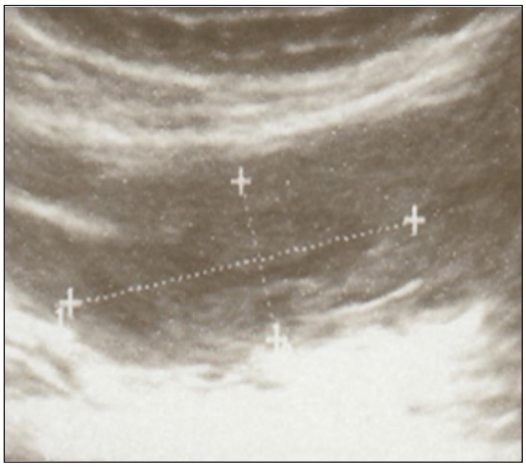


Figure 5C. Coronal T2-weighted SPAIR MR image, there is a decrease both number and the size of cysts in the 2nd year follow-up after treatment. In addition, a decrease is also observed in the size of the mass itself

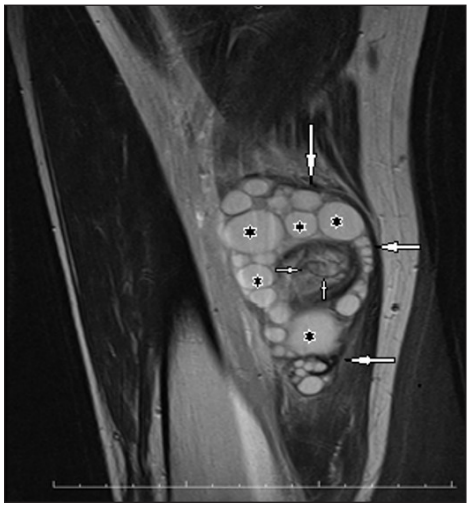


Figure 6A. 37-year-old man with cystic echinococcosis (CE 3b according to WHO). (Same case as Fig.4). A Sagittal T2 weighted turbo spin-echo MR image. The lesion containing cysts of various sizes (daughter cysts, asterisks) and floating membranes (arrows) is observed within the Vastus muscles. The peri cyst (long arrows) of the lesion which appears hypointense on T2-weighted image (rim sign). The daughter cysts appear homogeneous hyperintense on T2 weighted image

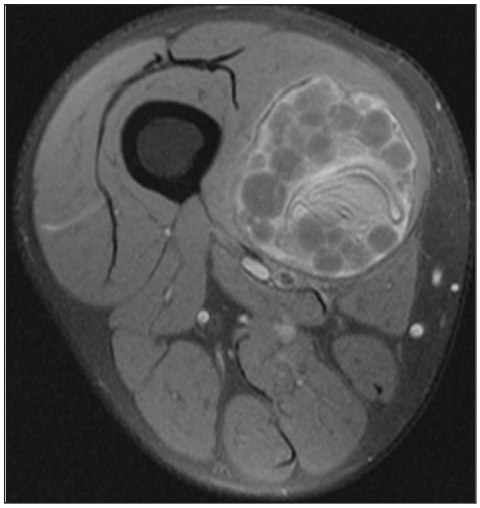


Figure 6B. Axial post-contrast fat sat T1-weighted turbo spin-echo MR image, the peripheral type of enhancement is seen on the cyst itself and the cystic components

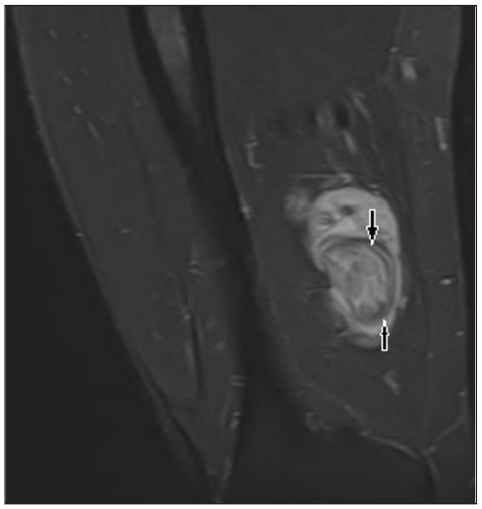


Figure 6C. Coronal T2-weighted SPAIR and Fig 6D: Axial post-contrast fat sat T1-weighted turbo spin-echo MR images, one year after the treatment, a decrease in lesion size and the detached endocyst (black arrows) are seen

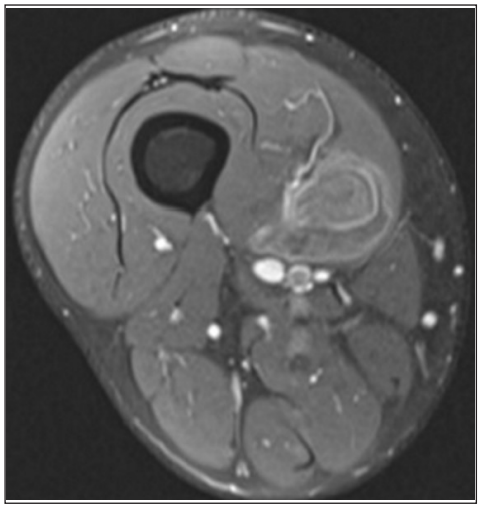


Figure 6D. axial post-contrast fat sat T1-weighted turbo spin-echo MR images, one year after the treatment, a decrease in lesion size and the detached endocyst (black arrows)are seen

DISCUSSION

Muscle hydatid disease is rarely seen in almost 0.5-5 % of all hydatid cysts 5. We found the incidence of muscle HD in 1.36 % among 1367 hydatid cysts in our series. The liver (70%) and the lungs (20%) are the most common locations of the disease because the liver and the lungs are natural mechanical filters of the oncosphere of hydatid cyst. If the oncosphere can pass through the liver and lungs, it can place in any organ like spleen, kidneys, muscles, brain, and bones by the systemic circulation. In primary muscle HD, the cyst located in only muscles. However, in the secondary HD, cysts place not only in muscles, but also in other organs such as the liver, the lungs or peritoneum. In this case series, it was found that the incidence of primary and secondary muscle hydatid cysts was 81.3% and 16.8%, respectively. It is difficult for oncosphere to place in the muscles because of lactic acidosis which occurs in working muscles. The need of

oxygen also increases when the hydatid cyst growing up and oxygenation of the cyst become difficult due to lactic acidosis. Another point is permanent contraction of muscles which gives rise to restrict of it to place in the muscles (2). The most common localizations of the hydatid cyst at the muscles are proximal part of the thigh, pelvis, paravertebral region, shoulder and humerus due to large volume of muscles and rich vascularization (2, 7) Most of the cysts (81.25%) located in proximal parts of the lower extremities in this series.

Usually, the patients with muscle hydatid cyst have no symptoms but one third of the patients may present palpable mass with pain on the thighs or hips. Serologic examinations for antigens of hydatid juice such as ELISA, LATEX agglutination test, immunoblotting test in muscle hydatid diseases is found negative on most of the cases (8).Ultrasonography is the method of choice for diagnosis of the disease. Gharbi (3) described the diagnostic criteria of hydatid disease on ultrasonographic examination in 1981.WHO-Informal Working Group on Echinococcosis improved Gharbi's classification for the diagnosis of echinococcosis in 2003. Ultrasonography has several advantages compared to CT or MR such as easy to find everywhere, no radiation, cheap, high diagnostic sensitivity and specificity (9).It can be decided easily stage of cyst, number of the cyst and distribution of the cyst(9).

Anechoic cyst with posterior acoustic enhancement and double wall sign, hydatid sand (it is dispersed in the cyst an appear as falling snowflakes "snowstorm sign" when the patient turn on right or left side), detachment of germinative membrane from the ectocyst "water lily sign", multivesicular cyst (multiple daughter cyst which are seen as anechoic, round cysts in the mother cyst are some of the characteristics of hydatid disease on the ultrasound examinations(3-5).(8) Daughter cysts locate at the peripheral areas in the mother cyst. After becoming mature, they fully filled inside of the mother cyst which is named "spoked wheel pattern(10).

On the CT examination, CE Type 1 cyst appears as water attenuation cyst with well-defined wall. CE type 2; daughter cysts appear as round, peripherally located cystic lesions in the mother cyst. After becoming mature, the high-density fluid surrounding the daughter cysts appears like "spoked wheel pattern". Small, round daughter cysts among the solid matrix in the mother cyst is described as CE type 3b. Detached germinative membrane from the ectocyst appear floating, thin and hypodense membrane which is called "water lily pattern" (CE type 3a). Calcification , infection in the cyst, peritoneal and bones invading of the cysts are shown best on CT examination.(5)

On the MRI examination, hydatid cyst appears hypointense on T1 weighted images and hyperintense on T2 weighted images. The hydatid matrix, daughter cysts, floating membranes in the cyst cavity are clearly seen compare to CT (10).The wall of hydatid cyst is shown as characteristically low signal intensity, as "rim" on T2 weighted MR images (5).

Differential diagnosis should be performed among hydatid disease and single or multiple hemangiomas, fungal, pyogenic or amoebic abscess, neoplasia with hemorrhage and/or necrosis, metastasis, post-surgical sequela and textiloma, lipoma, myositis, Tbc, aneurism, hernia synovial cyst etc. (9,11)

Stojkovic et al. (12) reported that 711 patients with 1308 liver and peritoneal hydatid cyst were treated by benzimidasole derivatives. They estimated that 40 % of the cysts are active or become active after two years. Similarly, Franchi C et al. (13) treated 448 patients with 929 hydatid cysts by mebendazole or albendazole for 3-6 months. After the long term (1-14 years) follow up, 74.1% of patients were shown degenerative changes, however almost 25 % of those cyst relapsed again. Benzimidazole derivatives are not recommended for the treatment of hydatid disease alone. They can be combined with before and after surgical treatment and Puncture, Aspiration, Injection, Re-aspiration (PAIR) Technique.

The combination of surgical and albendazole treatment is the method of choice for the treatment of hydatid diseases (1,11,14-18). Togral et al. (19) treated 5 patients with muscle and bone hydatid disease by the combination of surgical and albendazole treatment. One patient had relapse after 1-9 years follow up. Arazi et al. (11) treated 15 patients (n: 7 muscle, n:8 bone) with hydatid diseases by the combination of Albendazole and Surgical treatment. Four patients with bone hydatid disease had relapsed during the follow up for 30 months. However most of the case treated by surgery related the muscle HD reported cure without relapse (20).

Surgical treatment has some disadvantages such as large incision, longer hospital stays and expensive. Battelli G et al. (21) reported that while a patient who was operated on hydatid disease cost 14 000 USD, compared to the one who was not operated on hydatid disease cost 2500 USD.

PAIR technique had been using since 1985, as an alternative method compared to surgery for the treatment of hepatic hydatid diseases because of short hospital staying, less mortality and morbidity and cheaper than surgery. However, PAIR method and catheterization techniques for the treatment of hydatid disease with extrahepatic localization may have more complication and longer hospital stay. Arslan et al. (22) treated with 27 patients, PAIR (n:20) and Catheter technique (7 patients)

with extrahepatic localizations. Hospital staying was mean 2.3 days (1-14 days), occurrence of abscess was 16.6%.

Percutaneous treatment with Örmeci technique had been using since 1991 (2,23-26). This technique has some advantages such as, outpatient bases, no fistula occurrence, repeatable, less morbidity, cheaper, easy to use compared to PAIR method (27). We have published preliminary report related hydatid cysts in muscle in 2007(2).

In our best knowledge, this is the largest series about percutaneous treatment of muscle hydatid disease in the literature. Sixteen hydatid cysts in 12 patients were treated by percutaneous way. Fifteen out of 16 hydatid cysts (93.75%) cured. Two patients who had CE type 3 hydatid cysts had second puncture due to incomplete treatment of the first attempt. One patient who had CE type 3B hydatid cyst, 135×120×65 mm in diameter could not be cured. Ten CE type 1 and 3 CE type 3 were cured well after the treatment.

We had two complications of treatment. One patient had an abscess in the cyst after the percutaneous treatment. After the percutaneous drainage, patient was cured-well and he had no symptoms during the follow up. Another patient had torpidity in his leg after the treatment. After three months, he had no symptoms. The weak point of this study is to be retrospective study.

CONCLUSION

Percutaneous treatment with Örmeci technique, is useful in patients with muscle hydatid disease. It is successful, safe, repeatable, easy to perform and cheap. It can be an alternative treatment in patients who had contraindications to surgical treatment or who do not accept the surgical treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara University Faculty of Medicine Ethics Committee (Date: 26.10.2015, Decision No: 16-689-15).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Predictors of colonic diverticulosis in non-elderly patients

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ABSTRACT

Aim: To investigate the clinical and laboratory features patients under aged 65 years with diverticulosis and to compare them to subjects with no diverticula.

Material and Method: This retrospective case-control study included subjects aged under 65 years who underwent a colonoscopy in the period from January 2016 to June 2018 for diverse indications. Patients with diverticulosis as detected by a colonoscopy were compared to patients without diverticulosis. The comparison parameters included demographic data, comorbidities, and laboratory parameters, including a complete blood count, blood biochemistry, erythrocyte sedimentation rate (ESR), and C-reactive protein.

Results: The study included 129 patients with diverticulosis and age and sex-matched 130 patients with no diverticula. Diverticula were predominantly left-sided in 64.3%, right-sided in 9.3%, and bilateral in 26.4%. Hypertension was more prevalent among patients with diverticulosis compared to control subjects (31% vs 17%, p<0.01). The median ESR was higher in diverticulosis group than in control group (13±1.6 mm/h vs 9±0.9 mm/h, p=0.01). The mean creatinine was higher (0.86±0.25mg/dL vs 0.76±0.19 mg/dL, p<0.01) and the mean albumin was lower (4.3±0.4 g/dL vs 4.5±0.3 g/dL, p<0.01) in diverticulosis group compared to control group. Hypertension, ESR, creatinine and albumin were independent predictors of the presence of diverticulosis in univariate and multivariate logistic regression analysis.

Conclusion: It is important to determine the characteristic features of asymptomatic patients with diverticulosis in earlier ages. The presence of hypertension, in conjunction with high creatinine and low albumin values increase the probability of the presence of asymptomatic colonic diverticula.

Keywords: Albumin, colonic diverticula, creatinine, hypertension, non-elderly

INTRODUCTION

Colonic diverticulum is the herniation of the mucosal and submucosal layers outward due to deficiency in the muscle layer of colon. Although diverticulosis is an anatomical definition indicating the presence of a single or multiple diverticula in the colon in asymptomatic individuals, diverticular disease is defined as the presence of symptoms associated with diverticula and is observed in approximately 25% of patients(1). While several complications including diverticulitis, bleeding, obstruction, and perforation may develop in the course of disease in some of the cases, most of the symptomatic patients remain complication free for a long time(2). Older age, genetic predisposition, constipation, high intake of red meat, low-fiber diet, low level of physical activity, obesity and smoking have been linked as predisposing factors for the development of diverticulosis(3, 4).

Geographical location influences diverticulosis prevalence, and is more common in Western countries than in Africa and Asia. In Western countries, diverticulosis affects around 30% at 65 years, and up to 66% in those aged 80 years or older(5). On the other hand, the prevalence of colonic diverticulosis has been reported between 1.9% and 20% from Asian countries and the Mediterranean area(6, 7). Moreover, the location of diverticula varies among nations. While a dominancy of left-sided location is seen in Western populations, right-sided location is more prevalent in Asia.

Diverticulosis is an age-related disorder that is rare under age 40 years and is found in more than one-half of adults over the age of 65 years in Western countries. Diverticulosis can occur in younger ages in several connective tissue disorders like Marfan or Ehlers-Danlos

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syndromes(8). Right-sided diverticulosis can also appear in younger ages and presents predominantly with gastrointestinal bleeding.

Most of the studies about colonic diverticulosis have been carried out on geriatric population. There is little known about colonic diverticulosis in patients under 65 years old. In this study, we aimed to investigate the clinical and laboratory features of patients under 65 years who developed diverticulosis and to compare them to age and sex-matched subjects with no diverticula.

MATERIAL AND METHOD

Study Subjects

This retrospective case-control study included subjects aged between 18 and 64 years who underwent a colonoscopy for several indications, mostly for colorectal cancer screening, from January 2016 to June 2018. Patients who had diverticulosis on colonoscopic examination were recorded. Sex and age-matched subjects who had no diverticula on colonoscopic examination were also recorded. Subjects who had a history of colitis, colorectal cancer, and colonic surgery, subjects attended to the hospital with lower gastrointestinal bleeding or acute diverticulitis were excluded. Demographic data, co-morbidities including diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HL) and coronary arterial disease (CAD) were recorded. Laboratory parameters, including a complete blood count, blood biochemistry, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were collected from the patients' medical records.

Ethical Considerations

Ethics committee approval was obtained for the study from Firat University Non-Interventional Research Ethics Committee (Date: 19.07.2018, Decision No: 2018/13-18). All the procedures followed were in accordance with the ethical standards of the ethics committee and with the Declaration of Helsinki.

Statistical Analysis

All statistical analyses were performed using the SPSS® statistical package, version 22.0 (SPSS Inc., Amrok, NY, USA) for Windows®. Categorical variables are presented as frequencies and percentages. Categorical variables were appropriately analyzed by the Chi-square test or Fisher's exact test. The Shapiro-Wilk test was used to measure the normality of the data distribution for continuous variables. All the normally distributed continuous variables were presented as the mean and standard deviation while the non-normally distributed continuous variables were presented as the median and standard error of mean. The Mann-Whitney U test was applied for continuous variables without a normal distribution analysis, where appropriate. Univariate and multivariate logistic regression analyses were used to analyze the factors influencing the presence of colonic diverticulosis. In all analyses, the significance level was set at 0.05.

RESULTS

A total of 129 patients aged <65 years with diverticulosis and 130 patients aged <65 years who had no diverticula were recruited into the study. While the mean age of the diverticulosis group was 54.7±7.5 years, the mean age of control subjects was 54.1±7.8 years (p=0.52). Seventy-eight of subjects (61%) in diverticulosis group and 75 of control subject (58%) were male (p= 0.65). Diverticula were located at left colon in 83 subjects (64.3%), at right colon in 12 subjects (9.3%) and pancolonic in 34 subjects (26.4%).

Diabetes was found in 19 subjects with diverticulosis (15%) and, 23 subjects who had no diverticula (18%) (p=0.52). Likewise, hyperlipidemia did not differ between diverticulosis group and control group [24(19%) vs 16(12%), p=0.16]. Coronary arterial disease was another disorder that didn't differ between two groups [10(8%) vs 8(6%) respectively, p>0.61]. On the other hand, HT was more prevalent among subjects with diverticulosis compared to subjects without diverticulosis [40(31%) vs 22(17%), p<0.01].

The laboratory characteristics of the two groups of participants are presented in **Table 1**. The median erythrocyte sedimentation rate was higher in the diverticulosis group than in the control group (13±1.6 mm/hour vs 9±0.9 mm/hour, p=0.01) (**Figure 1**). We could not detect any difference between the groups in terms of CRP (3.4±2.3 mg/L vs 3.0±2.8 mg/L, p=0.15). The mean creatinine value was higher (0.86±0.25mg/dL) in the diverticulosis group when compared to the control group (0.76±0.19 mg/dL) (p<0.01) (Figure 1). On the other hand, albumin was lower in subjects with diverticulosis compared to control subjects (4.3±0.4 g/dL vs 4.5±0.3 g/dL, p<0.01) (**Figure 1**). Other laboratory parameters didn't differ between two groups (for all, p>0.05).

Univariate analysis for the presence of diverticulosis demonstrated that the presence of HT, erythrocyte sedimentation rate, creatinine and albumin were statistically significant independent predictive factors (**Table 2**). Variables that were statistically significant in the univariate logistic regression model were further subjected to a multivariate logistic regression analysis and all of them remained statistically significant independent predictors for the presence of diverticulosis (**Table 3**).

Table 1. Comparison of laboratory features between patients with diverticulosis and patients who had no diverticula

	Patients with tdiverticulosis (n=129)	Patients without diverticulosis (n=130)	p
WBC (x10³/uL)	7302±2259	7100±2011	0.45
Hemoglobin (g/dL)	13.9±1.8	14.3±1.5	0.09
Platelet (x10³/uL)	263±73	257±76	0.55
Fasting blood glucose (mg/dL)	104±30	107±33	0.53
ALT (U/L)*	23±1.3	24 ±1.2	0.43
AST (U/L)*	22±0.8 (18-27)	24±0.8	0.56
GGT (U/L)*	26±3.5	25±6.4	0.16
ALP (U/L)*	72±3.1	72±2.4	0.40
Bilirubin, total (mg/dL)*	0.5±0.04	0.6±0.03	0.11
Blood urea (mg/dL)	32.1± 9.5	30.2±9.8	0.12
Creatinine (mg/dL)	0.86±0.25	0.76±0.19	<0.01
Total protein (mg/dL)	7.3±0.7	7.4±0.6	0.06
Albumin (g/dL)	4.3±0.4	4.5±0.3	<0.01
Triglyceride(mg/dL)*	151±16.2	143±7.8	0.39
LDL (mg/dL)	129± 42	123±35	0.24
HDL (mg/dL)	44±12	45±15	0.69
ESR (mm/h)*	13±1.6	9±0.9	0.01
CRP (mg/L) *	3.4 ±2.3	3.0 ±2.8	0.15

(*) Data are presented as median±SE of mean, WBC, white blood cell count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein

Table 2. Univariate logistic regression analysis for predictors of diverticulosis

	B	OR	%95 CI OR	p
Age	0.010	1.010	0.979-1.043	0.524
Sex, male	0.115	1.122	0.683-1.841	0.650
Diabetes mellitus	-0.219	0.804	0.414-1.560	0.518
Hypertension	0.791	2.206	1.222-3.985	0.009
Hyperlipidemia	0.488	1.629	0.820-3.234	0.163
Coronary artery disease	0.248	1.282	0.489-3.358	0.614
White blood cell count	0.000	1.000	1.000-1.000	0.477
Hemoglobin	-0.127	0.881	0.762-1.019	0.088
Platelet	0.001	1.001	0.998-1.004	0.547
Fasting blood glucose	-0.002	0.998	0.990-1.005	0.533
Alanine aminotransferase	-0.006	0.994	0.977-1.011	0.477
Aspartate aminotransferase	-0.012	0.988	0.962-1.015	0.381
Gamma-glutamyl transferase	-0.001	0.999	0.995-1.004	0.759
Alkaline phosphatase	0.004	1.004	0.995-1.012	0.422
Bilirubin, total	-0.073	0.929	0.468-1.846	0.834
Blood urea	0.021	1.021	0.995-1.048	0.120
Creatinine	2.039	7.682	2.459-24.003	<0.001
Total protein	-0.405	0.667	0.439-1.014	0.058
Albumin	-1.072	0.342	0.173-0.677	0.002
Triglyceride	0.002	1.002	0.999-1.004	0.185
Low-density lipoprotein cholesterol	0.004	1.004	0.997-1.011	0.236
High-density lipoprotein cholesterol	-0.004	0.996	0.977-1.016	0.685
Erythrocyte sedimentation rate	0.033	1.033	1.012-1.055	0.002
C-reactive protein	0.005	1.005	0.992-1.018	0.444

Table 3. Multivariate logistic regression analysis for predictors of diverticulosis

	B	OR	%95 CI OR	p
Hypertension	0.876	2.401	1.242-4.642	0.009
Creatinine	2.628	13.852	3.729-51.457	<0.001
Albumin	-0.761	0.467	0.220-0.993	0.048
Erythrocyte sedimentation rate	0.034	1.035	1.010-1.061	0.007

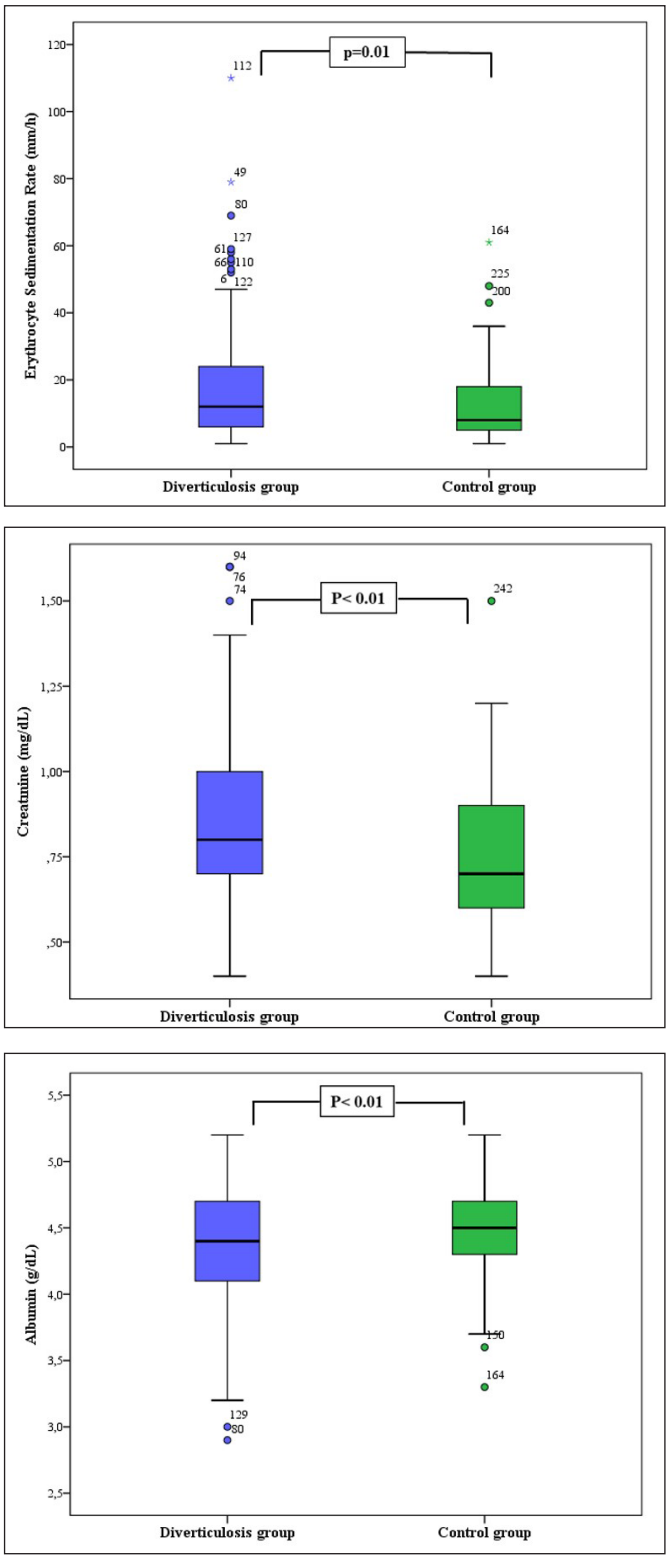

Figure 1. Comparison of erythrocyte sedimentation rate, creatinine and albumin values between diverticulosis group and control subjects

DISCUSSION

Diverticulosis is essentially considered an asymptomatic condition, frequently observed as an incidental finding in patients undergoing radiologic or endoscopic assessment for several indications. Technical advances in radiologic and endoscopic assessment of the colon and widespread use of these tools in the clinical assessment lead to increased diagnosis of colonic diverticulosis. The conventional idea about diverticular disease is that it typically affects older people. Several studies have reported an increased incidence of diverticular disease over time with a meaningful increase in younger aged people (9-11). The incidence of diverticulitis in people aged between 40 and 49 years old increased by 132% from 1980 through 2007 (12). Unlike these results, many time-trend studies demonstrated that there is no increase in the prevalence of symptomatic diverticular disease and hospitalization over time in younger ages (13). Though several studies have been carried out in younger people with symptomatic diverticular disease, especially acute diverticulitis, there is no satisfactory data on the frequency of asymptomatic diverticulosis.

In the current study, we assessed demographic clinical and laboratory features of asymptomatic patients with diverticulosis and, compared with subjects with no diverticula. As stated earlier, right-sided diverticulosis is related with the presentation of diverticulosis in earlier ages of the life, and several reports from Asia give information about the features of subjects with diverticulosis in non-geriatric population. In a prospective study from China on asymptomatic subjects undergoing colonoscopic evaluation that most of them under 70 years old, diverticula were right-sided in two-third of patients with diverticulosis (14). In our study, 64.3% of colonic diverticula were located in the left colon, 9.3% in the right colon, and 26.4% were located bilaterally. The distribution of colon diverticula varies geographically. Left-sided diverticulosis is more prevalent in Western world, Africa and the Mediterranean area. In a previous study from Middle East, 62% of colonic diverticula were located in the left colon and only 13% were located in the right colon (15). In a prospective study from Italy, diverticula were detected in 27.8% of 438 patients undergoing colorectal cancer screening and, 97.8% of diverticula was left-sided (16). Our findings were compatible with the results of studies form the Mediterranean area.

Hypertension is a well-known associated disorder with diverticulosis. In a Japan study on subjects undergoing colonoscopic evaluation aged between 51 and 59 years, HT and DM were observed more frequently in subjects with diverticulosis compared to those without diverticula (17). In the aforementioned study, HT was found to be 31% and 20% between patients with and without diverticula, respectively. Similar results were observed in the Middle East study, and HT, DM and HL were found at higher rates in patients with diverticulosis (15). In addition to the high rate of HT detected as 64% in those with diverticula, it was found that the presence of HT increased the likelihood of colonic diverticulosis by 2.3 times. On the other hand, in a population of aged under 70 years from China, HT was found more prevalent among patients with diverticulosis compared to those with no diverticula (14,17). In the current study, HT, but not DM, was found more prevalent among patients with diverticulosis. On the other hand, we couldn't detect any difference among patients aged over 65 years in terms of HT, DM and HL according to the presence of diverticulosis in our previous study (8). Moreover, a study from Israel showed that DM and hypothyroidism were associated with diverticulosis, while the presence of HT and HL were not (18). Further studies are needed to evaluate the relationship between colonic diverticulosis and associated disorders.

In the comparison of inflammatory markers, although CRP values were similar in both groups, ESR values were higher in patients with diverticulosis. CRP is a useful marker that can be used in the prediction of acute inflammatory conditions such as acute diverticulitis. The fact that our study group consisted of patients with asymptomatic diverticulosis and, the absence of acute inflammation in these patients explains normal CRP values. Leukocytosis, which is another indicator of acute inflammation, was not observed in patients with diverticulosis and, the leukocyte count was similar to the controls. The higher ESR values in patients with diverticulosis may be associated with the ongoing low level of systemic inflammation. C-reactive protein that is a direct acute phase reactant, shows short term alterations related to acute inflammation, while the change in ESR, which is an indirect indicator of inflammation, requires longer time.

In the current study, we found higher values of creatinine and lower albumin values among patients with diverticulosis than those had no diverticula. These results were compatible with our previous study on geriatric patients with diverticulosis (7). There is scarce data about laboratory parameters of subjects with diverticulosis on the literature. The underlying pathological mechanisms that cause the formation of colonic diverticula are likely to be the result of complex interactions among age, diet, colonic microbiota, genetic factors, colonic motility, and changes in colonic structure (19). Several metabolic alterations occur in extracellular matrix components of the colon as a result of aging process. Two major extracellular matrix components, collagen and elastin, have been

found to be altered in diverticulosis. The smaller, more densely packed collagen fibrils and an overexpressed cross linking of collagen molecules with increased rigidity and a subsequent loss of tensile strength occur (20). An increase of elastic fibers confined to the longitudinal muscle layer results in the thickening of the colonic wall (21). Chronic kidney disease (CKD) is characterized by the development of renal fibrosis, which is the inevitable consequence of continuous accumulation and activation of myofibroblasts and extracellular matrix deposition in interstitial space leading to organ dysfunction. Fibrosis occurs in every type of CKD, regardless of the cause, and it contributes to a progressive and irreversible loss of renal function (22). It is suggested that pathogenetic mechanisms that lead to the formation of diverticula are similar to the mechanisms that lead to the development of CKD. Thus, an identical fibrotic process may either cause the development of colon diverticulum and impairment of renal functions. Lower albumin values that were found in diverticular group may be associated with dietary factors or as a result of ongoing low grade inflammation.

There are some limitations to our study that is mainly associated with its retrospective nature. Several pathogenic life-style factors that reported to be associated with colonic diverticulosis; in particular, physical activity, familial and hereditary factors, obesity, and a detailed quantitative dietary history with regard to fiber, red meat and fat intake could not be incorporated into the analyses due to the study design. This is a single-center, retrospective, case-control study with a limited number of subjects undergoing colonoscopic examination. Future prospective studies are necessary to confirm the results of the present study. Third, self-election bias of the participants in the study might exist because, all of the study subjects intended to pay more attention to their health condition. Thus, the current findings cannot be generalized for all subjects under 65 years.

CONCLUSION

Consequently, the diagnosis of colonic diverticulosis has been increasing in younger aged people recently. It is important to determine the demographic, clinical and laboratory features that can help to indirectly identify asymptomatic patients with diverticulosis. Patients with HT was found to have an increased risk for the development of colonic diverticulosis in this study. Higher ESR and creatinine values in conjunction with lower albumin values were found as laboratory features of patients with diverticulosis. Further studies are needed to define the characteristic features that will enable the identification of patients with diverticulosis at an earlier age.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval was obtained for the study from Fırat University Non-Interventional Research Ethics Committee (Date: 19.07.2018, Decision No: 2018/13-18).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Retrospective evaluation of labial fusion in girls

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ABSTRACT

Aim: The aim of this study was to determine the factors affecting fusion in girls treated for labial fusion retrospectively over a three-year period.

Material and Method: The research has a cross-sectional design. The research sample consists of girls with ICD10 code Q52.5 who were brought to the hospital with labial fusion symptoms in a three-year period (January 2018-December 2020). The data were obtained from the hospital information system. Variables that were effective in labial fusion recurrence were determined by Chi-Square analysis, and the marginal effects of effective variables on recurrence were analyzed by Poison Regression analysis.

Results: 52.9% of 308 cases were younger than one year old (mean age 4.36±1.10 months). Symptom presentation is 10.4% in children younger than one year of age, and 84.83% in older. The most commonly presented symptoms are pain, burning, soiling of underwear, and bad odor during urination. Labial fusion recurrence is 14.1% in children younger than one year of age, and 62.8% in older. Manual opening was applied in all cases, and weekly follow-ups were performed with topical treatment. The Poison Regression analysis revealed that a history of allergy (1.31 times; z:3.61, p:0.000), winter (0.86 times; z:3.22, p:0.001), and diaper dermatitis (1.22 times; z:5.19, p:0.000) increased the number of labial fusion recurrence.

Conclusion: The findings of our study are similar to the literature in terms of factors causing labial fusion and treatment type. The recurrence rate was found to be higher in our study. It should be kept in mind that labial fusion is asymptomatic, especially in girls in the first year of life. Considering the possibility of recurrence of labial fusion, mothers and physicians examining the child should be aware of this issue.

Keywords: Labial fusion, labial adhesion, recurrence, manual opening, follow-up

INTRODUCTION

Labial fusion is the adhesion of the labia minora in the midline to cover the vaginal entrance and/or the urethral meatus, usually in girls between the ages of 3 months and 6 years (1). Labial fusion is reported in 0.6% to 5% of girls (1,2). Considering that the cases are asymptomatic and detected incidentally, the frequency of labial fusion may be higher (2).

In labial fusion, symptoms are associated with the pooling of urine behind the attached labia minora. Common symptoms are recurrent urinary tract infection, vulvovaginitis, activity-related perineal pain, post-void urine drip, and urinary retention resulting from complete adhesions of the labia minora (1,2). In girls presenting with these symptoms, physicians should keep in mind that there may be labial fusion, and the diagnosis can be easily made by careful physical examination (3,4).

There is no consensus on the etiology of labial fusion. However, it has been suggested that microtrauma and reepithelialization of the labium minora skin, vulvar irritation and hypoestrogenism may have an effect on adhesion (2,5).

There are opinions that labial fusion heals spontaneously with the production of estrogen at puberty, and that the treatment should be applied only to patients with symptoms in the prepubertal period (1). In the treatment of labial fusion, only case follow-up can be performed, but there are also treatment options with manual removal of adhesions and surgical intervention. The recommendation of gynecologists regarding the treatment of labial fusion cases is the use of estrogen-containing creams and follow-up (5).

Due to the increase in maternal observations and anxiety about adhesions in the labia minora, there has been a significant increase in the number of visits to the

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outpatient clinic compared to previous years. For this reason alone, more clinical attention should be given to the issue of labial fusion and requires a more detailed analysis of factors related to prevention.

The aim of this study was to determine the factors affecting fusion in girls treated for labial fusion retrospectively over a three-year period.

MATERIAL AND METHOD

The study was initiated approval by the Clinical Researches Ethics Committee of the Balıkesir University Medical Faculty (Date: 24.11.2021, Decision No: 2021/257). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The research has a cross-sectional design. The research sample consists of girls with ICD10 code Q52.5 who were brought to the hospital with labial fusion symptoms in a three-year period (January 2018-December 2020). The data were obtained from the hospital information system. For a three-year period, 308 patients with ICD10 Q52.5 code were identified in the hospital information system.

Data from the hospital information system were grouped (Group 1 and Group 2) by age, based on clinical observations of the abundant detection of labial fusion despite the absence of any symptoms in patients under one year of age. In Group 1, where asymptomatic but labial fusion cases were common, families were interviewed by telephone and a questionnaire was applied to determine the factors affecting labial fusion. In the questionnaire, such as patient's age, weight, allergy history, previous infection history, history of labial fusion in sibling, breastfeeding, frequency of diaper dermatitis, use of perineal cleaning products, adhesion season, labial fusion symptoms, number of hospital admission and treatment type variables were questioned.

In the obstetrics and gynecology literature, treatment with estrogen-containing creams is emphasized in labial fusion (6,7). Differently, treatments with estrogen-containing creams are not recommended in pediatric surgery due to side effects detected in infants or children (6,8-11). According to the treatment protocol applied in our clinic for patients presenting with labial fusion symptoms, manual opening is applied in all patients after the approval of the family in the first stage. In some cases, local anesthesia is applied with EMLA® 5% cream before manual opening. In many cases, the fusion can be easily opened manually without the need for this. In manual opening, the labia majora is gently pulled laterally to open the labia minora at the level of the commissura posterior. After manual opening, it is recommended to use epithelial cream (Fucidin 2% cream) and the patient is followed up on a weekly basis. In case of recurrence of labial fusion, manual opening is performed again and it is recommended to use 1% Betnovate cream during the follow-up. The recurrence of labial fusion is followed on a weekly basis. Patient follow-up ends with the disappearance of labial fusion and the family is informed about recurrence.

Statistical Analysis

In the analysis of the data, descriptive statistics were calculated. Variables that were effective in labial fusion recurrence (the number of hospital re-admissions with the same symptom) were determined by Chi-Square analysis, and the marginal effects of effective variables on recurrence were analyzed by Poison Regression analysis. SPSS statistical package (version:23) and Gretl program were used in data analysis.

RESULTS

When the hospital information system was filtered for the three-year research period, 308 patients with ICD10-Q52.5 code (labial fusion) were accessed. When grouped by age, 52.9% (n: 163) of the patients reached were under one year old (Group 1), and 47.1% (n: 145) were over one year old (Group 2).

The mean age of Group1 was 4.36±1.10 months, and only 10.4% (n:17) of these patients admitted to the hospital with labial fusion symptoms. It is noteworthy that labial fusion occurs without symptoms in this group of patients. The labial fusion recurrence rate in this group of patients is 14.1% (n:23) (**Table 1**).

Table 1. Characteristics of cases

	Group 1	Group 2
Number	163 (52.9%)	145 (47.1%)
Mean age	4.36±1.10 months	2±1.44 years
Symptom rate (%)	17/163 (10.4%)	123/145 (84.83%)
Recurrence rate (%)	23/163 (14.1%)	91/145 (62.8%)

The mean age of Group 2 was 2±1.44 years, 84.8% (n:123) of these patients admitted to the hospital with complaints such as pain, dysuria, staining on their underwear, and bad odor during urination. 22.06% (n:32) of these patients were diagnosed with the attention of their mothers or the examination of physicians. Despite treatment, labial fusion recurred in 62.75% (n:91) of this group of patients.

In Group 1, families were interviewed by telephone and a questionnaire was applied to determine the factors affecting labial fusion. The families of 37 patients (22.69%) were contacted by phone and a questionnaire was applied. These patients admitted to the hospital 2.78 times due to labial fusion. 38% of the patients have a history of allergy and the frequency of diaper dermatitis is 3.1 times higher. It was determined that all patients were

fed with breast milk and their perineum was cleaned with wet wipes. None of the patients had a history of urinary tract infection. Labial fusion occurred in 75.70% of patients during the winter months, and all patients were treated with manual opening.

Patients were admitted to the hospital an average of 2.78±0.82 (median:3) times with symptoms related to labial fusion. As a result of chi-square analysis, variables (diaper dermatitis, age, weight, allergy history, season) were found to be effective in labial fusion recurrence (Chi-square: 36.99, p:0.0000). The marginal effects of these variables on recurrence were calculated by Poison Regression analysis (**Table 2**). The analysis revealed that a history of allergy (1.31 times; z:3.61, p:0.000), winter (0.86 times; z:3.22, p:0.001), and diaper dermatitis (1.22 times; z:5.19, p:0.000) increased the number of labial fusion recurrence. The weight of the patients is not effective in the emergence of labial fusion. The age variable differed in the regression analysis. In other words, as the age of the patients increases, the labial fusion decreases and accordingly the admittance to the hospital decreases.

Table 2. Marginal effects of variables

Variables	Coefficient (Marginal effect)	Std. Error	z	p-value
Diaper dermatitis	1.221158	.2353893	5.19	0.000
Allergy history	1.307848	.3622907	3.61	0.000
Season (winter)	.8639268	.2684333	3.22	0.001
Weight	-.4480946	.451941	-0.99	0.321
Age	-.1217027	.0575402	-2.12	0.034

DISCUSSION

In this study, factors affecting patients treated for labial fusion were investigated retrospectively for a three-year period. Labial fusion is a clinical condition seen in the postnatal period (8). Although the etiology is not certain, it is widely believed that allergens, materials used in perineal cleaning and mechanical friction facilitate labial fusion, and urine pooling behind adherent labia is the cause of the symptoms (2). It has been reported that inflammation due to infection or trauma causes erosion and fusion in the epithelium of the labia minora (9,12). Bacon states that the most common reason for recurrence of labial fusion is dermatitis, and the history of allergy (38%) and the winter season (24%) facilitate recurrence (12). Wejde (13) emphasized that perineal ammonia dermatitis causes local inflammation and recurrence of labial fusion. Factors such as late changing of the baby's diaper, diarrhea, allergy and atopic nature, change in stool composition due to transition to complementary foods, zinc deficiency, antibiotic use, materials used in perineal cleaning are effective on perineal dermatitis (13). Similar to the literature, in our study, history of allergy, winter season and diaper dermatitis were effective on labial fusion.

In the literature, recurrence is reported in 7-55% of labial fusion cases (5,6). In our study, the recurrence rate (14-62%) was higher in girls older than one year.

There are different methods in the treatment of labial fusion. Acer (14) stated that labial fusion should be opened in symptomatic cases, but there is no consensus in asymptomatic cases. It has been reported that topical treatment should be the first choice treatment, but treatment failure is also high (6,9). Bacon (12) reported that with the onset of endogenous estrogen production with the adolescence period, the problem will resolve spontaneously and 0.05% betamethasone cream will be sufficient for treatment. Myers (9) states that follow-up with steroid cream is not sufficient and manual opening is necessary for labial fusion treatment. The most common treatment method is manual opening. A cotton papix or feeding tube can also be used to manually open the labial fusion (2,6). Repeated manual or surgical opening may cause labial fibrosis (9). In our study, in accordance with the literature, manual opening was applied in all cases and epithelializing and steroid creams were used gradually in the follow-up of the case. Estrogen-containing creams were not used in any of the cases in our study. Our cases were followed up on a weekly basis until there was no labial adhesions. No labial fibrosis was detected in the follow-ups.

Recurrence of labial fusion is a notable issue (15). Poor perineal hygiene, dermatitis, allergies and sexual abuse are reported as effective variables on recurrence (16-19). Kumetz (20), on the other hand, stated that the frequency of dermatitis, the duration of breastfeeding and the presence of infection were not associated with recurrence of labial fusion. According to Melek et al. (19) recommends medical treatment in recurrent or persistent labial fusion. Berkowitz (21) reports that treatment for relapse with topical estrogen is successful in 35% . In our study, similar to the literature, labial fusion recurrence was quite high and therefore the number of admissions to the hospital was high. Manual opening and topical therapy were used in the treatment of recurrences.

Although the study data are sufficient to determine the factors affecting labial fusion, the hospital-based data can be considered as a limitation of our study.

CONCLUSION

Our study findings have shown that labial fusion can be symptomatic or non-symptomatic. It should be kept in mind that labial fusion is asymptomatic, especially in girls in the first year of life. Considering the possibility of recurrence of labial fusion, mothers and physicians examining the child should be aware of this issue. Mothers should be informed about perineal hygiene, allergies, diaper dermatitis and the effect of changing

diapers frequently. Physicians, on the other hand, should carefully examine each case, be able to manually open the labial fusion when detected, and carefully investigate recurrent urinary tract infections by considering the possibility of labial fusion.

In the future study, it is aimed to inform family physicians about labial fusion, to detect labial fusion in girls early, and to inform mothers about preventing adhesions.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated approval by the Clinical Researches Ethics Committee of the Balıkesir University Medical Faculty (Date: 24.11.2021, Decision No: 2021/257).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Evaluation of preemptive interspace between the popliteal artery and the capsule of the posterior knee (IPACK) block for postoperative pain management in arthroscopic knee surgeries: a retrospective study

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ABSTRACT

Background: Interspace between the popliteal artery and the capsule of the posterior knee (IPACK) block is block, which is done by infiltration of local anesthetic to block terminal branches of genicular nerves and popliteal plexus that innervate the posterior capsule of the knee joint. In this study, we retrospectively reviewed our patient's data to which IPACK block was applied for arthroscopic knee surgery. Our aim was to evaluate the effectiveness of IPACK block on postoperative analgesia, effects on additional analgesic consumption and patient satisfaction.

Material and Method: The data of 60 patients who underwent arthroscopic knee surgery under spinal anesthesia with or without applied preemptive IPACK block for postoperative analgesia were collected between October 2019 and December 2020. Group I consisted of 30 patients with preemptive IPACK block, while 30 patients without block were classified as the control group (Group II). Postoperative 0-1-2-6-12 and 24th hour VAS scores, additional analgesic needs, patient satisfaction scores, were compared in groups as primary outcome.

Results: As a result of the comparison between the groups, it was seen that there was a significant difference between the groups in favor of the IPACK Block group in terms of postoperative VAS scores after 1st hour, postoperative analgesic needs and patient satisfaction ($p<0.001$).

Conclusion: Preemptive IPACK block, performed in patients who will undergo arthroscopic knee surgery, reduces the patient's pain in the postoperative period, shortens the length of hospital stay, positively affects patient comfort and increases patient satisfaction considerably. We believe that IPACK block alone or in addition to other blocks or methods will be effective in preventing arthroscopic knee surgery pain and it will be useful to conduct new studies with more patients.

Keywords: Interspace between the popliteal artery and the capsule of the posterior knee (IPACK) block, artroscopic knee surgery, preemptive, analgesia, peripheric block

INTRODUCTION

Anesthesia applications offer different options to anesthetists. Different techniques can be applied depending on the general condition of the patient, the operation area and the way it is done, the possibilities at hand and the wishes of the patient. In recent years, attempts to reduce health expenditures have been tried. The anesthesia method to be used in surgical procedures is aimed to be a method that will both accelerate the discharge of the patient and provide effective intraoperative and postoperative analgesia, increasing patient satisfaction (1,2).

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Postoperative pain is an acute pain that begins with surgery and gradually decreases with healing of the tissue. Despite the advances in anesthesia and surgery postoperative pain remains a challenging. It is critical to relieve pain, especially to prevent the development of chronic pain, and also to protect patient's cognitive functions and improve the quality of life. Postoperative analgesia for knee surgeries can be achieved by oral and systemic analgesics, intra-articular drug infiltration and regional anesthesia techniques (3-5).

A new technique for posterior knee joint pain is a block of interspace between the popliteal artery and the capsule of the posterior knee (IPACK), which is done by infiltration of local anesthetic to block terminal branches of genicular nerves and popliteal plexus that innervate the posterior capsule of the knee joint. This technique involves very selective blocking of the terminal sensory branches of the posterior aspect of the knee without the involvement of the motor branches of the tibial and peroneal nerves, resulting in reduced pain with no effect on muscle strength (6,7).

In recent years, studies evaluating the effectiveness of the IPACK block have begun to appear in the literature. We applied this relatively new and easy-to-do technique to our patients who were undergoing arthroscopic knee surgery in our training hospital; to learn the technique and to assess the effectiveness on postoperative analgesia.

In this study, we retrospectively reviewed our patient's data to which IPACK block was applied for arthroscopic knee surgery. Our aim was to evaluate the effectiveness of IPACK block on postoperative analgesia, effects on additional analgesic consumption and patient satisfaction.

MATERIAL AND METHOD

This retrospective study was conducted with the approval by the Non-Interventional Clinical Researches Ethics Committee of the Kırıkkale University (Date: 10/12/2020 Decision no: 2020.11.25). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The data of patients who underwent arthroscopic knee surgery under spinal anesthesia with or without applied preemptive IPACK block for postoperative analgesia were collected in our clinic between October 2019 and December 2020.

The preemptive analgesia block application procedure of our clinic is as follows: All patients are taken to the block room, routinely monitored before performing the blocks, nasal oxygen supply is provided, and sedated with 0.03-0.05 mg/kg IV midazolam. After the block is performed, the patients are taken to the operating room.

We perform IPACK block according to methods previously described in literature (8). After patient's knee flexed at supine position, ultrasound (USG) probe (Esaote MyLab 30, Geneva; Italy) is placed from the medial side; femur and popliteal artery are visualized. Then an 18 Gauge 50 mm needle (Pajung,Geisingen,Germany) is advanced in plane until it reaches the gap between popliteal artery and posterior knee joint capsule, where 3 mL isotonic saline is injected to ensure the correct placement; and then 20 mL of 0.5% bupivacaine (Buvasin 0.5%,Vem, İstanbul, Turkey) solution is injected into this space. The cephalic and caudal distribution of local anesthetic is observed by USG (**Figure 1**).

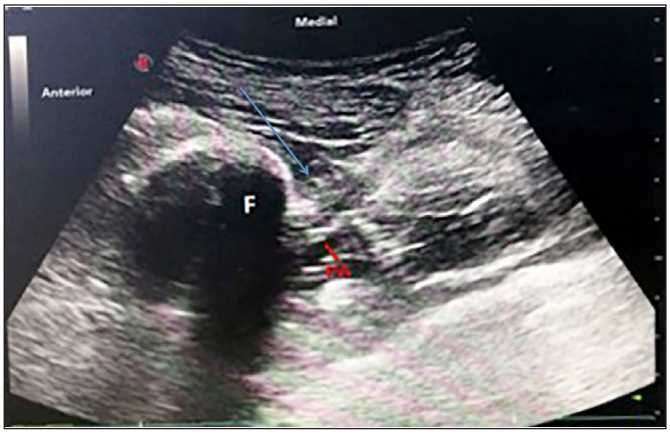

Figure 1. IPACK Block USG image (F: Femur, PA: Popliteal artery)

In accordance with the spinal anesthesia application procedure for arthroscopic knee surgery of our clinic, the procedure is performed in the operating room; with 2.5-3 mL of 0.5% hyperbaric bupivacaine (Buvasin 0.5% Spinal Heavy, Vem; İstanbul, Turkey) at the L3-L4 or alternatively L2-L3 interspaces. For postoperative pain, if there are no contraindications, patients receive tramadol 100 mg IV immediately before the end of surgery.

For our study, the files of patients who underwent arthroscopic knee surgery under spinal anesthesia between October 2019 and December 2020 were scanned first. Among these patients, it was determined that there were 30 patients who had preemptive IPACK block and had the necessary data for the study in their file and these patients were grouped as Group I (IPACK Block). Among scanned patients, those who had spinal anesthesia as mentioned above, but no block was made for preemptive analgesia; and whose files had complete data required for the study were identified. Thirty of them were randomly selected and grouped as Group II (Control).

Demographic data, ASA scores, postoperative 0-1-2-6-12 and 24th hour VAS scores, additional analgesic needs, patient satisfaction scores, presence of complications and adverse effects, and length of hospital stay were collected from the anesthesia and service follow-up forms of the

patients. While assessing patient satisfaction, we use a 5-point Likert scale (9), in which 5: Very satisfied 4: Satisfied 3: Not sure 2: Not satisfied 1: Not at all satisfied.

The data obtained in the study were evaluated using the IBM SPSS Statistics 16 (IBM SPSS, Turkey) (Statistical Package of Social Science) program. Assessment of whether the data are normally distributed was made using the Kolmogorov-Smirnov test. Continuous variables were analyzed using the Student's t Test. Categorical data were analyzed using Chi-square test and expected count analysis were interpreted using Fisher's exact test, Continuity Correction or Likelihood Ratio as appropriate. VAS values are given as median, and all other values are given as mean±standard deviation. P<0.05 was considered statistically significant in all results.

RESULTS

Between October 2019and December 2020, 60 patients scheduled in this study in which 30 patients received IPACK block+spinal anesthesia (Group I) and 30 patients received only spinal anesthesia alone (Group II) for arthroscopic knee surgery. The entire study group included 33 male and 27 female patients of which 16 males and 14 females were in IPACK block group (Group I) and 17 males and 13 males in control group (Group II). The overall demographic perioperative characteristics in both groups were similar (p>0.05) and given in **Table 1**.

Table 1. The patient characteristics in groups

	Group I (IPACK Block) n=30	Group II (Control) n=30
Age (years)	47.8±16.5	42.5±17.4
Sex (M/F)	16 / 14	17 / 13
Weight (kg)	79.1±13.5	78.9±13.2
Height (m)	1.68±0.1	1.69±0.1
Duration of surgery (min)	87.7±22.7	100.7±27.7
ASA (I/II/III)	6 / 20 / 4	4 / 20 / 6

The values are mean±SD, number as appropriate.

As a result of the comparison between the groups, it was seen that there was a significant difference between the groups in favor of the IPACK Block group in terms of postoperative VAS scores after 1st hour, postoperative analgesic needs and patient satisfaction. It was determined that the VAS scores at the time of the patient's removal from the surgery (VAS 0) did not differ significantly between the groups; while the VAS scores were lower in the IPACK Block in all other time periods, which were also statistically significant (**Table 2**).

Patient satisfaction scores in the postoperative period were higher in IPACK Block group and this difference was statistically significant. All of the patients in the Control group needed additional analgesics, while none of the patients in IPACK Block group needed additional analgesics (p<0.001). In addition, when the discharge times between the groups were compared, it was seen that patients in IPACK Block were discharged earlier, which was also statistically significant (**Table 3**).

Table 2. VAS scores in groups

	Group I (IPACK Block) n=30	Group II (Control) n=30	p
VAS 0.hr	0 (0-2)	0 (0-9)	0.550
VAS 1.hr	2 (0-5)	6 (1-9)	<0.001*
VAS 2.hr	2 (0-4)	6 (2-9)	<0.001*
VAS 6.hr	2 (0-3)	5 (2-10)	<0.001*
VAS 12.hr	1 (0-3)	4 (1-8)	<0.001*
VAS 24.hr	0 (0-1)	2 (0-5)	<0.001*

The values are median (range), *: statistically significant

Table 3. Patient satisfactions, additional analgesic needs and discharge times, and in groups

		Group I (IPACK Block) n=30	Group II (Control) n=30	p
Patient satisfaction	1	0	0	<0.001*
	2	0	3	
	3	0	18	
	4	8	9	
	5	22	0	
Additional analgesic need (n of patient)		0	15	<0.001*
Discharge time (days)		2.5±0.9	3.1±1.2	0.013*

The values are mean±SD, number as appropriate. *: Statistically significant

No reported complications and adverse effects were detected in either group.

DISCUSSION

In this retrospective study, we aimed to evaluate the effect of IPACK block applied for preemptive analgesia on postoperative pain and patient comfort in patients undergoing arthroscopic knee surgery. At the end of our study, we determined that preemptive IPACK block applied to arthroscopic knee surgery patients operated under spinal anesthesia resulted in a significant decrease in postoperative VAS scores, increased patient satisfaction and shortened the discharge times.

Preemptive analgesia is defined as alleviating the severity of pain and reducing the pain-induced stress response by using an analgesic medication or performing a nerve block before a painful stimulus, as well as reducing the need for postoperative analgesics (10). IPACK block, which is a relatively new block, is a block that we apply as a preemptive analgesia method for patients who will undergo arthroscopic knee surgery. In this study, we wanted to evaluate the effectiveness of this block for this purpose.

The increase in the number of knee surgeries performed worldwide increases the importance of postoperative pain control. This has led to the emergence of various postoperative pain management strategies, where peripheral nerve blocks have gained popularity (11). If the literature is reviewed, it is seen that various drugs and methods like systemic analgesic administration, neuraxial blocks, peripheral nerve blocks, local anesthetic infiltration and intra-articular injections are applied for knee surgery to reduce postoperative pain and increase patient satisfaction (12).

Peripheral nerve blocks like femoral nerve block (FNB), adductor canal block (ACB) and sciatic nerve block (SNB) have been used to control pain after knee surgeries (13-15). One of the peripheral nerve blocks, ACB is a nerve block that has been reported to provide significant pain relief and earlier mobilization in patients as it preserves quadriceps muscle strength (16). However, this technique provides pain relief only in anterior and medial areas since it has no effect on deep genicular nerves and is not effective in posterior knee pain. Therefore, full knee extension and early ambulation are prevented, resulting in a delay in rehabilitation (14,17-18). FNB may provide effective pain control, but it may also cause weakness in the quadriceps muscle after surgery (13). Neither of these blocks is effective in relieving posterior knee pain (19-21). To alleviate posterior knee pain, periarticular multimodal drug injection (PMDI), sciatic nerve block (SNB) and IPACK can be applied (8). PMDI can be performed easily and quickly without risk of injury to nerves and blood vessels. However, direct injection into the knee joint has a potential risk of infection (22). Also, PMDI can only be performed during surgery and additional injections are not possible. SNB can also reduce posterior knee pain but foot drop in 65-68% of cases reported in literature (19,23).

IPACK is the administration of a local anesthetic agent into the space between the popliteal artery and the posterior capsule to block the deep genic nerves supplying the posterior aspect of the knee joint. The technique involves very selective blocking of the terminal sensory branches of the posterior aspect of the knee without involvement of the motor branches of the tibial and peroneal nerves, resulting in reduced pain with no effect on muscle strength (21).

When the literature on the use of IPACK block after knee surgeries for postoperative analgesia is reviewed, it is seen that studies are mostly performed in addition to ACB. In the study of Zhen et al. (15) postoperative 72-hour VAS scores were evaluated after ACB and FNB were applied to patients who had undergone total knee artroplasty operations. While the VAS scores measured in the first 24 hours were found to be significantly lower in the FNB group, they found that the VAS scores at the 48th and 72nd hours were similar.

Elliot et al. (22) in their study on knee surgeries showed that IPACK block applied together with ACB shortened the hospital stay and improved the response to physical therapy. They found that it had positive effects on pain scores, opioid consumption, physical therapy performance and discharge times.

Sahaneani et al. (23) reported significantly increased range of motion and walking distance as well as reduced visual analog scale scores after total knee arthroplasty in the ACB and IPACK block combined group compared to a group receiving ACB alone. Grevstad et al. reported that the femoral nerve catheter can provide significant postoperative analgesia, but on the other hand, it causes significant weakness in the quadriceps, which prevents early physical activity (13).

Amer et al. (24), in their study investigating the efficacy of peripheral nerve blocks in meniscal surgeries, documented that ACB and IPACK blocks are more effective than combined ACB and intra-articular infiltration for postoperative pain following surgery. They showed that their patients' VAS values were low in ACP and IPACK blocks.

Our study has some limitations. It is a retrospective study & the sample size is not large. We frequently apply spinal anesthesia as neuraxial block and ACB as peripheral block in our patients who have approved for arthroscopic knee surgery in our clinic. IPACK block is a relatively new block and we wanted to evaluate the effectiveness of this block, which we applied alone and in a limited number of patients for preemptive analgesia in our education clinic; and also compare it with the control group.

As a result of this evaluation, we found that the VAS scores of the patients, who had IPACK block, were lower from the 1st postoperative hour to the 24th hour. Although IPACK block is effective on the pain of the posterior side of the knee, we have also seen that this decrease in VAS scores increases the satisfaction of the patients and reduces the use of additional analgesics. The absence of any complications and the shortening of the discharge times were also considered as positive effects.

In conclusion; in this study, we observed that preemptive IPACK block, performed with ultrasonography in patients who will undergo arthroscopic knee surgery, reduces the patient's pain in the postoperative period, shortens the length of hospital stay, positively affects patient comfort and increases patient satisfaction considerably. We believe that IPACK block alone or in addition to other blocks or methods will be effective in preventing arthroscopic knee surgery pain and it will be useful to conduct new studies with more patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kırıkkale University Hosptial, Non-invasive Clinical Researches Ethics Committee (Date: 10/12/2020, Decision No: 2020.11.25).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Original Article

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The comparison of treatment with orlistat and orlistat plus metformin in relation to insulin resistance and weight loss

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ABSTRACT

Introduction: Obesity is a growing health problem. Many drugs have been developed to treat obesity. Orlistat is a widely used drug to treat this disease. Metformin is an antidiabetic drug. Clinicians often prescribe it to treat insulin resistance and achieve weight loss. Our research aims to compare the effects of orlistat alone and its combination with metformin on weight loss and insulin resistance.

Material and Method: This retrospective study was conducted by scanning the data of patients who presented to Antalya Training and Research Hospital Endocrinology, and General Surgery Clinics between 2016 and 2021. 42 morbidly obese patients who met inclusion and exclusion criteria and were prescribed orlistat plus metformin (group 1, n: 28) or orlistat alone (group 2, n: 14) along with a low-calorie diet for three months and were taking it regularly were included. Subsequently, weight, body mass index, fasting blood glucose, fasting insulin, and HOMA-IR (homeostasis model assessment for insulin resistance) were recorded and analyzed at baseline and after three months of taking the medications.

Results: After 3 months of treatment, significant weight loss was achieved in both groups compared to baseline weight (p=0.001 group 1, p=0.003 group 2). HOMA-IR values decreased significantly in both groups (p=0.001 group 1, p=0.01 group 2). Both groups lost the same amount of weight after three months (p=0.06).

Conclusion: In morbidly obese patients without prediabetes or diabetes, the addition of metformin to orlistat therapy did not add benefits in terms of weight loss or insulin resistance.

Keywords: Orlistat, obesity, metformin, insulin resistance

INTRODUCTION

Obesity is a growing health problem (1,2). The risk of diabetes, metabolic syndrome, hypertension, cardiovascular and cerebrovascular disease, gastrointestinal disease, and various malignancies increases with obesity (3). Weight loss of 5% to 10% of body weight is sufficient to reduce morbidity and mortality (1,4,5). Some patients who have difficulty adapting to diet and exercise and do not lose enough weight require medical or sometimes surgical treatment. Orlistat is one of the weight loss agents approved for the treatment of obesity (6). It specifically and effectively inhibits gastric and pancreatic lipase activity in the intestinal lumen and reduces fat absorption from food (7). It binds to the active serine region of gastric and pancreatic lipases via a covalent bond and thus exerts its effect. Therefore, it indirectly leads to weight loss by preventing the formation of monoacylglycerols and free fatty acids and providing a caloric deficit (6). Orlistat exerts all of its pharmacological effects in the gastrointestinal tract and does not alter neurotransmitter levels, as it has no effects on the central nervous system (6). However, the main adverse effects are gastrointestinal. Since orlistat decreases the absorption of fat-soluble vitamins, some associated health problems may occur if the necessary vitamin supply is not provided (6). Through weight loss, orlistat improves lipid profile, blood pressure, fasting blood glucose (FBG), and insulin concentration (8-12). The potential for gastrointestinal discomfort and moderate weight loss may limit the clinical usefulness of the agent. Metformin is an antidiabetic agent that promotes tissue utilization of glucose and decreases insulin resistance (13). Abdominal discomfort, decreased appetite, diarrhea, or constipation are some of the side effects of this drug (13). Weight loss has also been noted

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in patients treated with metformin. However, there are conflicting studies on the effects of metformin on weight loss (13-16). While significant weight loss was achieved with metformin in some studies (13,14), no consistent weight loss was observed in other studies (15,16). The aim of our study is to compare orlistat alone with combined therapy with metformin on body weight and insulin resistance.

MATERIAL AND METHOD

This retrospective study was conducted by scanning data from patients who presented to the Endocrinology and General Surgery Clinics at University of Health Science Antalya Training and Research Hospital between 2016 and 2021. The Ethics Committee of the University of Health Science Antalya Training and Research Hospital approved the study protocol dated June 24, 2021 and No. 9/19, and the report followed the Declaration of Helsinki. Patients were between 18 and 70 years of age, had a BMI (body mass index) ≥40 kg/m², no malignancies, cardiovascular disease, diabetes, or prediabetes (subjects with HbA1c (glycated hemoglobin) <5.7% and FBG <100), who had been taking orlistat regularly for three months or, in the case of insulin resistance, orlistat and metformin, were included in the study. To determine insulin resistance, we used the HOMA-IR (Homeostasis Model Assessment for Insulin Resistance) index and calculated it according to the following formula: HOMA-IR=insulin x FBG (mg/dl)/405. If the value of HOMA-IR was ≥2.7, insulin resistance was assumed (17). As exclusion criteria, we specified that patients were under 18 or over 70 years of age, had a BMI of < 40, were pregnant or breastfeeding, had chronic kidney disease, malignancy, a history of significant cardiovascular disease, diabetes mellitus or prediabetes (FPG ≥100 or HbA1c ≥5.7%), were taking medications that affect glucose metabolism, or had not used orlistat or combination therapy regularly for 3 months. We retrospectively obtained the sex, age, chronic diseases, medications, height, weight, BMI, and laboratory results of patients presenting to our outpatient clinics from their records. We included 42 morbidly obese patients in our study who met the inclusion and exclusion criteria. 28 patients had insulin resistance and were prescribed orlistat 3x120 mg and metformin 2x500 mg and took these drugs regularly for 3 months along with a low-calorie diet (group 1). 14 patients without insulin resistance according to the HOMA-IR index were prescribed orlistat 3x120 mg and took this medication regularly for 3 months along with a low-calorie diet (group 2). We then recorded baseline weight, BMI, FBG, HbA1c, fasting insulin level and after three months of medication. Biochemical tests, including FBG, creatinine, AST, ALT and others, were analyzed by spectrophotometric method using Beckman coulter AU5800 (Beckman coulter Inc. CA, USA) autoanalyzer. Insulin and other necessary hormone tests were analyzed by chemiluminescence method on Beckman coulter DxI800 (Beckman coulter Inc. CA, USA) analyzer. The reference range for fasting insulin in our hospital is 5-35 µU\ml.

Statistical Analysis

We used IBM SPSS version 20 to analyze the data. We used descriptive statistics to define continuous variables (number (n), percentage (%), mean±standard deviation). We performed Student's t-test or Mann-Whitney U-test to compare the two independent groups depending on whether the data were parametric or not. A 'p' value < 0.05 was considered statistically significant.

RESULTS

According to the exclusion and inclusion criteria, the data of a total of 42 patients were fully accessed. The mean age of the patients was 40.6±7.3 years. Thirty cases were female and twelve were male. Twenty-eight subjects were found to have insulin resistance in addition to obesity as established by the HOMA-IR result, and these patients were assigned to group 1. The remaining 14 patients were not found to have insulin resistance, and these patients were assigned to group 2. The mean age of group 1 was 39.9±8.2 years and that of group 2 was 41.1±5.6 years. The age and gender of the two groups were similar (p=0.51 and p=0.92, respectively). The baseline weight of group 1 was 112.6±9.4 kg and that of group 2 was 109.0±11.5 kg. There was no significant difference in baseline weight between the two groups (p=0.06). The baseline value of HOMA-IR was 3.16±1.2 for group 1 and 1.98±0.5 for group 2. The HOMA-IR value was significantly higher in group 1 (p=0.001) (**Table 1**). At the end of the third month, no significant difference was found between the two groups in terms of weight and BMI (p=0.09 and p=0.07, respectively). While weight loss in group 1 was 4.6±2.1 kg, it was 5.2±2.5 kg in group 2. There was no significant difference between the two groups in the amount of weight loss (p=0.06). The HOMA-IR value of group 1 was significantly higher than that of group 2 (2.11±1.5 group 1, 1.45±0.7 group 2) at the end of 3 months (p=0.002) (**Table 2**). Patients in both treatment groups significantly lost weight with these therapies compared to baseline (p=0.001 group 1, p=0.003 group 2). As expected, a significant decrease was observed in HOMA-IR (p=0.001) and FBG (p=0.02) values at the end of 3 months in the group using metformin. However, a significant decrease was achieved in FBG (p=0.04) and HOMA-IR (p=0.01) values at the end of the 3rd month in group 2 who received only orlistat. HOMA-IR was significantly reduced in both groups (p=0.001 group 1, p=0.01 group 2) (**Table 3**).

Table 1. Demographic characteristics and baseline parameters of the patients

	Group 1 (n=28)	Group 2 (n=14)	p
Age (years) (mean±sd)	39.9±8.2	41.1±5.6	0.51
Gender (n/%)			
Male	8 (%28)	4 (%28)	
Female	20 (%72)	10 (%72)	0.92
Body weight (kg) (mean±sd)	112.6±9.4	109.0±11.5	0.06
Body mass index (kg/m²) (mean±sd)	45.5±3.7	44.2±3.1	0.08
Fasting blood glucose (mg/dl) (mean±sd)	90.1±7.9	87.8±9.3	0.98
Fasting insulin (µU/ml) (mean±sd)	13.8±6.6	10.1±3.2	0.002*
HOMA-IR	3.16±1.2	1.98±0.5	0.001*

*<0.05 statistically significant. HOMA-IR; homeostasis model assessment for insulin resistance

Table 2. Control parameters of the patients at the 3rd month after treatment

	Group 1 (n=28)	Group 2 (n=14)	p
Body weight (kg) (mean±sd)	106.0±8.1	104.2±9.5	0.09
Weight loss after 3 months (mean±sd)	4.6±2.1	5.2±2.3	0.06
Body mass index (kg/m²) (mean±sd)	43.2±4.1	42.3±2.9	0.07
Fasting blood glucose (mg/dl) (mean±sd)	85.2±7.5	84.4±8.9	0.62
Fasting insulin (µU/ml) (mean±sd)	9.7±6.3	7.5±2.1	0.008*
HOMA-IR (mean±sd)	2.11±1.5	1.45±0.7	0.002*

*<0.05 statistically significant. HOMA-IR; homeostasis model assessment for insulin resistance

Table 3. Comparison of the parameters of the groups at baseline and after 3 months

	Baseline	After 3 months	p
Body weight (kg) (mean±sd)			
Group 1	112.6±9.4	106.0±8.1	0.001*
Group 2	109.0±11.5	104.2±9.5	0.003*
Body mass index (kg/m²) (mean±sd)			
Group 1	45.5±3.7	43.2±4.1	0.001*
Group 2	44.2±3.1	42.3±2.9	0.005*
Fasting blood glucose (mg/dl) (mean±sd)			
Group 1	90.1±7,9	85.2±7.5	0.02*
Group 2	87.8±9,3	84.4±8.9	0.04*
Fasting insulin (µU/ml) (mean±sd)			
Group 1	13.8±6.6	9.7±6.3	0.001*
Group 2	10.1±3.2	7.5±2.1	0.002*
HOMA-IR (mean±sd)			
Group 1	3.16±1.2	2.11±1.5	0.001*
Group 2	1.98±0.5	1.45±0.7	0.01*

*<0.05 statistically significant. HOMA-IR; homeostasis model assessment for insulin resistance

DISCUSSION

Our study shows that in patients with morbid obesity who do not have prediabetes or diabetes, the addition of metformin to orlistat therapy has no benefit in treating obesity and insulin resistance. As expected, FBG and HOMA-IR levels decreased significantly at the end of the third month in the group receiving combination therapy. However, we observed a significant improvement in insulin resistance and FBG levels, possibly as a result of weight loss in the patients receiving orlistat alone. Obesity is a major cause of hypertension, diabetes, dyslipidemia, and insulin resistance (3). Many drugs have been developed to treat obesity. Orlistat is a drug for the treatment of morbid obesity that reduces cardiovascular risk in these individuals (9,10). This benefit has been reported in some studies as a result of weight loss, but it has also been observed in other studies in which weight loss was not a factor (9,10). In diabetic patients, Kelley et al. (9) observed significant improvement in glycemic parameters and other risk factors for cardiovascular events after 1 year of orlistat treatment, in addition to substantial weight loss. In studies investigating the effects of short-term orlistat use on the risk of cardiovascular events, Bloch et al. observed significant reductions in diastolic blood pressure, FBG, weight, and total cholesterol levels in obese hypertensives after 3 months of orlistat use (12). In previous studies, orlistat treatment increased insulin sensitivity and significantly decreased HOMA-IR (4,5,15). In their study, Heymsfield et al. demonstrated that orlistat prevented the progression of prediabetes and diabetes in obese individuals (5). In the study of Song et al. (18) in obese and overweight women with polycystic ovary syndrome, it is shown that orlistat has fewer side effects and is better tolerated than metformin and provides more benefits in terms of lipid profile and weight loss. Berne et al. (19) showed that orlistat administration in patients with type 2 diabetes resulted in significant improvement in glycemic parameters, insulin resistance, apolipoprotein B levels, and beta cell function, as well as weight loss after approximately 13 months of treatment. We could not investigate other cardiovascular risk factors in our study because it was a retrospective study and patient data were insufficient, but our results are consistent with these studies regarding the benefits of orlistat on weight loss, FBG, and insulin resistance. Metformin may promote weight loss in obese individuals, although some studies have shown no effect in terms of weight. In the study by Kay et al. (23), the metformin group achieved more significant weight loss than the group taking the placebo while in another study, it was observed that taking metformin for six months did not result in noticeable weight loss (16). In another study, weight loss was observed after three months of taking metformin, but it was not statistically significant

(20). Insulin-sensitising drugs such as metformin have been shown in studies to reduce hyperinsulinemia and increase hepatic insulin sensitivity in individuals with obesity, diabetes, or polycystic ovary syndrome, all of which are associated with insulin resistance (16,20). The study conducted by Gokcel et al. (14) showed a significant decrease in weight and HOMA-IR values in individuals treated with orlistat or metformin for six months. In this study, orlistat resulted in a 9.06% weight loss and metformin alone resulted in a 9.90% weight loss. HOMA-IR decreased by 32.73% with orlistat and 39.28% with metformin. We did not include patients taking metformin alone in our study. This is a shortcoming of our study. Therefore, we could not compare the metformin use alone with the orlistat use alone, but we indirectly note that we did not detect any additional effect of metformin in terms of weight, FBG, and insulin resistance. The prospective study by Sari et al. (15) in obese patients showed similar results in terms of insulin resistance or weight loss with the use of orlistat alone and the combined use of metformin and orlistat. These results were consistent with ours. Our study had several limitations. These include its retrospective nature, low metformin dose, small study population, short treatment duration, and lack of overweight and obese groups. Because of the lack of a patient group taking metformin alone and the lack of analysis of other cardiovascular risk factors, it is not possible to comment on the superiority of orlistat over metformin and the benefit of orlistat on other cardiovascular risk factors.

CONCLUSION

Orlistat therapy alone resulted in weight loss and reduced insulin resistance. The addition of metformin to orlistat treatment showed no additional benefit in these parameters. Considering that combination therapy increases costs and side effects, treatment with diet and orlistat might be sufficient to improve insulin sensitivity and lose weight in morbidly obese patients who do not have prediabetes or diabetes.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Antalya Training and Research Hospital Ethics Committee (Date: 24.06.2021, Decision No: 9/19).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Fetuin A level in advanced placental calcification at term pregnancies

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ABSTRACT

Aim: Fetuin A is a multifunctional protein which is a marker of pathological calcification in several diseases. This study aimed to evaluate serum fetuin A level in term pregnancies with grade 3 placental calcification.

Material and Method: Fifty-seven pregnant women who applied obstetrics outpatient clinic for routine pregnancy follow-up at term were included in this study. The study was designed prospectively. Patients with grade 3 placental calcification (n=29) were compared to patients with non-calcified placenta (n=28) in terms of serum fetuin A levels.

Results: Maternal serum calcium levels of pregnant women with grade 3 calcified was significantly increased compared to pregnant women with non-calcified placenta. There was no significant difference between the fetuin A levels of study and control groups. The fetuin A level was not found to be correlated with maternal serum calcium level.

Conclusion: Fetuin A has been targeted as a marker for pathological calcification. The findings of the current study may support the thought that term placental calcification may be physiological rather than a pathological process.

Keywords: Placental calcification, term pregnancy, calcium, fetuin A

INTRODUCTION

Fetuin A is a plasma glycoprotein which exists in all vertebrates and takes role in metabolic events as mineralization, inflammation, cell adhesion, proliferation, and differentiation. It is mainly synthesized in liver and sequestered at physiological and pathological calcification areas (1-3). Fetuin A is a potential inhibitor of ectopic calcification, as well as takes role in normal osteogenetic activity of bone tissue (2, 4). Increased serum fetuin A levels were shown to be significantly related with gestational and type 2 diabetes mellitus (GDM and DM), preeclampsia, cardiovascular diseases (CVD), chronic kidney disease, hypertension (HT), metabolic syndrome and cigarette smoking (5-8).

Placental calcification is a widespread calcium deposition of placenta. The calcification can be clinically detected through ultrasonographic evaluation. Grannum et al. established the grading scale for placental maturity. Advanced degree (grade 3) placental calcification was indicated to be related with pulmonary maturity (9). The placental calcification at third trimester was shown to indicate adverse pregnancy outcomes including preeclampsia, intrauterine growth restriction and stillbirth. Besides, its clinical significance was reported as more prominent when it is detected at early weeks of the third trimester (10-14).

Despite the studies reporting the adverse outcomes of grade 3 placental calcification at the third trimester of pregnancy, the pathophysiology of placental calcification at term is not clearly elucidated yet. In this study, we aimed to evaluate the change in serum fetuin A levels in grade 3 placental calcification at term pregnancies.

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MATERIAL AND METHOD

This study was performed by participation of 57 Caucasian singleton pregnant women who consecutively applied to Ankara Dr. Zekai Tahir Burak Women Health and Research Hospital, Ankara, Turkey, for routine pregnancy follow-up. Approval for the study was granted by Ankara Zekai Tahir Burak Women Health Training and Research Hospital Ethics Committee (Date: 26.06.2014, Decision No: 8). The study design was prospective, and data collection was completed in six months starting from June 2014. Informed consent was taken from all participants before the data collection. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Healthy pregnant women at term were included in this study. The study group consisted of pregnant women with grade 3 placental calcification (n=29). The control group included pregnant women without grade 3 placental calcification (n=28). Detailed medical history was taken from all participants. Physical examination was performed including systolic blood pressure measurement and body mass index (BMI) calculation. Routine laboratory parameters including complete blood count, liver and kidney function tests were also evaluated whether they are in normal ranges. The clinical characteristics and demographic features of the participants were recorded. Pregnant women with the history of smoking, comorbidities including DM, GDM, HT, preeclampsia, cardiovascular diseases, metabolic syndrome, thyroid dysfunction, connective tissue disorders, inflammatory diseases, active infectious conditions, rupture of membranes, and presence of structural/chromosomal fetal abnormalities were excluded.

Placental calcification was assessed through Grannum's classification (9). Ultrasonographic evaluation was performed with MindrayM5 brand ultrasound scanner (Mindray, Shenzhen, China) by the same obstetrician. Fetuin A level was examined from blood serum samples taken from antecubital vein. Boster brand ELISA kit (USA, Catalog #EK0757) based on quantitative sandwich ELISA principle was used for serum analyses. The results were presented as ng/ml.

Statistical Analyses

Statistical analyses were carried out by IBM-SPSS for Windows V21 (IBM-SPSS, Armonk, NY, USA). Categorical variables were expressed as number and percentages, numerical variables were expressed as mean, median and standard deviation. Independent samples t-test was used for normally distributed numerical variables. Chi-Square test was used to compare categorical variables. Spearman's rho correlation analysis was used to investigate the relationship between not normally distributed numerical variables. Two-tailed P-value <0.05 was considered statistically significant. Binary logistic regression analysis was used to investigate the variables affecting the placental calcification through forward stepwise LR method. G-Power Version 3 was used in for sample size analysis (Universitat Kiel, Kiel, Germany), before the study start. The sample size was found to be 25 for each group with assumed power of 0.84, a significance level of 0.05 and effect size of 0.5. A p value lesser than 0.05 was defined as statistically significant.

RESULTS

This study included 60 pregnant women. However, 3 participants were excluded from the study due to preeclampsia development after their participation in the study. The data of 57 pregnant women were evaluated at final analysis. Demographic features and clinical characteristics of study and control groups were presented in **Table 1**.

Table 1. Demographic features and clinical characteristics of study and control groups

Variables	Placental calcification (n=29)	Control Group (n=28)	P-value
Maternal age (years) Mean±SD	25.03±5.15	27.14±4.9	0.12
BMI (kg/m²) Mean±SD	27±2.66	27.65±3.40	0.87
Gestational age (weeks) Mean±SD	39±1.62	39±1.95	0.93
Birth weight (g) Mean±SD	3417.93±374.26	3281.78±364.42	0.17
APGAR score 5th min <7 n (%)	4/29 (13)	3/28 (10)	0.75

BMI: Body mass index; SD: Standard deviation P-value <0.05 was considered statistically significant

The fetuin A levels were similar for the study and control groups (350.72±63.68 vs. 336.27±56.96, respectively; p=0.370). Maternal serum calcium level was significantly higher in pregnant women with grade 3 placental calcification (p=0.022) (**Table 2**).

Table 2. Maternal serum calcium level and maternal serum fetuin A level of the study group compared to control group

Variables	Placental calcification (n=29)	Control Group (n=28)	P-value
Maternal serum calcium level (mg/dl) Mean±SD	8.51±0.42	8.15±0.70	0.02*
Maternal serum Fetuin A level (ng/ml) Mean±SD	350.72±63.68	336.27±56.96	0.37

SD: Standard deviation, *P-value <0.05 was considered statistically significant

There correlation of fetuin A level with the maternal serum calcium level was not significant (p >0.05) (**Table 3**).

Table 3. Correlation between maternal serum Fetuin A level and the related parameters

Variables	r	P-value
Birth weight (g)	-0.16	0.21
Maternal serum calcium level (mg/dl)	-0.21	0.11

P-value <0.05 was considered statistically significant

Logistic regression analysis indicated that 1 unit (mg/dl) increase at maternal serum calcium level was resulted in 3.58-fold increase at possibility of placental calcification development [CI 95%=3.58 (1.08-11.86), p=0.037].

DISCUSSION

Fetuin A is a marker for pathological calcification in several pregnancy related comorbidities. Grade 3 placental calcification at last trimester of pregnancy may be related with adverse pregnancy outcomes. The current study evaluated serum fetuin A level in grade 3 placental calcification in term pregnancies. Serum fetuin A level was slightly decreased in study group however, the difference was not significant. On the other hand, maternal serum calcium level was found to be an independent estimator of placental calcification with a 3.58-fold increase regarding to 1 unit increase in calcium level.

Actual mechanism of placental calcification is unclear and may be physiological in course of placental aging (15). On the other hand, placental calcification may be the result of a pathological process such as dystrophic changes under ischemic conditions or metastatic calcification related with mineral supersaturation (16, 17). In the current study, maternal serum calcium level of the pregnancies with grade 3 placental calcification was significantly higher than the control group. Maternal serum calcium level was detected as a determinant of placental calcification. However, serum calcium levels of both the study group and control group were within the normal range. Therefore, the significant difference should not be assumed as a pathological increase in calcium level in the study group. The correlation analysis between fetuin A level and maternal serum calcium level was also not reveal a significant relation.

Previous studies on fetuin A have been concentrated on preeclampsia and GDM in pregnant population. Fetuin A executes some cell protective functions by inhibiting receptor tyrosine kinases. However, the inhibitory pathways triggered by increased amount of fetuin A may impair trophoblast invasion in the early weeks of gestation, consequently leading to preeclampsia development (18). Through the inactivation of receptor tyrosine kinase activity, fetuin A also takes role in pathogenesis of GDM and liver diseases (19-21). In addition, fetuin A levels also increase in normal course of pregnancy by advancing gestational age (22). Subsequent studies also shown that fetuin A levels decrease by young maternal age and morbid obesity (18, 21, 23). In this study, the study and control groups were similar in terms of gestational age, maternal age, and maternal BMI.

Fetuin A is known to be a negative acute phase reactant in inflammatory diseases and infectious conditions besides to be a calcification marker (24, 25). Previous studies have shown that serum fetuin A level is significantly lower in preeclamptic patients with hemolysis-elevated liver enzymes-low platelets (HELLP) syndrome compared to the healthy controls and compared to preeclamptic cases without HELLP syndrome. This situation was explained through that fetuin A is a negative inflammatory mediator (26, 27). In this regard, the current study included the pregnant cohort without any systemic inflammatory or infectious condition.

Advanced placental calcification before 36 weeks of gestation is defined as preterm placental calcification. It was indicated to be related with pregnancy complications as preeclampsia, placental abruption, and stillbirth (11, 13, 15). Advancing gestational age, that is related with the increasing fetuin A level is as well as responsible for increasing amount of placental calcium deposition. On the other hand, prominent placental calcification at term thought to be a physiological process with 39.4 % incidence (28). Similar serum fetuin A levels between the groups also appear as evidence for the physiologic basis of grade 3 calcification at term. The birthweight and 5th minute APGAR score were also similar between the study and control groups. The results of the current study appear to be consistent with the general approach which is towards to accept placental calcification at term as physiological.

The similarity of clinical, and demographic features between the groups and inclusion of healthy subjects appears to be the strongest aspects of the study. On the other hand, the study has a number of limitations. In this study, ELISA method was used to was measure serum fetuin A level. However, some previous studies reported that quantitative measurement of fetuin-mineral complexes may better exhibit calcification stress and give more accurate results (29, 30). Another limitation of the current study is that umbilical cord fetuin A level was not evaluated. Due, possible relations between of umbilical cord fetuin A level with placental calcification could not be evaluated. This study did not examine preterm placental calcification, which may lead to adverse pregnancy outcomes, can be also considered as a limitation. A third study group composed of pregnancies with preterm placental calcification might give us some notable findings regarding the role of fetuin A in pathogenesis of placental calcification.

The study findings supported the general thought that placental calcification at term may not be accepted as a component of fetal wellbeing. This study did not show any significant relation between the placental calcification and serum fetuin A level. There is need for studies designed in pregnant cohort with preterm placental calcification to evaluate the role of fetuin A in pathophysiology of calcified placenta.

CONCLUSION

In this current study, fetuin A, which is a marker of dystrophic calcification, was not found to be increased in advanced degree placental calcification at term. The current findings indicate that the development of placental calcification at term may be based on a physiological course.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for the study was granted by Ankara Zekai Tahir Burak Women Health Training and Research Hospital Ethics Committee (Date: 26.06.2014, Decision No: 8).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Overview of the angiogenic effect of probiotics (*Lactobacillus acidophilus* and *Lactobacillus rhamnosus*) at human umbilical vein endothelial cells

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ABSTRACT

Introduction: Angiogenesis (neovascularization), which means new vessel construction, is normal and physiologically, wound healing, embryogenesis, a necessary menstrual cycle it's a mechanism. When taken in appropriate amounts together with or separately with nutrients, mucosal and by regulating systemic immunity, ensuring nutritional and microbial balance in the intestines living nonpatogenic microorganisms that positively affect the health of the host it is called "probiotics". Lactic acid bacteria, the most probiotic microorganisms it constitutes its important group. Where probiotics have an effect on angiogenesis, and it is thought to help heal wounds through the road. With this research indicated that roles of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* on angiogenesis if present to demonstrate in vitro methods and the gene expression responsible for the formation of these effects it is intended to reveal.

Material and Method: This study is an experimental study conducted in vitro human umbilical cord vein endothelial cell (HUVEC) MTT test in cell culture with (3-[4,5-Dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) evaluation of viability and proliferation wound healing model, tube formation method and gene expression with real rime-polymer chain reaction (RT-PCR) methods of appointment were used.

Results: HUVEC cells *L. acidophilus* 10^9 CFU\ml after extract application statistical of mRNA expression of VEGF and FGF genes by control group 24 per hour it was found to increase significantly. *L. rhamnosus* 10 6 CFU\ml and 10^9 CFU\ml after application of extracts VEGF gene mRNA by control group 24 per hour its expression was found to be statistically significantly increased. Also *L. rhamnosus* extracts cell proliferation and migration of in vitro wound model it was found to increase statistically significantly.

Conclusion: In this study, in vitro *L. acidophilus* 10^9 CFU\ml extract and 10^6 CFU\ml and 10^9 CFU\ml extract of *L. rhamnosus*, VEGF gene mRNA revealed to be effective on angiogenesis in HUVEC cells by increasing expression it is.

Keywords: Probiotics, angiogenesis, HUVEC, wound healing model

INTRODUCTION

Angiogenesis (neovascularization), which means new vessel construction, is actually a necessary mechanism for normal and physiological wound healing, embryogenesis, menstrual cycle. Angiogenesis is a very complicated process that progresses with endothelial cells proliferation, migration and new vessel formation. Angiogenesis as a dynamic event occurs as a result of releasing proteolytic enzymes to the environment in certain proportions, breaking the extracellular matrix and reconstructing the endothelial cells by migration from the microvascular field. Provided to all these processes, new capillaries and blood vessels are formed from the existing microvascular bed by proliferation of endothelial cells in the capillaries. The quality of all angiogenic interactions has not yet been clarified. The greatest possibility is that the balance between angiogenic stimulants and angiogenesis inhibitors ensures that the vascular components normally remain silent. Increased angiogenic stimuli and decreased angiogenesis inhibitors initiate angiogenesis (1).

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Stimulation of endothelial cells by some factors also initiates a series of events that cause angiogenesis. The occurrence of pathological angiogenesis is responsible for many diseases such as diabetic retinopathy, hemangioma, psoriasis and collagen tissue diseases, as well as tumor growth and metastasis development. In triggering angiogenesis, which plays an important role in cell proliferation; by gaining importance again, especially in the field of industrial microbiology, it has brought probiotic bacteria to the agenda, which take part in many biological processes such as wound healing. Although vascular endothelial cells in adult humans are typically at low turnover rate, they have the capacity to proliferate to form new blood vessels throughout their lifetime (2-3).

Angiogenic stimulation activates endothelial cells shortly after proteolytic destruction. Endothelial cells migrate to the extracellular matrix and multiply. The most effective angiogenic factor in this process is vascular endothelial growth factor (VEGF). Angiogenesis begins with the formation of hemostatic buffer with the release of PDGF, TGF-β and FGF from platelets. VEGF is released in combination with other cytokines. Thus, neovascularization begins by increasing endothelial cells. When the angiogenesis process progresses, a rich vascular network forms from the healthy vessels to the wound area. The oxygen levels in the tissues regulate the angiogenesis process by interacting with oxygen proteins that regulate the transcription of angiogenic and anti-angiogenic genes.

Wound healing is a natural physiological process and microflora is one of the important factors that can affect this process both negatively and positively. In the case of wounds or burns, bacterial colonization occurs due to disruptions in the skin barrier and an infection-prone condition occurs. The wound is a stressful condition and causes the release of neuroendocrine and stressors such as cortisol, epinephrine, norepinephrine, acetylcholine, catectatin, substant P, α-melanotropin. These molecules increase the risk of infection and complicate wound healing (4-5).

Probiotics are thought to be effective in the expression of various growth factors such as ornithine decarboxylase (ODC), VEGF, fibroblast growth factor (FGF), B-cell lymphoma 2 (Bcl-2) and epidermal growth factor receptor (EGF receptor) by affecting these mechanisms. These molecules activate mechanisms such as chemotaxis, cell proliferation, angiogenesis, extracellular matrix deposition and reconstruction (6-9).

Probiotic bacteria can survive in the gastrointestinal environment without being damaged. When taken with food, probiotics can maintain up to 1-4 hours in an stomach with an pH between 2.0 and 3.0 in an enzymatic environment. Probiotic bacteria can grow in the mucous substance secreted from the mucosa. Can use mucin in this secretion as an energy source (10-12).

L. acidophilus is naturally present in the human intestinal microflora. These microorganisms, which have a facultative anaerobic feature, develop in the colonic intestinal epithelial tissue they form, and prevent them from adhering to the surface and show antagonistic effect. In particular, dairy products containing *L. acidophilus* have been found to decrease serum cholesterol levels and increase antibody responses, and have effects to increase the number of fecal lactobacilli (13-16).

Lactobacillus rhamnosus is a type of beneficial bacteria that produces L (+) lactic acid and ethanol in an oxygen-free environment, is naturally found in the human intestinal flora, resistant to low pH environments, and can attach to the wall of the gastrointestinal tract. Some studies show that *L. rhamnosus* can stimulate natural barrier mechanisms in patients with atopic dermatitis and food allergy and can be an effective therapy method in the treatment of diseases such as food allergy (17,18).

The aim of our study is to show the effects of lactic acid bacteria commonly used in the field of industrial microbiology such as *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* in vitro methods and to analyze the gene expressions responsible for the occurrence of these effects.

MATERIAL AND METHOD

Ethics committee approval is not required for cell culture research in preclinical studies. All procedures were performed adhered to the ethical rules and principles of the Helsinki Declaration.

Cell Culture

HUVEC cell line (supplied by ATCC) was used and in vitro analysis were performed at vascular biology lab in the study. HUVEC is in stem cell structure, it has the basic features of vascular endothelial cells, and are frequently used in angiogenesis studies the reason for preferred.

The medium for HUVEC cells was prepared as 90% DMEM (L-glutamine), 10% FBS. Cells were produced in 25 cm^2 and 75 cm^2 flasks containing this nutrient medium by keeping them in an incubator with a 5% CO_2, 95% air mixture and humidity inside the 37°C and routine passage 3 times a week cell model in studies related to angiogenesis.

After the cells were evaluated cytotoxicly with the MTT test, it was decided to study the bacterial extracts with the most appropriate proliferation property on HUVEC cells in the wound model. In order to evaluate the effects on wound healing, 4 test groups and a control group were created for HUVEC cells.

Would Healing Assay

Petri dishes (35 mm, high), which are physically suitable for cell adhesion and proliferation, with a highly hydrophobic unbedded surface, have used. In addition, a silicone structure was placed on the graders at the base of these flasks in order to mimic the shape of the wound edges just before cultured the cells. After the cells were expected to completely cover the bottom of the wells, the previously placed silicone structures were carefully removed. Then, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* were treated which has determined optimum concentrations and 0, 8, 24 h incubation period. In the in vitro mimic wound healing model, the effects of bacteria species on cellular activities were compared with the control group.

Tube Formation Model

M-Slide Angiogenesis and M-Plate Angiogenesis Tube Formation plates, which were placed in a 37°C incubator with BD Matrigel gel solution 12 hours before, were removed from the incubator and 10 microliter gel solution was added to each in middle of well. Bacterial extracts and cell suspensions were prepared and cultivated on the matrigel stabilized in the wells. Within 12-24 hours, the results were visualized by phase contrast microscope and scoring was taken considering the tube length, number and density.

Gene Expression by RT-PCR

In order to perform gene expression study mRNA molecule (QIAGEN Cat.No.74104 RNeasy Mini Kit) from each cell application group, the Transcriptor First Strand cDNA synthesis kit (ROCHE-Germany) was used primarily for obtaining cDNA. Quantitative RT-PCR method followed by cDNA synthesis used Light Cycler FastStart DNA Master SYBR Green I kit (Roche-Germany) in gene expression analysis. "Betaactin" gene was studied as a reference gene. Target mRNA normalization rates were determined using the Lightcycler software 4.0 program version. Normalization rates were calculated automatically from the amplification curves of the program samples. ROCHE Lightcycler 480 device was used in the study. Statistical evaluations were made using target mRNA primers, FGF, VEGF, whose expression level want to be measured.

RESULTS

MTT cell viability test was performed to determine the effect of different concentrations (10^6-10^9 CFU\ml) of bacterial extras on the HUVEC cell line. Significant results were obtained after 24-hour incubation of bacterial extras on the HUVEC cell line. Changing cell viability effects at different concentrations and different incubation points are shown in **Figure 1**.

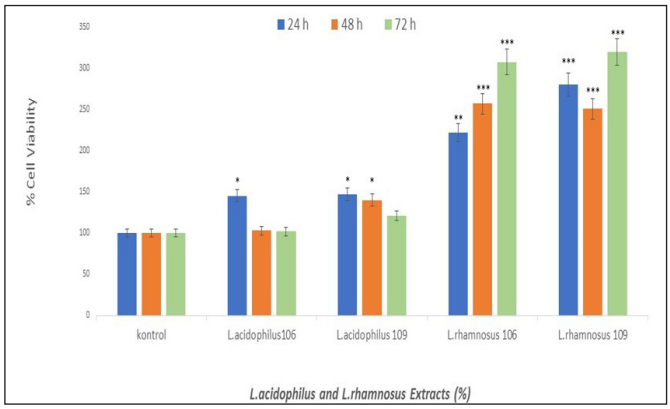

Figure 1. MTT cytotoxic assay of *L. acidophilus* and *L. rhamnosus* on HUVEC cells (different time point)

According to datas, 10^9 of *L. acidophilus* concentration had more effective. Compared to *Lactobacillus acidophilus*, *L. rhamnosus* has more dominant role on endothelial cell functions.

Images of the cell culture wound model created with *L. acidophilus* 10^9 CFU/ml extract were taken under the inverted microscope at 0, 8, and 24 hours. No statistically significant effect on cell migration compared to the control group. However, *L. rhamnosus* 10^9 CFU/ml extract were taken under an inverted microscope at 0, 8 hours and observed that it increased the cell migration statistically significantly compared to the control group and closed the wound model at 8 hour time point (**Figure 2**).

Figure 2. Wound healing cell migration rate on HUVEC cells

Genes involved in angiogenesis and wound healing in HUVEC cells were evaluated by RT-PCR method. While *L. acidophilus* 10^6 did not show a significant effect at the dose, in the 10^9 CFU/ml group, 5 times more gene expression was observed among VEGF gene expressions compared to the control group, and FGF gene expression increased 2-fold in this group (**Table 1**). For *L. rhamnosus* 10^9 CFU/ml group, there was a statistically significant difference in VEGF gene expression 2 times more than in the control group, but no statistically significant difference was found for FGF gene expression.

Table 1. RT-PCR results of VEGF and FGF on *Lactobacillus* application group

	Control group	*L. acidophilus* 10^6	p Value	*L. acidophilus* 10^9	p Value
VEGF	0.105±0.001	0.125±0.022	p>0.05	0.525±0.021	p<0.05
FGF	0.040±0.012	0.040±0.223	p>0.05	0.095±0.011	p<0.05
	Control group	***L. rhamnosus* 10^6**	**p Value**	***L. rhamnosus* 10^9**	**p Value**
VEGF	0.105±0.001	0.250±0.022	p<0.05	0.019±0.021	p<0.05
FGF	0.040±0.012	0.040±0.012	p>0.05	0.045±0.013	p>0.05

The effect of *L. acidophilus* and *L. rhamnosus* bacteria on tube formation was investigated by using tube formation analysis (Wimasis) on matrigel, which was stabilized in the wells by preparing bacterial extracts and cell suspensions. It could not determined tube formation effect that *L. acidophilus* bacteria, which applied 106 CFU/ml dose. While a slight effect of *L. rhamnosus* bacteria administered 10^6 CFU\ml dose was observed on tube formation, a statistically significant difference was found for *L. acidophilus* and *L. rhamnosus* bacteria at 109 CFU\ml dose (**Figure 3**)

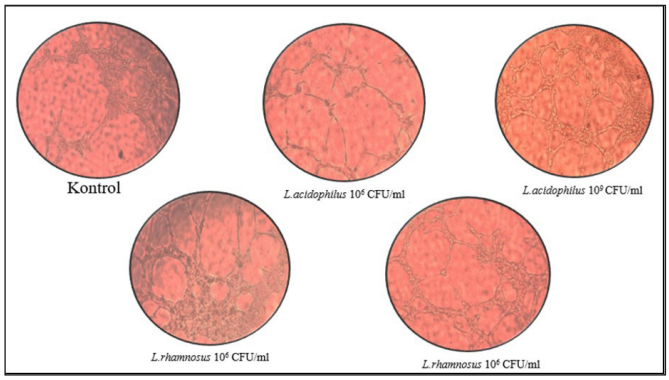

Figure 1. MTT cytotoxic assay of *L. acidophilus* and *L. rhamnosus* on HUVEC cells (different time point)

DISCUSSION

In recent studies in the field of angiogenesis and wound healing, it is aimed to find new molecules that increase cell proliferation, support migration and regulate cellular activities. In this respect, biological molecules, namely probiotics, which do not show cytotoxic properties, do not damage other organs and systems of the body, come to the fore. Live non-pathogenic microorganisms that provide microbial balance in the intestines and positively affect the health of the host in this way by regulating mucosal and systemic immunity when taken together with the nutrients or separately, are called "probiotic" (19-21). According to the increasing number of scientific research results, it is pointed out that living microorganisms can be used in the prevention and even treatment of some diseases. In this context, the importance of probiotics is increasing day by day. It has been proven that human and animal model studies that probiotics have many beneficial effects in the organism, especially in the GI system. Therefore, the place of probiotic bacteria has become indisputable in the treatment of a healthy life and diseases (22,23).

Lactobacillus and *Bifidobacterium* are the most important and most frequently used group of probiotic cultures. Factors such as delivery method, antibiotic use, age, nutrition, genetic factors, stress and pregnancy affect the richness of microflora. Because probiotics are sensitive to factors such as pH, gastric and pancreatic fluids, bile, and intestinal mucosa, studies are often geared towards stabilizing bacteria. It is thought that probiotics may also have an effect on angiogenesis and in this way can help wound healing (24).

In our study, the effect of probiotics on migration on HUVEC cells was investigated by in vitro wound model. According to the studies in the literature, Halper et al. (25) reported that *Lactobacillus* supernatants have angiogenesis and wound healing properties in vitro and in vivo. In another study, *L. acidophilus* was reported to form reepithelialization in HaCaT cells. According to the study of Eunok et al. (26) probiotic B.polyfermenticus increases migration and proliferation on endothelial cells. Studies using *L. acidophilus* encapsulated with ginger extract have shown that gastric ulcer heals in rats without signs of mucosal damage. In our study, it was found that *L. rhamnosus* 10^6 CFU\ml and 10^9 CFU\ml extracts significantly increased cell proliferation and migration compared to the control group. While the proliferative effect of *L. acidophilus* extracts in HUVEC cells was observed, no effect on migration was observed in the in vitro wound model (27). In a study, it was reported that probiotic B. polyfermenticus is effective in tube formation in human intestinal endothelial cells. Besides our study, a statistically significant difference was found in tube formation at 18 hours after application of *L. acidophilus* 10^9 CFU\ml and *L. rhamnosus* 10^9 CFU\ml extracts to HUVEC cells. These results are also consistent with the findings of VEGF and FGF gene expression at the final stage of the study (28,29).

VEGF is an effective substance, from early vascular development to tube formation. After this stage, angiopoietin (Ang) interferes with the endothelium for vascular stabilization, collects periendothelial cells and vascular stabilization is achieved. In the literature, *L. acidophilus* has been shown to significantly accelerate wound healing and provide a significant increase in collagen content, which is one of the markers that show wound healing. In a study, it has been reported that probiotic culture [*Lactobacillus acidophilus*, bulgaricus, casei, plantarum, Bifidobacteria breve, infantis, longum and Streptococcus], which increase the angiogenesis by VEGF, have a statistically positive effect on wound healing in gastric ulcers (30).

It is stated that probiotics act on angiogenesis in the inflammatory process by VEGF receptor signaling in the gastrointestinal tract. By angiogenesis, VEGF stimulates new microvesel formation and granule formation. Simulation of angiogenesis has a wound healing effect. In our study, the fact that VEGF and FGF genes expressing a role in angiogenesis in the experimental group where *L. acidophilus* 10^9 CFU\ml extract was applied to HUVEC cells compared to the control group supports the literature findings (31-33).

Although many studies have been conducted on probiotics for the treatment of gastrointestinal infections and cancer prevention, the results of these studies cannot be compared due to differences in many factors such as the type of probiotic microorganism used, its dosage, or whether the study was in vitro or in vivo. There are also significant differences between the in vitro effects of two different *L. acidophilus* and *L. rhamnosus* probiotic microorganisms we use. In addition, this shows that not only the variety but also the concentration changes applied may affect the results.

CONCLUSION

These findings support that probiotic microorganisms may differ depending on the type and nature. It is thought that it would be beneficial to support the data in this area and to reach new data by using different probiotics. It is also considered that it would be appropriate to try a variety of combinations including probiotics, prebiotics or their relationships with each other. After our research, realized that evaluate the probiotic effect of angiogenesis more detaily, it must be treated with other strains of *Lactobacillus*. The results of this in vitro study should be also shown in vivo and must be enriched with clinical applications.

Where the effects of probiotics on angiogenesis and human health are examined, we think that the results will provide an important data for the more comprehensive and more detailed studies to be conducted.

In addition, human studies are currently underway that strongly support the use of probiotics. After all these studies, it seems possible to benefit from probiotics in the diagnosis and treatment of diseases in the near future.

ETHICAL DECLARATIONS

Ethics Committee Approval: No interventional procedure was performed with the method and study protocol infrastructure of the study. Due to the absence of clinical studies, ethics committee approval is not required for cell culture research in invitro study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest: The authors have no conflicts of interest for declaration.

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Correlation of vitamin D level with the clinical-radiological severity of COVID-19 in geriatric patients

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ABSTRACT

Objective: This study was planned to investigate the effect of 25-OH-Vitamin D (Vit D) deficiency on clinical and radiological findings of coronavirus disease-2019 (COVID-19) in geriatric patients hospitalized due to COVID-19.

Material and Method: Seventy-five patients who were treated for COVID-19 were reviewed retrospectively, and grouped in relation with their ages [(1) 65-74, (2) 75-84, (3) >84 years] and the severity of Vit D deficiency [(1) severe deficiency: <10 ng/mL, (2) moderate deficiency: 10-20 ng/mL, (3) minor deficiency: 21-30 ng/mL, (4) normal: >30 ng/mL]. The complaints on admission, comorbidities, intensive care unit (ICU) need, length of hospital stay, laboratory data, and mortality of the ones who had and did not have Vit D replacement (n=18/75) were recorded. The patients were analyzed for COVID-19 severity using radiological and clinical markers.

Results: Moderate Vit D deficiency (10-20 ng/mL) was frequently detected. When the disease severity and Vit D levels were analyzed, it was found that the disease was more severe (46.6%) in the Vit D <10 ng/ml group, and milder (37.5%) in the >30 ng/ml group, but there was no statistically significant difference among the groups. Low or high Vit D levels did not show any significant correlations with the severity of pneumonia or the thorax CT findings. The intensive care unit (ICU) admission rate was significantly lower in those who had Vit D replacement ($p<0.001$).

Conclusion: The ICU admission rate was lower in patients who had Vit D replacement, however, serum Vit D concentrations were not correlated with COVID-19 severity or mortality risk.

Keywords: COVID-19, vitamin D, pneumonia, elderly, intensive care unit

INTRODUCTION

Individuals of all ages have been affected by the COVID-19 pandemic worldwide; however, the COVID-19-related mortality rate is significantly higher in the elderly. Several researchers investigated potential models to reduce infection rates and reported various molecules, including Vit D (1). A European study reported an inverse correlation between serum Vit D levels and the number of COVID-19 cases and mortality, however, another study reported the correlation between Vit D supplementation with less severe disease and a lower mortality rate in the elderly hospitalized patients (2,3).

The European Food Safety Authority (EFSA) has determined that six vitamins are important for maintaining a healthy immune system: vitamins D, C, A, ß-carotene, and B vitamins (B6, folate, and B12) (4).

Vit D plays an important role in the immune system: Vit D reduces the risk of microbial and viral infections by maintaining cellular physical defense and enhancing cellular immune responses (5,6).

Vit D contributes to humoral defense and has a regulatory role in proinflammatory cytokine production. Vit D can reduce cytokine storm and improve cellular immunity in severe COVID-19 patients (7-9).

Considering the hyper-inflammatory immune response induced by COVID-19, Vit D regulates vital anti-inflammatory mediators, and current data suggest that patients with Vit D deficiency may be more susceptible to being infected by SARS-CoV-2, and they may more likely develop severe symptoms (10). The high mortality rates observed in the elderly care facilities, particularly in the Northern countries with little exposure to sunlight, suggest that Vit D deficiency increases the severity of COVID-19 (11).

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Old age is an independent risk factor for Vit D deficiency (12). A recent study reported that although Vit D deficiency is common in older people, the difference between older and oldest adults is negligible. Therefore, all seniors may carry a high risk for Vit D deficiency and should have Vit D supplementation regardless of their age (13).

COVID-19 infection affects all age groups, but the elderly appear to be more severely affected. Cytokine storm and high pro-inflammatory cytokine release appear to be an important pathophysiological mechanisms in the elderly COVID-19 patients (14).

In 2020, Ebadi and Montano-Loza reported that Vit D could suppress the expression of pro-inflammatory cytokines including IL-1α, IL-1β, and TNF-α, found Vit D deficiency in 50% of COVID-19 cases and approximately 70% of the ones who died due to COVID-19 (15).

Ilie et al. investigated the relationship of mean Vit D levels with morbidity and mortality of COVID-19 in 20 European countries and reported negative correlations of Vit D levels (mean 56 mmol/L) with the number and mortality of COVID-19 cases (2).

A study on nutritional status in COVID-19 patients reported that Vit D deficiency was the most common deficiency. The mean Vit D level was reported as 15.73 ng/dl, and there was severe deficiency (≤10 ng/dl) in 24% of the patients. 100% of mechanically ventilated COVID-19 patients had Vit D deficiency (16). It was reported that the severity of Vit D deficiency was correlated with the prognosis of COVID-19 (relative risk 1.59 if <30 ng/mL) and risk of death (relative risk 1.56) (17,18). In another study, it was reported that the 14-day mortality rate was 31.3% in older adults who did not have Vit D supplements (3).

Vit D deficiency appears to be correlated with infection severity and mortality. However, findings cannot be generalized, and there is a need for randomized controlled trials (19).

In a study on COVID-19 patients who had and did not have Vit D replacements, only 1 patient (2.7%) died in the Vit D replacement group while 24 patients (14.1%) died in the non-replacement group (p=0.038). It has been reported that a single dose of 300,000 IU Vit D may be useful in the treatment of COVID-19 (20). Unlikely, another study reported that there was no link between serum Vit D concentrations and the risk of severe COVID-19 infection or death (21).

We investigated the effect of the severity of Vit D deficiency and high dose replacement therapy on the clinical and radiological findings of COVID-19 in geriatric patients hospitalized with the diagnosis of COVID-19.

MATERIAL AND METHOD

The ethics committee approval for this study was obtained by Health Science University Keçiören Education and Training Hospital Clinical Studies Ethics Board (Date: 28/12/2021, Decision No: 2012-KAEK-15/2440). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The data of the elder patients (>65 years old) with a confirmed diagnosis of COVID-19 [clinical-radiological diagnosis or with polymerase chain reaction (PCR)] and hospitalized between April 01, 2020, and March 01, 2021, were reviewed retrospectively from the electronic database of our hospital. The age groups were determined as (1) 65-74 years, (2) 75-84 years, and (3) >84 years. There were 75 patients (28 women and 47 men) included in the study. Whether the patients received Vit D replacement during the hospitalization period, their initial symptoms, comorbid diseases, hospital admission, intensive care unit (ICU) admission, length of hospital stay, mortality status, and their laboratory and radiological data were obtained from the electronic database.

The inclusion criteria were being ≥65 years old and the presence of measured Vit D levels. The ones who were younger than 65 years old and the ones who did not have Vit D level measurements were excluded.

Laboratory Data

Serum Vit D, ferritin, CRP, LDH, D-dimer, and troponin levels of the patients hospitalized for COVID-19, measured on the day of hospitalization, were recorded (previous Vit D supplementation status was unknown). The patients who had and did not have 300,000 IU Vit D replacement during hospitalization were determined. The severity of Vit D deficiency was categorized as; (1) severe Vit D deficiency: <10 ng/mL, (2) moderate deficiency: 10-20 ng/mL, (3) minor deficiency: 21-30 ng/mL, (4) normal: >30 ng/mL (22,23).

Clinical evaluation of COVID-19 severity was classified into mild, moderate, severe, or critical (WHO 2020. "Clinical management of COVID-19) [mild disease: Symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia, moderate disease: clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) but no signs of severe pneumonia, including SpO_2 ≥90% on room air, severe disease: with clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following: respiratory rate >30 breaths/min; severe respiratory distress; or SpO_2 <90% on room air, critical disease: respiratory failure not fully explained by cardiac failure or fluid overload. The patients admitted to the second or tertiary intensive care units, requiring high flow oxygen (HFO) or non-invasive/invasive mechanical ventilation (NIMV/IMV) were regarded to have critical disease].

Radiological Evaluation

Postero-anterior chest X-rays (CXR) and thorax computerized tomography (CT) performed at the first admission to the hospital were examined. Radiologic classification on high-resolution CT (HRCT) (typical, indeterminate, atypical, negative for pneumonia) and CXR grading of lung involvement (mild, moderate, severe) (RSNA Chest CT/ CXR findings related to COVID-19) were recorded (24).

Statistical Analysis

For the distribution of all numerical values, the Kolmogorov-Smirnov or Shapiro-Wilk test, skewness-kurtosis values, coefficient of variation, histogram, and detrended plot graphs were examined. Categorical data were analyzed with Chi-square or Fisher test, as appropriate. Categorical data were displayed as n / %. In our study group, 65-74-year-old, 75-84-year-old, and ≥85 years old groups were analyzed with ANOVA test, according to the Vit D levels. The relationship between gender and Vit D level was analyzed with Student's t-test. In our study group, the correlation between Vit D levels and COVID-19 severity (mild, moderate, severe, critical) was analyzed with an ANOVA test. Similarly, analyses of the numerical values in more than two groups were performed with Anova or the Kruskal Wallis tests, as appropriate. All analyses were performed using SPSS version 22. P values smaller than 0.05 were considered statistically significant.

RESULTS

There were 75 patients in total; 37.3% (n=28) were females and 62.7% (n=47) were males. Among the age groups, the 65-74-year-old age group comprised 48.0% (n=36) of all patients, and it was the most crowded group (Table 1). Each patient had at least one comorbid disease (%90, n=68). The most common comorbidity in women was cardiovascular disease (CVD) in 92% (n=26), and diabetes mellitus (DM) in 50% (n=14) of the females. In men, CVD was present in 78% (n=34), DM in 34% (n=14); non-pulmonary cancer 19% (n=8) and cerebrovascular disease (CVD) 21% (n=9) were also more common in men (not shown in the **Table**).

Dyspnea was present in 91.6% (n=31), 83.8% (n=26) and 87.7% (n=7), cough in 38.8% (n=14), 29% (n=9) and 62.5% (n=5), and fever was seen in 47.2% (n=17), 29% (n=9) and 12.5% (n=1) of the age groups, respectively. Dyspnea was more frequent in patients who needed ICU care (93.1%) (n=41) and in the ones who died (94.2%) (n=33), and fever was more frequent in those who did not need ICU care (41.9%) (n=13) and in those who survived (35.9%) (n=14), but there was no statistically significant difference among the groups (not shown in the **Table**). Mortality (47.2% (n=17), 46.6% (n=14), 50% (n=4), respectively) and ICU admission [(61.1% (n=22), 58% (n=18), and 50% (n=4)], respectively) were similar in the age groups studied, and no statistically significant correlations were found (not shown in **Table**).

Table 1. The distribution of the age groups and genders

	Number (n)	Percent (%)
Gender		
Female	28	37.33
Male	47	62.67
Age (years)		
65-74	36	48.00
75-84	31	41.33
85+	8	10.67

Overall, the Vit D levels were low in all age groups, and the Vit D level did not significantly decrease in parallel to aging. Moderate Vit D deficiency (10-20 ng/mL) was frequently detected. The mean Vit D was level 22.25±20.1 ng/mL (median: 16.46 ng/mL) in the 65-74-year-old age group, but the levels were not significantly different among the age groups. The mean Vit D levels were similar in males and females showing moderate Vit D deficiency, however, there was no statistically significant difference (**Table 2**).

Table 2. Vitamin D levels by age group and gender

	Vitamin D levels (ng/ml)		
	Mean	Median	p
Age (years)			0.095
65-74	22.25±20.1	16.46	
75-84	14.78±9.73	11.33	
85+	18.35±11.16	16.72	
Gender			0.322
Female	18.94±20.64	12.15	
Male	18.63±12.58	15.74	

When the disease severity and Vit D levels were analyzed, it was found that the disease was more severe (46.6%) (n=14) in the Vit D <10 ng/ml group, and milder (37.5%) (n=3) in the >30 ng/ml group, but there was no statistically significant difference among the groups. Due to the small number of patients in the groups, disease severity and Vit D levels data were divided into two subgroups to increase the number of patients and analyzed with Fisher's exact test. No statistical significance was found between the groups (**Table 3**).

Vit D level groups did not show any statistically significant correlations with the severity of pneumonia or the thorax CT findings. CXR, chest CT findings, and Vit D level data were divided into two subgroups and analyzed with Fisher's exact test. No statistical significance was found between the groups (**Table 4**).

Table 3. The clinical severity of COVID-19 and the Vitamin D levels

Vitamin D levels (ng/ml)					p*
Clinical stage	0-10 ng/ml (%)	10-20 ng/ml (%)	20-30 ng/ml (%)	>30 ng/ml (%)	
Mild n (%)	0 (0)	4 (50)	1 (12.5)	3 (37.5)	0.083
Moderate n (%)	2 (16.67)	8 (66.67)	2 (16.67)	0 (0)	
Severe n(%)	14 (46.67)	8 (26.67)	5 (16.67)	3 (10)	
Critical n(%)	7 (28)	12 (48)	3 (12)	3 (12)	
Vitamin D levels (ng/ml)					**p****
Clinical stage	<20ng/m		>20ng/m		
	n	%	n	%	
Mild-Moderate	14	25.45	6	30.00	0,452
Severe-Critical	41	74.55	14	70.00	

*Chi-square test **Fisher test ***WHO 2020. "Clinical management of COVID-19, grouped as mild, moderate, severe, or critical)

Table 4. Vitamin D level groups, pneumonia severity, and chest CT findings

Vitamin D levels (ng/ml)					**p value***
Chest X-ray pneumonia severity	0-10	10-20	20-30	30+	0.265
Normal n(%)	1(33.33)	0(0)	1(33.33)	1(33.33)	
Mild n (%)	3(12)	16(64)	4(16)	2(8)	
Moderate n(%)	14(48.28)	9(31.03)	2(6.9)	4(13.79)	
Severe n (%)	5(27.78)	7(38.89)	4(22.22)	2(11.11)	
Chest CT findings					
Typical n (%)	15(30)	23(46)	7 (14)	5(10)	
Indeterminate n (%)	3 (60)	2(40)	0 (0)	0(0)	0.265
Atypical n (%)	1(16.67)	2(33.33)	1(16.67)	2(33.33)	
Negative n (%)	0 (0)	1(100)	0 (0)	0(0)	
Chest X-ray pneumonia severity	**Vitamin D levels (ng/ml)**				**p value***
	<20 ng/m		**> 20 ng/m**		
	n	%	n	%	
Normal- Mild	20	36.36	8	40.00	0.488
Moderate-Severe	35	63.64	12	60.00	
Chest CT findings	**Vitamin D levels (ng/ml)**				**p value****
	< 20 ng/m		**> 20 ng/m**		
	n	%	n	%	
Typical-Indeterminate	43	78.18	12	60.00	0.102
Atypical-Negative	12	21.82	8	40.00	

*Chi-square test; **Fisher test; CT, Computerized tomography

Vit D groups did not show any statistically significant correlations with the lymphocyte or eosinophil counts, or CRP, D-dimer, ferritin, and troponin levels (not presented in the **Table**).

Vit D levels did not have any statistically significant correlations with mortality or ICU admission. ICU admission, mortality, and Vit D level data were divided into two subgroups and analyzed using Fisher's exact test. No statistical significance was found between the groups (**Table 5**).

Table 5. Vitamin D levels, ICU admission, and mortality

		Vitamin D levels (ng/ml)				**p***
		0-10	**10-20**	**20-30**	**30+**	
ICU n(%)	Not admitted	5(16.13)	16 (51.61)	6(19.35)	4(12.9)	0.141
	Admitted	18(40.91)	16(36.36)	5(11.36)	5(11.36)	
Mortality n(%)	Died	12(34.29)	14(40)	5(14.29)	4(11.43)	0.956
	Survived	11(28.21)	17(43.59)	6(15.38)	5(12.82)	
ICU admission, and mortality		Vitamin D levels (ng/ml)				p**
		<20ng/m		>20ng/m		
		n	%	n	%	
ICU		26	48.15	9	45.00	0,509
Mortality		28	51.85	11	55.00	

*Chi-square test **Fisher test, ICU: Intensive care unit

The days of hospitalization were determined as 13.7 days, 14.5 days, 11.3 days, and 10.2 days, in the Vit D level groups <10 ng/mL, 10-20 ng/mL, 21-30 ng/mL, and >30 ng/mL, respectively. There was no significant difference among the Vit D groups (not shown in the Table).

In the first hospitalization period, high-dose vit D replacement was given to only 18 patients with low serum Vit D levels. The analysis of Vit D replacement status (n=18/75) and ICU admission showed a statistically significant difference (p<0.001). Those who had Vit D replacement had a lower ICU admission rate, however, no statistically significant difference was observed in the mortality rates (**Table 6**). The number of days of hospitalization did not show any statistically significant correlation with the laboratory parameters (CRP, LDH, D-dimer, troponin) when the groups that had and did not have Vit D replacement were compared (not shown in the table).

Table 6. ICU admission and mortality in patients who had and did not have Vitamin D supplementation.

		No Vitamin D supplementation		**Had Vitamin D supplementation**		**p**
		n	**%**	**n**	**%**	
Survival	Died	28	50.00	7	38.89	0.292
	Survived	28	50.00	11	61.11	
ICU admission	No	18	31.58	13	72.22	<0.001
	Yes	39	68.42	5	27.78	

ICU: Intensive care unit

DISCUSSION

Vit D plays a key role in modulating the immune system, and its deficiency is associated with immune system disorders (5). Vit D reduces the risk of bacterial and viral infections with both the continuity of cellular physical defense and the strengthening of innate and adaptive cellular immunity.6 Bulut et al. found that Vit D deficiency is a frequent occurrence in COPD and is correlated with the frequency of exacerbation and hospitalization in COPD patients (25).

Due to the hyper-inflammatory nature of COVID-19, the anti-inflammatory mediator regulatory capacity of Vit D is noteworthy. In terms of regulation of anti-inflammatory mediators, one may suggest that patients with Vit D deficiency may be more susceptible to COVID-19, and the disease may be more severe (10). Higher number of cases and higher mortality were reported in the ones with Vit D deficiency and COVID-19, however higher disease severity and higher survival rates were reported in the ones who had Vit D supplementation (3,4).

In our study, the Vit D levels were generally low in all age groups. Consistent with the literature, moderate Vit D deficiency (10-20 ng/mL) was determined. The mean Vit D level was the highest at 22.25±20.1 ng/ml (median: 16.46 ng/ml) in the 65-74-year-old age group, however, there was no significant difference among the age groups. The mean Vit D levels were similar in males and females, and both genders had moderate Vit D deficiency (26).

The age groups (the old and the oldest) did not show severe Vit D deficiency or decrease in Vit D levels parallel to aging, however, there are dissimilar results in the literature (13).

Bassatne et al. (27) reported that they observed an increasing trend in COVID-19 disease severity in patients with serum 25(OH) D <30 ng/ml (RR=3.0, 95% CI) in their meta-analysis.

There was no statistically significant difference among the Vit D groups for COVID-19 severity, but the number of severe stage patients was higher in the severe Vit D deficiency (<10 ng/ml) group, and the number of mild stage patients was higher in the normal Vit D level group (11).

Although a negative correlation was reported between Vit D level and radiological pulmonary involvement in the literature (26), no statistically significant correlation was found between Vit D level and CXR pneumonia severity or chest CT findings in our study.

D-dimer is one of the most important indicators associated with mortality risk in COVID-19. Studies have reported a negative correlation between Vit D and D-dimer levels. In our study, Vit D groups did not show any negative or positive correlations with D-dimer, CRP, lymphocyte, eosinophil, ferritin, or troponin values (28,29).

Bassatne et al. (27) pooled data from three individual studies (30-32) (n=480) and found that the risk of intensive care unit admission was increased in COVID-19 patients with low 25(OH) D levels (<20 ng/ml). However, in conclusion, none of the outcomes evaluated showed a clear and strong correlation between vitamin D status on COVID-19 health-related outcomes.

Similar to literature data, the ICU admission rate was higher (40%) (n=18) in the group with severe Vit D deficiency (0-10 ng/ml), but the difference among the Vit D groups was not statistically significant (16,33).

The highest mortality rate in the Vit D subgroups was observed in the moderate (10-20 ng/ml) 40% (n=14) and severe deficiency (0-10 ng/ml) 34.2% (n=12) groups. However, no significant difference was found among the Vit D subgroups in terms of mortality. Our results are not in line with the previous studie (18,19,29).

Hernandez et al. (31) reported significantly longer hospital stays in COVID-19 patients with low serum 25(OH)D (<20 ng/ml) than in the normal group (p=0.013). But Baktash et al. (28) found no significant difference between Vit D groups in COVID-19 patients in terms of mean hospital stay.

In our study, the number of hospitalization days was 13.7 days in the severe Vit D deficiency group and 10.2 days in the normal Vit D level group. There was no significant difference among the Vit D groups for this parameter.

In a meta-analysis involving hospitalized COVID-19 patients, it was found that there was no statistically significant difference in mortality in patients who received vitamin D supplementation compared to patients who did not receive it (p=0.87) (34).

Our results (mortality rate 38% (n=7) in the group that had Vit D replacement, 50% (n=28) in the group that did not have it) were not similar to the previously reported results in terms of mortality, hospitalization days, and laboratory values (CRP, LDH, D-dimer, troponin) when the patients who had and did not have Vit D replacement were compared (35).

In a meta-analysis (n=532) of data from two randomized controlled trials and a retrospective case-control study involving hospitalized COVID-19 patients, it was reported that patients who received vitamin D supplementation had a statistically (p<0.0001) lower ICU requirement than patients who did not (34).

Tan et al. (36) In their prospective cohort study, it was observed that 17 patients with COVID-19 who received 1000 IU of vitamin D3, magnesium, and B complex daily for up to 14 days had a reduced risk of admission to ICU compared to 26 patients who did not receive.

Similar to the studies in the literature, a statistically significant difference was found when the status of having a Vit D replacement (n=18/75) and ICU admission were compared (p<0.001). Those who had Vit D replacement had a lower ICU admission rate (ICU admission rate was 27.7% (n=5) in the group that had Vit D replacement, 68.4% (n=39) in the group that did not have Vit D replacement) (3).

However, contrary to the results of these studies; there is also a study reporting that a single oral supplement of vitamin D3 (200,000 IU of vitamin D3) (n=120/120) did not improve COVID-19-related health outcomes, such as mortality, intensive care unit admission, and need for mechanical ventilation, compared to placebo (37).

It is not possible to generalize the results of this single-center study. We could not obtain data related to Vit D deficiency including obesity, socioeconomic status, population demographics, and exposure to sunlight. We believe that these factors would influence the results of the study (12). We did not have any information on the Vit D replacement statuses of the patients before they had COVID-19. We were unable to distinguish whether Vit D deficiency was a pre-existing deficiency in patients with COVID-19, or the decrease was due to the inflammatory process as a result of rapid metabolism of Vit D, and/or because Vit D is a negative acute-phase reactant (19).

CONCLUSION

The results of the systematic review and meta-analysis revealed inconclusive evidence of a relationship between low serum 25(OH) D levels (<20 ng/ml) and the risk of mortality, admission to the intensive care unit, mechanical ventilation, and the need for non-invasive ventilation. puts it. In addition, no associations were found between disease severity and risk of ARDS, and length of hospital stay.

Although the ICU admission rate was found to be low in patients who had Vit D replacement in our study, our findings do not support a potential link of Vit D concentrations in general with disease severity or mortality risk of COVID-19 infection in hospitalized elderly patients. To achieve statistical significance in disease severity, mortality, and the benefit of Vit D replacement, expanding the sample size at the national level may be helpful. New studies should be conducted to determine whether a low Vit D level is the cause or consequence of the COVID-19-related inflammatory process.

ETHICAL DECLARATIONS

Ethics Committee Approval: The ethics committee approval for this study was obtained by Health Science University Keçiören Education and Training Hospital Clinical Studies Ethics Board (Date: 28/12/2021, Decision No: 2012-KAEK-15/2440).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The effect of main pulmonary artery diameter on the prognosis of COVID-19 patients in the ICU

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ABSTRACT

Aim: The aim of this study was to examine the effect of main pulmonary artery diameter (MPAD), which is evaluated in patients when first admitted to the intensive care unit due to COVID-19, on mortality.

Material and Method: Thoracic computed tomography examinations performed during the initial admission to hospital of patients who were treated in the intensive care unit between October 1, 2020, and June 1, 2021, were evaluated retrospectively. Cox regression analysis was performed with the program R-Project to evaluate the relationship between MPAD and mortality.

Results: No significant correlation was found between MPAD and mortality in models used with or without adjusting for age and sex (respectively P: 0.890 and P: 0.920).

Conclusion: The MPAD value measured at the initial admission of COVID-19 patients hospitalized in the intensive care unit is not a parameter that can be used to predict mortality.

Keywords: COVID-19, main pulmonary artery diameter, intensive care unit, mortality

INTRODUCTION

Coronavirus disease is contracted through a newly identified viral infection caused by SARS-CoV-2, which can cause respiratory tract disease (1). This disease was first seen in the city of Wuhan located in Hubei Province in the People's Republic of China in the last days of 2019 (2). Due to the abundance of asymptomatic cases, it spread rapidly around the world (3). On March 11, 2020, this situation was declared a pandemic by the World Health Organization(WHO) (4). According to WHO data, as of January 15, 2022, the number of cases in the world exceeded 326 million and deaths exceeded 5.55 million (https://www.worldometers.info/coronavirus/). Although SARS-CoV-2 infection usually affects the lung and respiratory system, it can disrupt the structural integrity of the endothelial wall of the vascular structures and disrupt the functionality of the vascular structure. Endothelial cells provide vascular vasodilation and anticoagulation in the circulatory system through the cytokines they secrete in physiological state. When an inflammatory process such as COVID 19 is experienced, this situation reverses and causes vasoconstriction and procoagulation (microthrombus, etc.). This situation affects the entire vascular system as well as the pulmonary artery system, which plays an important role in the cardiopulmonary circulation. It can lead to increased pressure in the pulmonary artery, that is, pulmonary hypertension (5, 6).

Pulmonary hypertension can be regarded as the main reason for increases in the diameter of the pulmonary artery. Pulmonary hypertension is the case when mean pulmonary artery pressure, usually measured by cardiologists, is above 25 mmHg in right heart catheterization (7). During this procedure, the patient should be at rest (7). If the mean pulmonary artery pressure is between 21 and 24 mmHg, it is called borderline pulmonary hypertension (8). Diseases causing pulmonary hypertension are classified into five groups according to clinical, physiopathologic, and hemodynamic characteristics. Generally, primary lung pathologies are included in group 3 (9). The diagnosis of pulmonary hypertension is made by electrocardiogram, chest X-ray, pulmonary function tests, arterial blood gas, Doppler echocardiography, lung ventilation-perfusion

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scintigraphy, and thoracic computed tomography (CT). The increase in pulmonary pressure causes an increase in the diameter of the pulmonary artery. Pulmonary hypertension is suspected if the main pulmonary artery diameter (MPAD) is greater than or equal to 29 mm or the ratio of the diameter of the main pulmonary artery to the diameter of the ascending aorta is greater than one in thoracic CT (10, 11). It has been shown by previous studies that the increase in pulmonary diameter is associated with prognosis in patients other than COVID-19 (5, 6, 11). In line with all this information, the effect of MPAD in predicting the probability of mortality due to SARS-CoV-2, the biggest pandemic this century, was examined.

MATERIAL AND METHOD

This study was approved by the Ministry of Health of the Republic of Turkey and Adıyaman University Non-Invasive Clinical Researches Ethics Committee (Date: 15.03.2022, Decision No: 2022/3-31). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patient Selection

Patients hospitalized in the intensive care unit of our hospital between October 1, 2020 and June 1, 2021 due to SARS-CoV-2 infection were included in this retrospective study. Those over the age of 18 were included. Patients who were confirmed by real-time polymerase chain reaction (RT-PCR) for the SARS-CoV-2 RNA test and who had involvement congruent with COVID-19 pneumonia on thoracic CT imaging were included. Those with a negative RT-PCR for the SARS-CoV-2 RNA test and with no involvement in the thoracic CT examination were excluded. Patients with chronic liver disease, chronic kidney disease, malignancy, chronic obstructive pulmonary disease (COPD), and/or hematological diseases were excluded (**Diagram 1**). The criteria for admission to the intensive care unit were set according to those included in the COVID-19 adult patient treatment guideline of the Ministry of Health of the Republic of Turkey (12).

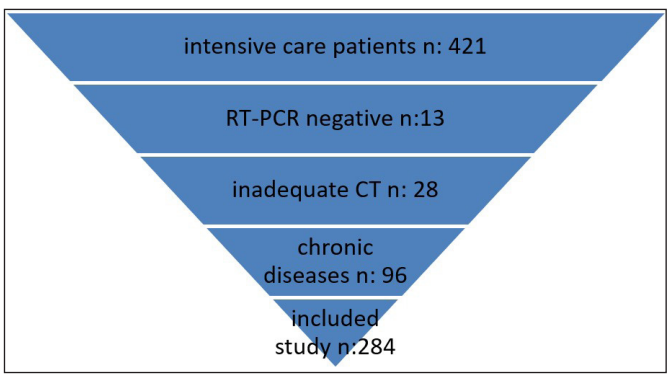

Diagram 1. Flow-chart for patient selection

Image Acquisition

Patients included in the study were screened using a 16-detector CT scanner (MX16, Philips Medical Systems, Eindhoven, the Netherlands). Pulmonary artery diameter was measured using the program Oracle Database version 1.10.48.299 on the images obtained. These patients were evaluated by a radiologist (M.Ç.) with at least 10 years of experience in the field of thoracic CT images upon initial admission to the hospital. Patients were excluded from the study in the event of conditions that prevented the main MPAD from being evaluated in the thoracic CT examination (artifact, inappropriate extraction, scoliosis, etc.). As recommended in the literature, MPAD was measured from wall to wall of the pulmonary artery at the level where it was seen at its widest level in the axial plane (13, 14) (**Figure 1**).

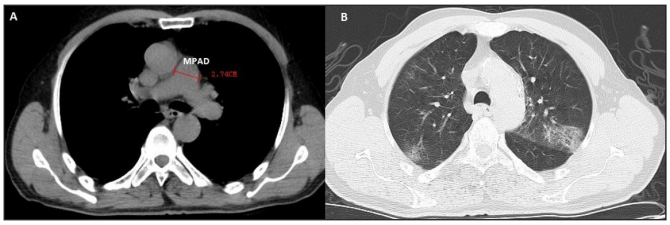

Figure 1. Axial noncontrast thoracic computed tomography images of a 63-year-old male patient A) Measurement of the main pulmonary artery diameter (27 mm) B) Peripheral ground-glass areas due to COVID-19 involvement in the upper lobes of both lungs at the time of initialadmission

Statistical Analysis

Frequency analysis, descriptive statistics, and survival analysis of the study were performed. Cox regression analysis was also performed to examine the effects of pulmonary artery diameter on the survival period of the patients. The results were obtained separately for the adjusted model and the unadjusted model, which was obtained by removing the effects of age and sex in Cox regression analyses. The margin of error was taken as 5% and the significance level was considered $P < 0.05$. The entire analysis was conducted with the program R-Project (R CoreTeam, 2020) (15).

RESULTS

In total 284 patients were included in the study. The results of the patients were calculated according to the descriptive statistics. Of the patients, 169 (59.5%) were male and 115 (40.5%) were female. Of these followed-up patients, 182 (64.1%) died and 102 (35.9%) were discharged alive. The mean age of the patients was 69.82 (range: 27-95) years (**Table 1**).

The mean MPAD of the patients was 29.33 mm. The narrowest MPAD was 21 mm and the widest was 44 mm (**Table 2**). No significant correlation was found between MPAD and mortality in the model unadjusted for age

and sex (P: 0.890). No significant correlation was found between MPAD and mortality in the model adjusted for age and sex either (P: 0.920). According to these results, an increase in MPAD did not cause a statistically significant increase in mortality (**Table 3**).

Table 1. Patients included in the study and their outcomes

	n	%
Sex		
Male	169	59.5
Female	115	40.5
Outcome		
Exitus	182	64.1
Alive	102	35.9

Table 2. Descriptive statistics of MPAD

	Mean	SD	Min	Max
MPAD	29.33	3.88	21.00	44.00

Table 3. Cox regression analysis results used as age and sex factor adjustment and established without correction effect

	Adjusted Model				Unadjusted Model			
	OR	Lower limit	Upper limit	P	OR	Lower limit	Upper limit	P
MPAD	1.003	0.961	1.047	0.890	1.002	0.959	1.047	0.920

DISCUSSION

It was demonstrated that there was no significant relationship between MPAD, which was evaluated in the thoracic CT examination obtained during the initial admission of patients who needed intensive care due to COVID-19 pneumonia and were followed up in the intensive care unit, and mortality. Although there are many studies in the literature evaluating the relationship between MPAD and COVID-19, the present study is unique in terms of study population and study design, since patients in intensive care units were evaluated and there were a large number of patients.

In the study by QQ Zhu et al. (16) that investigated the prediction of mortality using the pulmonary artery diameter of 180 COVID-19 patients, the contribution of pulmonary artery diameter to the increase in mortality was significant. In that study, the MPAD cut-off value used was 29 mm and a pulmonary artery diameter above 29 mm was considered large. However, in the present study, the effect of MPAD increase on mortality was examined without using a cut-off value. In addition, the mortality rates in the present study were quite high compared to those in the study by QQ Zhu et al. (16) (64.1% vs. 7.8%) due to the high mean age of the patients included in the present study (69.82 years vs. 46.99 years) and the patients requiring intensive care treatment.

In the study conducted by Erdoğan et al. (17) investigating the effect of MPAD, ascending aorta diameter, and the ratio of these two diameters on the prognosis of COVID-19 patients, the increase in the diameter of these vessels had a significant effect on mortality. They showed that MPAD was the most significant in relation to mortality among the three parameters they evaluated. In the study by Erdoğan et al., in which they included 255 patients hospitalized for COVID-19, the mean age was 55 and the mortality rate 21.9% (17). However, the patient profile included in the present study differed from the patient profile of Erdoğan et al.'s study, which caused the results obtained in the two studies to be different.

In their study conducted with 101 patients, Yıldız et al. (18) showed that the correlation of MPAD with the severity of COVID-19 pneumonia was significant. In that study with a small group of patients, they did not evaluate the relationship between MPAD and mortality (18). The relationship between pneumonia severity and MPAD was not examined in the patients included in the present study. Since the patient group in the present study consisted of intensive care unit patients, evaluation of pneumonia severity was not considered. The mortality status of the patients was evaluated as the prognosis.

In a COVID-19 study using the data of 1469 patients hospitalized in seven tertiary hospitals in Italy, the relationship between MPAD and mortality was examined (19). In that study, MPAD equal to or greater than 31 mm was the parameter that increased the mortality rates of COVID-19 patients. Although the border diameter length referenced was greater, it was determined that the mortality rate increased as MPAD increased. The mean age of the patients in that study was 69 years, which is similar to that of the present study. The mortality rate was 21%, which is lower than the rate in the present study (19). However, in that multicentric study, thoracic CT examinations were not obtained during the initial admission of the patients to the hospital, and thoracic CT examinations obtained 72 hours after admission were evaluated. Another difference from the present study is that only intensive care patients were included. In addition, cut-off values were not used to evaluate the diameter of the pulmonary artery in the present study.

Although the primary outcome revealed that it had no significant effect on mortality due to MPAD and COVID-19, this study has some limitations. One of the main limitations is that it was carried out as a single-center, retrospective, and nonrandomized study. The effect of COVID-19 disease could not be evaluated because MPAD was not evaluated during the intensive care period. The lack of data on the history of the patients in terms of pulmonary thromboembolism and heart diseases is another limitation of the study. In addition, thoracic CT images included in the study were without contrast and were obtained without using electrocardiography gating.

In the present study, information about the dynamic state of the pulmonary artery could not be obtained, since additional examinations such as echocardiography and right heart catheterization were not performed. Finally, the long-term effect of COVID-19 disease was not investigated, since MPAD follow-up could not be performed after treatment.

CONCLUSION

Despite the limitations of this study, it was shown that MPAD obtained at the time of initial admission to the hospital in intensive care units due to COVID-19 disease is not a parameter that can be used to predict mortality.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Ministry of Health of the Republic of Turkey and Adıyaman University Non-invasive Clinical Researches Ethics Committee (Date: 15.03.2022, Decision No: 2022/3-31).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Does your sleeping position affect your shoulder pain?

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ABSTRACT

Aim: This study aimed to evaluate patients with shoulder pain according to their sleeping positions based on their clinical and magnetic resonance imaging (MRI) findings and to determine possible factors affecting shoulder pain.

Material and Method: A total of 115 patients were included in the study. The severity of shoulder pain was evaluated with the visual analog scale (VAS), shoulder function was evaluated with the simple shoulder test, and the ability to perform physical activities was evaluated with the QuickDASH questionnaire. The biceps tendon, rotator cuff (RC), subacromial-subdeltoid bursa, glenohumeral joint (GHJ), and acromioclavicular joint (ACJ) were evaluated using MRI.

Results: Of the patients with shoulder pain, 66.1% were female, 50.4% were primary school graduates, 53.9% were housewives, and 41.7% had a systemic disease. The mean age of the patients was 50.48±13.61 years while the median BMI and VAS values were 26.1 (18.2-41.4) and 8 (2-10), respectively. Considering the sleeping positions, it was found that 39.1% (most common) of the patients were sleeping in the fetus position, and considering the results of patients' MRI examinations, the most common problem was related to the pathologies of the supraspinatus tendon (42.6%). It was found that sleep quality, which was poor in all patients, was worse in females (p=0.311), in those over 50 years of age (p=0.007), and those with a systemic disease (0.325). It was discovered that Pittsburgh's sleep quality index score was generally worse in those who slept in the soldier position and in the log position (p>0.05). The rates of pathologies of the supraspinatus tendon were found to be the highest in those that slept in the fetus position (p=0.931). It was also found that the rates of impingement, bicipital tendinitis, combined problems, and adhesive capsulitis did not differ significantly according to sleeping positions. Although occupational variables for supraspinatus degeneration remained significant in the model, having a desk job statistically significantly increased the probability of supraspinatus degeneration by 3.38 times when compared to being a housewife (95% CI=1.143-9.996; p=0.028) and it was identified that the probability of acromioclavicular degeneration increased by 1.16 times for every 1-unit increase in BMI.

Conclusion: Different sleeping positions may predispose to different shoulder pathologies and shoulder pain, and shoulder pathologies may lead to deterioration of sleep quality, especially in older patients. For this reason, suggesting correct and appropriate sleeping positions may be a useful treatment method in reducing pain and disability and increasing sleep quality.

Keywords: Sleep position, shoulder pain, magnetic resonance

INTRODUCTION

The shoulder joint is the joint that anatomically connects the thorax and the upper extremity. The shoulder joint has a wide range of motion but little stability, and therefore, it is frequently exposed to traumas and injuries. Shoulder pathologies are very painful conditions due to the rich sensory innervation network (1). Prevalence studies indicate that the prevalence of shoulder pain affects 7-10% of the society (2). The most common causes of pain are rotator cuff (RC) pathologies originating from the tendon and bursa. Subacromial impingement syndrome (SIS) occurs as a result of the compression of the structures within the RC between the acromion, the coracoacromial ligament, the coracoid process, and the acromioclavicular joint (ACJ) through glenohumeral joint (GHJ) movements (especially during flexion and rotation) (3). SIS is the most common cause of shoulder pain with a prevalence of 44-65% (4). Vascular, degenerative, traumatic, mechanical, and anatomical causes are believed to lead to etiopathogenesis (5). Additionally, adhesive capsulitis, calcific tendinitis, ACJ degeneration, GHJ osteoarthritis, glenohumeral instability are among the causes of shoulder pain. Even though shoulder pain increases during the day due to activities of daily living

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and sports activities (swimming, volleyball, handball, etc.) that involve shoulder overhead mobility (6), night pain is one of the most common symptoms of shoulder pain. Some patients first complain of the pain that wakes them up in the middle of the night or that they feel when they wake up in the morning (7-9).

Considering that people spend most of their lives sleeping, sleeping positions can entail a risk for shoulder pain. In this study that was based on the view that some sleeping positions may trigger shoulder pain by increasing the subacromial pressure and disrupting the blood supply of anatomical structures, we aimed to evaluate patients with shoulder pain according to their sleeping positions using clinical and magnetic resonance imaging (MRI) findings and to determine possible factors affecting shoulder pain.

MATERIAL AND METHOD

The study protocol was approved by the Adana Çukurova University Balcalı Hospital Clinical Trials Ethics Committee (Date: 12.02.2021, Decision No: 55). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. We retrospectively reviewed the medical records of this patients, who applied to the Physical Medicine and Rehabilitation Polyclinic of Adana City Training and Research Hospital with the complaint of shoulder pain from February to May 1, 2021, and who underwent MRI. Patients with a history of shoulder surgery and trauma, malignancy, and neurological, systemic, endocrine, metabolic, and rheumatic diseases were not included in the study. Demographic, clinical, and MRI findings of the patients were recorded. The patients were divided into 6 different groups according to their sleeping positions as fetus, log, yearner, soldier, prone, and starfish (Figure 1). The severity of shoulder pain (at motion/rest) was evaluated via the VAS. Shoulder function was evaluated with the SST, the ability to perform physical activities with the QuickDASH questionnaire, and the quantitative measurement of sleep quality was conducted with the Pittsburgh sleep quality index (PSQI).

QuickDASH allows evaluating a patient's ability to perform physical activities, taking their condition within the last week into account. This questionnaire consists of 11 questions scored between 1-5 according to the degree of difficulty. A special formulation is used in the calculation and the total QuickDASH score ranges from 0 to 100.

SST is a questionnaire consisting of 12 items concerning shoulder function. The total score that can be obtained from the questionnaire is between 0 and 12, and the items are scored using "Yes" or "No". The lower the score is, the greater the disability is.

PSQI provides an assessment of sleep quality, amount of sleep, presence, and severity of sleep disturbance in the previous month. This index consists of 7 subscales, namely subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime drowsiness. Total PSQI score is obtained by summing 7 subscales and can range from 0-21 points. A total PSQI score of ≤5 indicates good sleep quality, and that of >5 indicates poor sleep quality. The biceps tendon, RC, subacromial-subdeltoid (SA-SD) bursa, GHJ, and ACJ of the patients were evaluated in MRI examination.

Statistical Analysis

Continuous variables were expressed as mean±standard deviation and median (min-max) while categorical data were expressed in numbers and percentages. Normality analyzes of the continuous variables were performed with the Kolmogorov-Smirnov Goodness of Fit Test. When the data did not normally distribute, the Mann Whitney U Test was used for comparisons between two groups, and the Kruskal Wallis Test was employed for comparisons between three or more groups (the Mann Whitney U Test with Bonferroni correction was used for further analysis). The categorical data were analyzed through the Chi-Square Test. To predict the presence of findings evaluated by MRI, possible risk factors examined within the scope of the study and having a significance level of $p<0.25$ as a result of univariate analyzes were evaluated with the multivariate (Binary) Logistic Regression Model. The Hosmer Lemeshow Test was used for model fit. Analyzes were performed using the IBM SPSS version 22.0 (IBM Corporation, Armonk, NY, USA). The cases in which the type 1 error level was below 5% were considered significant.

RESULTS

The demographic and clinical characteristics of the 115 patients included in the study are shown in **Table 1**. When the sleeping positions were examined, it was observed that most of the patients (45%) were sleeping in the fetus position.

When the nominal demographic data were compared in terms of QuickDASH and PSQI scores, it was observed that the PSQI scores were significantly higher in the patients with shoulder pain aged 50 years and over compared to those aged 50 years and below in both genders [15 (3-21) vs. 9 (1-21)], and that the QuickDASH test scores were significantly higher in females than in males [61.3 (22.8-95.0) vs. 45.4 (13.6-90.9)] (p=0.007 and p=0.003, respectively) (**Table 2**).

The VAS, QuickDASH, and PSQI scores were found to be significantly higher in housewives (p=0.007) (**Table 2**).

The QuickDASH test scores [61.3 (22.8-95.0) vs. 45.4 (13.6-90.9)] were found to be significantly higher in females than in males (p=0.003) (**Table 2**).

The SST scores were found to be significantly higher in patients with supraspinatus degeneration (p<0.05) (**Table 2**).

The VAS scores were found to be significantly higher in patients that had combined problems (more than one disorder in the shoulder) (p<0.05) (**Table 2**).

The PSQI scores were found to be significantly higher in those with adhesive capsulitis (p<0.05) (**Table 2**).

It was observed that in those sleeping in the starfish position, the median values of the SST [7 (1-8)] were generally higher compared to those sleeping in the other positions, and the differences were statistically significant compared to those sleeping in the fetus position [2 (0-8)] and the soldier position [2 (0-4)] (p<0.05) (**Table 3**).

While those sleeping in the soldier position [18 (2-21)] and the log position [17 (2-21)] had the highest PSQI scores, those sleeping in the yearner position [10 (1-21)] and the fetus position [12 (2-19)] had the lowest PSQI scores. However, there was no statistically significant difference between the sleeping positions regarding the PSQI scores (p>0.05) (**Table 3**).

The rates of acromioclavicular degeneration were found to be the highest in those sleeping in the starfish position (16.7%) (p=0.618). The supraspinatus degeneration rates were found to be the highest in those sleeping in the fetus position (46.7%) (p=0.931). Similarly, the rates of impingement, bicipital tendinitis, combined problems, and adhesive capsulitis did not show a significant difference according to the sleeping positions (**Table 3**).

In order to predict the presence of findings evaluated by MRI, possible risk factors examined within the scope of the study and having a significance level of p<0.25 as a result of univariate analyzes were evaluated with multivariate logistic regression analysis. Although the variables of occupation and SST for supraspinatus degeneration remained significant in the model, when compared to being a housewife, having a desk job increased the probability of supraspinatus degeneration statistically significantly by 3.38 times (95% CI=1,143-9.996; p=0.028) (**Table 4**), and each 1-unit increase in BMI increased the probability of acromioclavicular degeneration by 1.16 times (**Table 5**).

Table 1. Demographic and clinical characteristics of the patient group experiencing shoulder pain

	Patient group (n=115)
Age (years) (Avg.±SD.)	50.48±13.61
BMI (kg/m²) [median (min-max)]	26.1 (18.2-41.4)
VAS [median (min-max)]	8 (2-10)
Gender (n, %)	
Female	76 (66.1)
Male	39 (33.9)
Educational background (n, %)	
Illiterate	13 (11.3)
Primary School graduate	58 (50.4)
Secondary-High School graduate	31 (27.0)
University graduate	13 (11.3)
Occupation (n, %)	
Housewife	62 (53.9)
Blue-collar	14 (12.2)
Desk job	20 (17.4)
Retired	19 (16.5)
Systemic disease (n, %)	
Yes	48 (41.7)
No	67 (58.3)
Shoulder pain (n, %)	
At motion	76 (66.1)
At rest	39 (33.9)
Sleeping positions (n, %)	
Fetus	45 (39.1)
Prone	25 (21.7)
Starfish	6 (5.2)
Soldier	7 (6.1)
Yearner	24 (20.9)
Log	8 (7.0)
Acromioclavicular degeneration (n, %)	
No	105 (91.3)
Yes	10 (8.7)
Supraspinatus degeneration (n, %)	
No	66 (57.4)
Yes	49 (42.6)
Impingement (n, %)	
No	104 (90.4)
Yes	11 (9.6)
Bicipital tendinitis (n, %)	
No	100 (87.0)
Yes	15 (13.0)
Combined problems (n, %)	
No	96 (83.5)
Yes	19 (16.5)
Adhesive capsulitis (n, %)	
No	111 (96.5)
Yes	4 (3.5)
Total	115 (100.0)

Table 2. Comparison of the VAS, SST, Quick dash test, and PSQI scores of the patient group with shoulder pain according to specific demographic and clinical characteristics

	VAS	P	SST	p	Quick dash test	p	PSQI	p
Age (year)		0.470*		0.086*		0.329*		0.007*
≤50 (n=57)	7 (3-10)		4 (0-8)		55.8 (20.4-95.0)		9 (1-21)	
>50 (n=58)	8 (2-10)		2 (0-10)		56.3 (13.6-90.9)		15 (3-21)	
BMI (kg/m²)		0.281*		0.685*		0.853*		0.251*
<30 (n=87)	8 (3-10)		3 (0-8)		55.8 (18.1-95.0)		10 (1-21)	
≥30 (n=28)	8 (2-10)		3 (0-10)		57.9 (13.6-90.9)		15 (2-21)	
Gender (n, %)		0.111*		0.310*		0.003*		0.311*
Female	8 (3-10)		2 (0-8)		61.3 (22.8-95.0)		14.5 (2-21)	
Male	7 (2-10)		4 (0-10)		45.4 (13.6-90.9)		10 (1-21)	
Educational attainment (n, %)		0.093**		0.059**		0.307**		0.335**
Illiterate	8 (7-10)		2 (0-8)		63.4 (18.8-90.9)		14 (3-21)	
Primary school	8 (3-10)		2 (0-8)		58.0 (18.1-95.0)		15 (1-21)	
Secondary-High school	7 (2-10)		4 (0-10)		47.7 (13.6-86.3)		10 (2-21)	
University	7 (3-10)		3 (0-7)		52.2 (25.0-72.7)		10 (3-17)	
Occupation (n, %)		0.004**		0.125**		0.001**		0.016**
Housewife	8 (5-10)a		2 (0-8)		63.4 (22.8-95.0)a		15 (2-21)a	
Blue-collar	7 (3-9)a		4 (0-8)		44.2 (20.4-75.0)a		6 (1-21)a	
Desk job	7 (3-10)a		4.5 (0-8)		52.2 (25.0-78.5)		8 (3-18)	
Retired	7 (2-10)		3 (0-10)		43.1 (13.6-90.9)a		12 (5-21)	
Systemic disease (n, %)		0.100*		0.561*		0.016*		0.325*
Yes	7 (2-10)		2.5 (0-10)		52.8 (13.6-86.3)		15 (2-21)	
No	8 (3-10)		3 (0-8)		59.0 (20.4-95.0)		12 (1-21)	
Shoulder pain (n, %)		0.686*		0.049*		0.125*		0.785*
At motion	8 (2-10)		2 (0-10)		60.9 (13.6-90.9)		14 (1-21)	
At rest	7 (5-10)		4 (0-8)		53.4 (18.1-95.0)		10 (2-21)	
Acromioclavicular degeneration (n, %)		0.023*		0.082*		0.113*		0.521*
No	8 (2-10)		2 (0-10)		56.8 (13.6-95.0)		14 (1-21)	
Yes	6.5 (4-8)		3 (2-8)		38.6 (25.0-68.1)		11 (2-21)	
Supraspinatus degeneration (n, %)		0.127*		0.028*		0.469*		0.095*
No	8 (3-10)		2 (0-8)		58.0 (18.1-95.0)		14 (2-21)	
Yes	7 (2-10)		4 (0-10)		55.4 (13.6-86.3)		10 (1-21)	
Impingement (n, %)		0.614*		0.943*		0.552*		0.360*
No	8 (3-10)		3 (0-10)		56.3 (13.6-90.9)		12 (1-21)	
Yes	7 (2-10)		4 (0-7)		47.7 (18.1-95.0)		15 (2-21)	
Bicipital tendinitis (n, %)		0.865*		0.775*		0.871*		0.505*
No	8 (2-10)		3 (0-10)		56.8 (13.6-95.0)		14 (1-21)	
Yes	8 (6-10)		2 (0-7)		47.7 (25.0-86.3)		8 (3-21)	
Combined disorders (n, %)		0.001*		0.004*		0.226*		0.623*
No	7 (2-10)		3 (0-10)		54.5 (13.6-95.0)		12 (1-21)	
Yes	9 (7-10)		1 (0-8)		61.3 (22.7-90.9)		14 (2-21)	
Adhesive capsulitis (n, %)		0.043*		0.829*		0.813*		0.016*
No	8 (2-10)		3 (0-10)		55.8 (13.6-95.0)		12 (1-21)	
Yes	6 (5-7)		2.5 (0-7)		54.5 (25.0-68.1)		19 (17-21)	

* Mann Whitney U Test, ** Kruskal Wallis Test (aMann Whitney U Test with Bonferroni correction; the difference between the two groups is statistically significant, p<0.01)

Table 3. Comparison of some clinical and MRI findings according to sleeping positions

	n	Fetus position	n	Prone Position	n	Starfish position	n	Soldier position	n	Yearner position	n	Log position	p
Pain at motion (VAS) [median (min-max)]	33	8 (5-9)	16	8 (4-10)	3	5 (3-7)	3	8 (7-10)	16	7 (2-10)	5	7 (7-8)	0.084*
Pain at rest (VAS) [median (min-max)]	12	7.5 (5-9)	9	7 (6-10)	3	5 (5-9)	4	7.5 (7-9)	8	7.5 (6-9)	3	9 (5-10)	0.798*
SST [median (min-max)]	45	2 (0-8)a	25	3 (0-8)	6	7 (1-8)a	7	2 (0-4)a	24	4 (0-10)	8	3 (0-5)	0.057*
Quick dash test [median (min-max)]	45	61.3 (22.8-86.3)	25	56.8 (18.1-90.9)	6	32.1 (27.2-95)	7	51.5 (43.1-77.2)	24	44.2 (13.6-86.3)	8	59.7 (25-75)	0.226*
PSQI [median (min-max)]	45	12 (2-19)	25	15 (4-21)	6	14 (2-21)	7	18 (2-21)	24	10 (1-21)	8	17 (2-21)	0.626*
Acromioclavicular degeneration (n, %)													0.618**
No	41	91.1	22	88.0	5	83.3	6	85.7	24	100.0	7	87.5	
Yes	4	8.9	3	12.0	1	16.7	1	14.3	0	0.0	1	12.5	
Supraspinatus degeneration (n, %)													0.931**
No	24	53.3	15	60.0	4	66.7	5	71.4	13	54.2	5	62.5	
Yes	21	46.7	10	40.0	2	33.3	2	28.6	11	45.8	3	37.5	
Impingement (n, %)													0.179**
No	42	93.3	23	92.0	4	66.7	6	85.7	23	95.8	6	75.0	
Yes	3	6.7	2	8.0	2	33.3	1	14.3	1	4.2	2	25.0	
Bicipital tendinitis (n, %)													0.135**
No	42	93.3	21	84.0	6	100.0	5	71.4	18	75.0	8	100.0	
Yes	3	6.7	4	16.0	0	0.0	2	28.6	6	25.0	0	0.0	
Combined problems (n, %)													0.707**
No	35	77.8	21	84.0	5	83.3	6	85.7	21	87.5	8	100.0	
Yes	10	22.2	4	16.0	1	16.7	1	14.3	3	12.5	0	0.0	
Adhesive capsulitis (n, %)													0.166**
No	44	97.8	25	100.0	6	100.0	7	100.0	23	95.8	6	75.0	
Yes	1	2.2	0	0.0	0	0.0	0	0.0	1	4.2	2	25.0	

* Kruskal Wallis Test (aMann Whitney U Test with Bonferroni correction; the difference between the two groups is statistically significant, p<0.008), ** Chi-square Test

Table 4. Logistic regression analysis for supraspinatus degeneration

	B	SE**	OR**	%95 CI**	p
Occupation (ref=housewife)					0.120*
Occupation (blue-collar)	0.912	0.615	2.488	0.745-8.314	0.139*
Occupation (white-collar)	1.218	0.553	3.380	1.143-9.996	0.028*
Occupation (retired)	0.368	0.544	1.445	0.497-4.200	0.499*
Simple shoulder test	0.119	0.082	1.127	0.960-1.322	0.144*
Constant	-1.068	0.358	0.344		0.003

* Multivariate (Binary) Logistic Regression Test (Backward: LR), (Omnibus Tests of Model Coefficients =0.037, Nagelkerke R Square=0.114, Hosmer and Lemeshow Test=0.627), ** SE=Standard error, OR=Odds Ratio, CI=Confidence interval, *** Age, gender, educational attainment, occupation, VAS, Simple shoulder test and Pittsburg sleep quality test were included in the model.

Table 5. Logistic regression analysis for acromioclavicular degeneration

	B	SE**	OR**	%95 CI**	p
VAS	-0.373	0.180	0.689	0.484-0.980	0.038*
BMI	0.156	0.076	1.169	1.007-1.356	0.040*
Constant	-4.168	2.417	0.015		0.085

* Multivariate (Binary) Logistic Regression Test (Backward: LR), (Omnibus Tests of Model Coefficients =0.016, Nagelkerke R Square=0.155, Hosmer and Lemeshow Test=0.177), ** SE=Standard error, OR=Odds Ratio, CI=Confidence interval, *** Age, gender, BMI, VAS, Simple shoulder test, Quick dash test were included in the model

DISCUSSION

When the shoulder pathologies were examined according to the sleeping positions of patients with shoulder pain, it was found that the rates of acromioclavicular degeneration were higher in those sleeping in the starfish position, and the rates of supraspinatus lesions were higher in those sleeping in the fetus position. Moreover, it was determined that age was predictive in all shoulder pathologies, pain scale scores were higher in patients with combined shoulder disorders, and sleep quality was worse in patients diagnosed with adhesive capsulitis.

Pathologies of the supraspinatus tendon are the most common causes of shoulder pain and dysfunction. The incidence of shoulder pain is increased in activities in which the arms are held above the head. Therefore, different sleeping positions may affect shoulder pain and pathologies in different ways. Holdaway et al. (10) believe that prone and starfish sleeping positions are the most risky because keeping the arms above the head leads to the narrowing of the subacromial space and increases intra-articular pressure, and the RC mechanism is compressed between the acromion, coracoacromial ligament, coracoid process, and ACJ. Zenian reported that the risk of pain in the shoulder

on which one lies increases in the lateral recumbent position (11). In their study, Werner et al. (12) defined 4 different sleep positions and measured the subacromial pressure with the catheter inserted in the SA-SD bursa, and observed the lowest pressure in the supine sleeping position (soldier position) and the highest pressure in the supine position with the arms next to the head, prone (prone-starfish) and lateral recumbent positions. Karabay et al. (13) found that the risk of pain caused by shoulder movement, SIS, and partial supraspinatus tendon rupture increased in the most preferred "fetus" position, which is a lateral recumbent position. In our study, in compliance with the literature, supraspinatus degeneration rates were found to be highest in those sleeping in the fetus position. There is a smaller contact area between the body and the bed in the lateral recumbent position than in the supine or prone positions, so more pressure is placed on the shoulders. Measuring skin pressure, Seiler et al. (14) showed that body weight creates more pressure on the shoulder contact area in the lateral recumbent position than in the supine position.

It was observed in our study that adhesive capsulitis did not develop in any of the participants sleeping in the "soldier" position, which did not increase the subacromial pressure, and of those sleeping in the prone and starfish positions. In addition, it was found that biceps tenosynovitis did not develop at all in those sleeping in the starfish and log positions. Holdaway et al. (10) reported that sleep positions that do not increase subacromial pressure are preferred in patients with shoulder pain. These results suggest that shoulder pathologies may occur due to the sleeping position and that patients may choose a sleeping position in which they will feel less pain. Therefore, it is possible to say that there is an inevitable relationship between sleeping positions and shoulder pain: sleeping position may be the cause of pain or it may be used as a measure to protect/recover from pain, or the sleeping position may be a result of shoulder pain.

According to Neer, osteophytes that develop due to degenerative changes that occur with age in ACJ cause impingement syndrome by extending into the subacromial space (15-16). In line with the literature, our study shows that the incidence of shoulder pathologies increases with age.

At the beginning of the twentieth century, Ernest Amory Codman reported in his studies that pain and movement limitations in shoulder abduction without trauma were caused by complete and partial RC muscle tears, as well as SA bursitis (17-20). Similarly, in our study, impingement and supraspinatus pathologies were observed at a higher rate in those sleeping in the starfish position in which the arms were slightly abducted.

The most well-known and widely accepted association between sleep and shoulder pain is that shoulder pain reduces sleep quality (21-22). Patients with shoulder pain experience sleep problems in a spectrum between feeling tired when they wake up in the morning, and waking up in the middle of the night and taking painkillers to relieve the pain (23). Our study found that most of the patients had poor sleep quality and this rate was higher in those over 50 years of age. Apart from sleep quality, another possible relationship between shoulder pain and sleep is due to sleep posture. In the study published by Werner et al. in 2010 on the subject, a pressure-measuring catheter was inserted into the SA bursa in 20 healthy volunteers, and the amount of SA pressure and pressure changes were recorded during four different arm positions and body posture combinations. The results revealed that SA pressure was higher on the shoulder on which the participant was lying and on the shoulder with the arm being abducted (24). It can be thought that having pain in the shoulder will prevent the person from lying on that shoulder, and they should turn to the side of the shoulder that does not hurt in order to protect themselves. However, the pain in question here is not a rapidly transmitted one that is observed in acute situations such as fractures, which undergo rapid changes when moving. The pain occurring with SIS is blunt and is slowly transmitted. Since the pain does not occur suddenly or increase with pressure, it does not prevent the person from lying on the aching shoulder (25). Our study revealed that most of the patients with poor sleep quality have increased pain at night. These findings suggested that sleep disorder could effect daily life of patients negatively and the patients with shoulder pain need to be further researched in terms of night pain.

The limitations of our study are as follows: the sleeping positions of the patients were recorded based on their own statements, and more objective and indicative data such as polysomnography were not obtained, the distribution within the groups was heterogeneous and there was no control group. On the other hand, among the strengths of the study are the patients were evaluated based on their MRI findings and tests assessing shoulder pain and function in detail.

In conclusion, it should be taken into consideration that evaluating sleep positions can be guiding in providing treatment for shoulder pain, which is one of the problems that can significantly affect daily life activities and the labor force and recommending correct and appropriate sleeping positions can be a useful treatment method in reducing pain and increasing sleep quality.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for the study was granted by the Adana Çukurova University Balcalı Hospital Clinical Trials Ethics Committee (Date: 12.02.2021, Decision No: 55).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed. Conflict of Interest Statement: The authors have no

conflicts of interest to declare.

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Evaluation of the alteration in retinal features following bariatric surgery in patients with morbid obesity

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ABSTRACT

Aim: The aim of the study is to evaluate the effect of bariatric surgery on the retina and choroid in non-diabetic and non-hypertensive patients with morbid obesity using optic coherence tomography (OCT) retrospectively.

Material and Method: Seventy-four eyes of seventy-four patients who have been underwent sleeve gasterectomy for morbid obesity in Balıkesir University Medicine Faculty & February 2019 and November 2020 were evaluated. All participants has a detailed ophthalmologic examination including best-corrected visual acuity (BCVA), intraocular pressure (IOP), slit-lamp biomicroscopy, retinal examination through non-dilated pupil via 90 D fundus lens, the retinal thickness (central, perifoveal, and parafoveal superior/nasal/inferior/temporal quadrants), and choroidal thickness through optic coherence tomography (RTVue XR Avanti, Optovue) in immediate preoperative and postoperative sixth-month. All values were compared.

Results: The macular thickness was increased significantly in all quadrants in postoperative visits ($p<0.05$). There was no change in choroidal thickness with surgery (p:0.898). The change in BMI was found correlated with the postoperative paranasal macular thickness (R:0.273, p:0.024) and mean macular thickness (R: 0.244, p: 0.045). There was no correlation between preoperative BMI, and preoperative/postoperative macular thickness ($p>0.05$). The correlation between preoperative BMI and preoperative choroidal thickness was significant (R: 0.416, $p<0.05$).

Conclusion: In patients with morbid obesity, the retinal thickness increases with an effective BMI change after bariatric surgery while choroidal thickness was not affected. The effective weight loss has a significant effect on retinal structure.

Keywords: Morbid obesity, sleeve gastrectomy, retinal thickness, choroidal thickness, optic coherence tomography

INTRODUCTION

The prevalence of obesity has been a huge burden worldwide in recent years. There are more than 1.9 billion overweight adults over the age of 18, and approximately 650 million of them are obese (1). The diagnosis of obesity and morbid obesity is depended on body mass index (BMI), which is calculated as weight/height2 (kg/m²). The definition of obesity is a BMI over 29.99 kg/m², obese class I BMI 30.00-34.99, obese class II BMI 35-39.99, and obese class III or morbidly obese BMI ≥40 kg/m². Another definition of morbid obesity is a BMI ≥35 kg/m² with concomitant health problems (2). Morbid obesity is associated with significant comorbidities, such as systemic diseases (diabetes mellitus, cardiovascular diseases, hypertension, etc.), and also ocular diseases such as glaucoma, age-related macular degeneration, and cataracts (3,4).

The treatment of morbid obesity with bariatric surgery has been popular in recent years. Besides the effect of weight loss, bariatric surgery provides an improvement in other comorbidities (5). The frequently used technique is sleeve gastrectomy, which is carried out by removing 80% of total stomach volume including the fundus and major curvature of the stomach. As a result of the surgery, the amount of food intake is reduced, and the hormone secretion that increases appetite decreases, and weight loss occur (6).

Obesity is a multisystemic disease, and it affects all tissues as well as the eye. The effects of obesity on ocular tissues have been reported in previous studies (7-9). Especially, the changes in the retina due to microvascular damage and inflammation may be observed. The effect of bariatric surgery on the retina and choroid is still

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controversial. In the light of these information, the aim of this study is to evaluate the changes in retina and choroid after bariatric surgery in patients with morbid obesity at the time of effective weight loss (10). In our opinion, this study has the largest case number in literature which obtain the alterations in retinal structure.

MATERIAL AND METHOD

The study followed the tenets of the Declaration of Helsinki, and it was approved by the Balıkesir University Faculty of Medicine Clinical Researches Ethics Committee (Date: 11.08.2021, Decision No:2021/168). The written informed consent was obtained from all participants. In this study, seventy-six (n:76) patients who have been diagnosed with morbid obesity (BMI >40 or ≥35 kg/m² when associated with comorbidities such as arterial hypertension, dyslipidemia, sleep apnea, or diabetes) and performed bariatric surgery between February 2019 and November 2020 at Department of General Surgery in Balıkesir University were evaluated. In each visit, BMI, abdominal circumference (AC), heart-rate, systolic and diastolic blood pressure, blood sugar were recorded. The patients who were candidate for sleeve gastrectomy was consulted to the department of ophthalmology for routine ophthalmic examinations at the immediate preoperative period and postoperative sixth months. The patients who have been performed sleeve gastrectomy were included while two patients who underwent Roux-en-Y gastric bypass and biliopancreatic diversion with duodenal switch were excluded. Other exclusion criteria were being under the age of 18, the refractive error above 2.00 Diopters (D), best-corrected visual acuity (BCVA) under 8/10, axial length more than 24 mm or less than 21 mm, diabetes mellitus, diabetic retinopathy, systemic hypertension, any type of retinopathy history, drug usage that may cause retinopathy (amiadoron, plaquenil, etc.), glaucoma or glaucoma suspect, history of retinal photocoagulation, history of ocular trauma or intraocular surgery, media opacity that may block the imaging of the retina. In the preoperative period, the blood sugar and Haemoglobin A1c (HbA1c) have been investigated, and the patients with blood sugar more that 110 mg/dL or HbA1c more than %5.5 were also excluded.

All included cases were directed to the Department of Ophtalmology and underwent a detailed ophthalmological examination including refractive error, BCVA, IOP, slit-lamp biomicroscopy, retinal examination through non-dilated pupil via 90 D fundus lens. Additionally, the retinal thickness (central, perifoveal, and parafoveal superior/nasal/inferior/temporal quadrants), and choroidal thickness were recorded via optic coherence tomography (RTVue XR Avanti, Optovue). The retinal thickness measurements were automatized while the choroidal thickness was measured manually as described in previous studies. The choroidal images were taken in "Enhanced deep imaging-optical coherence tomography" (EDI-OCT) mode. The cursor was placed above the hyperreflective retina pigment epithelium in the subfoveal area and lined towards the choroid-scleral junction. The vertical length of the line was calculated. Three repeated measurements were applied, and the average value was accepted as choroidal thickness. The choroidal thickness evaluation was performed between 9-10 A.M without smoking cigarettes or alcohol/caffeine consumption at least for two hours to prevent being affected by diurnal rhythm, and environmental factors. The data obtained from patients in the pre&post operative period were compared.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows V.23 (SPSS, Inc, Chicago, Illinois, USA). All values were reported as mean±standard deviation (SD). The normality of the data distribution was evaluated by the Shapiro-Wilk test. For the difference between the parameters in the first and last examination, paired t-test was used for dependent groups, and the chi-square test was used for parametric values. The relationship between the parameters at the first examination and the anatomical changes was evaluated by regression analysis. Spearman correlation analysis was used to understand the relationship between BMI change and retinal thickness. P-value under 0.05 was accepted statistically significant.

RESULTS

The study was initiated with 77 patients with morbid obesity, but the routine ophthalmological examination data of three patients was absent, so three patients were excluded. Finally, the study was included 74 eyes of 74 cases (F/M:52/22). The mean age of the patients was 31.66±9.65 (21-59) years. In the preoperative and postoperative period, the mean BMI was 43.12±2.13, and 31.71±4.82 kg/m² ($p<0.05$), and AC was 132.97±6.17 and 108.38±6.26 cm ($p<0.05$), respectively. The mean BMI change was 14.71±5.32 (6-26) kg/m². The mean BCVA was 0.98±0.11 and 1.00±0 (p:0.170), IOP was 18.07±3.35 mmHg and 15.40±3.00 mmHg ($p<0.05$) in preoperative and postoperative visit, respectively. The macular thickness was increased significantly in all quadrants in postoperative visits ($p<0.05$). There was no change in choroidal thickness with surgery (p:0.898) (**Table 1**).

Table 1. The change of visual acuity, intraocular pressure, BMI, AC, retinal and choroidal thickness in preoperative and postoperative sixth-months after bariatric surgery in morbidly obese patients

	Preoperative (n:74)	Postoperative (n:74)	p value
Body-mass Index (BMI)	43.12±2.13	31.71±4.82	<0.05*
Abdominal Circumference (AC)	132.97±6.17	108.38±6.26	<0.05*
Visual Acuity	0.98±0.11	1.00	0.170
Intraocular Pressure (IOP)	18.07±3.35	15.40±3.00	<0.05*
Central Macular Thickness	231.18±27.31	242.56±29.71	<0.05*
Parafoveal Macular Thickness			
•Superior	305.65±13.74	320.44±16.92	<0.05*
•Nasal	300.76±17.10	309.43±17.64	<0.05*
•Inferior	303.00±20.72	313.81±20.09	<0.05*
•Temporal	295.41±20.07	307.94±19.10	<0.05*
Perifoveal Macular Thickness			
•Superior	276.44±14.27	283.90±12.90	<0.05*
•Nasal	278.59±19.96	274.94±17.88	<0.05*
•Inferior	265.54±19.16	274.94±17.88	<0.05*
•Temporal	267.85±22.33	278.57±21.60	<0.05*
Mean Retinal Thickness	279.40±9.92	289.81±11.19	<0.05*
Subfoveal Choroidal Thickness	250.99±34.35	251.59±22.31	0.898

p value: statittically significance ratio

The change in BMI was found correlated with the postoperative paranasal macular thickness (R:0.273, p:0.024) and mean macular thickness (R: 0.244, p: 0.045). There was no correlation between preoperative BMI, and preoperative / postoperative macular thickness (p>0.05). The correlation between preoperative BMI and preoperative choroidal thickness was significant (R: 0.416, p<0.05).

DISCUSSION

In this study, we investigated the effect of the change in BMI after bariatric surgery on retina and choroid in patients with morbid obesity and resulted that the retinal thickness increases with an effective BMI after surgery while choroidal thickness was not affected. Doğan et al. (11) showed a significant decrease in central and total macular thickness in the third and sixth months after surgery, and the increase was in the normal range. They commented that the increase in macular thickness may be related to the decrease in macular pigment density in the foveal area. Lutein and zeaxanthin locate in the fovea, and play a role as a blue-light filter to block ultraviolet (UV) light. In patients with obesity, the density of macular pigments decreases due to accumulation in the adipose tissue, and the density returns to normal levels after weight loss with bariatric surgery and the macular thickness increases. Similarly, Laigin et al. (8) detected increased macular thickness and no change in choroidal thickness after bariatric surgery. Additionally, they examined the inner and outer layers of the retina and resulted that the main reason for increase in the retinal thickness was related to inner retinal layers while outer retinal layers have not an influence. As known, the balance between vasoconstrictor and vasodilator mediators is disturbed in favor of vasoconstrictors in patients with obesity (12). The vasoconstrictor mediators such as angiotensinogen and endothelin-1 (ET-1), which increase in patients with obesity, cause vasospasm in the microcirculation and return to normal levels with metabolic control after bariatric surgery (13). We think that there is an increase in retinal thickness associated with the increase in microvascular flow. As proof of this thesis, it has been shown that there is an increase in central retinal artery flow after bariatric surgery with Doppler ultrasonographic studies (7). To reveal the effects of vasoactive substances on retinal blood flow, OCT-Angio studies that show the vascular structure of the retina in more detail should be helded. Since the increase in retinal thickness after surgery was still within the normal range, we should consider this not as macular edema, but an atrophic retina recovering normal limits. Thus, systemic inflammation in patients with obesity may cause atrophy in the retina as well as all tissues. The level of systemic inflammatory markers is increased in obesity (14-17). Since we did not compare the data of patients with obesity with controls, it is not possible to claim whether the retina was atrophic in the preoperative period. However, we can comment that the inhibition of inflammation after surgery leads to improvement in the retina.

According to the results, there was no significant change in choroidal thickness after surgery. Since we did not compare choroidal thickness measurement in the preoperative period with the control group, we could not exactly claim about the changes in choroidal thickness in obesity. Gönul et al. (9) compared the patients with morbid obesity with control group and found that choroidal thickness was increased in patients with obesity compared to the normal population. Similarly, in this study, although patients with obesity were not compared with the control group, a significant correlation was found between preoperative BMI and subfoveal choroidal thickness even if we could not study on controls (R: 0.416, p<0.05). The increased choroidal thickness may be associated with venous congestion. Consistent with our results, Bulus et al. (18) showed that the choroidal thickness increase in patients with obesity, and that BMI and subfoveal choroidal thickness were correlated. The authors attributed this to the increased leptin and obesity-related inflammatory factors in patients with obesity.

Yumusak et al. (19) also found a positive correlation between BMI and choroidal thickness. In patients with obesity, the intraorbital fat volume may expand likewise visceral adipose tissue, and it may lead to an increase in venous pressure, and an increase in choroidal thickness which is fed by the veins. After surgery, the decrease in choroidal venous congestion with decreased intraorbital pressure is expected (20). In this study, the change in choroidal thickness was not significant even the IOP was decreased significantly. In contrast, it has been shown that the choroidal thickness is not different from the normal population (21), or thinner (11,22,23) in previous studies. This result can be associated with the follow-up duration. The postoperative measurements were recorded at sixth-month when the structural changes initiate (10). Since the changes in choroidal thickness may occur in a long time (24), we think that the different results may be obtained if a longer follow-up time was required. In contrast to our results, Dogan et al. (11) found a significant increase in choroidal thickness even in the immediate postoperative period. The controversial results may be related to the difference in choroidal measurement methods, and the choroidal thickness is also affected by many environmental factors (19). We measured choroidal thickness in accordance with the diurnal rhythm, and without smoking cigarettes or alcohol/caffeine consumption in order to minimize the effect of choroidal thickness. Additionally, the multivariate regression analysis was helded to reveal the relationship between preoperative choroidal thickness, and preoperative BMI, AC, central macular thickness to support our results. The preoperative BMI was found significantly correlated with preoperative BMI and AC (respectively; R:0.383 p:0.024, R: 0.264 p: 0.015). The evaluation with new methods, such as OCT-A may be useful in determining the vascular density and flow index to reach more reliable results.

There are a few limitations of this study. First of all, the relatively small number of patients with single-center limits the diversity of data. Also, the sub-analyses about the retinal layers (inner/outer) were not performed. We have not compared the preoperative data of patients with morbid obesity with the control group, so we could not comment that whether the preoperative results were in the normal range. Additionally, the choroidal thickness measurements were made manually, because the device has not an automatic software to quantify choroidal thickness. To deal with this, three repetitive measurements in two different visits were recorded, and the average of values was accepted as correct. Although the follow-up period was six months when the effective weight loss was achieved, the long-term results were not concluded.

CONCLUSION

We evaluated the effect of bariatric surgery on the posterior segment in patients with morbid obesity by excluding the influence of diabetes mellitus and hypertension which are frequently encountered as risk factors for retinopathy. The thickening in all retinal quadrants was detected after bariatric surgery while choroidal thickness had no change. The multi-center studies with larger series are needed for more reliable results.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Balıkesir University Faculty of Medicine Clinical Researches Ethics Committee (Date: 11.08.2021, Decision No:2021/168)

Informed Consent: The written informed consent was obtained from all participants.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Early and late results of intravenous immunoglobulin as potential adjuvant therapies in critically ill COVID-19 patients: a retrospective cohort study

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ABSTRACT

Introduction: Intravenous immunoglobulin (IVIG), which is one of the adjuvant therapy strategies, has been started to be used in critically ill COVID-19 patients due to its anti-inflammatory and immunomodulatory effects.

Material and Method: In our study, it was aimed to evaluate the effect of IVIG used in critically ill COVID-19 patients in the intensive care unit on early laboratory findings and late lung damage. Twenty-two critically ill COVID-19 patients who met the inclusion criteria were included in the study. Laboratory data of the patients who received 0.4 gr/kg/day IVIG for 5 days were analyzed before the treatment and on the 1st and 5th days of the treatment. For the percentage of injured lung areas was evaluated with chest CT.

Results: Respiratory rate and CRP decreased with IVIG, while an increase was observed in PaO_2/FiO_2, WBC, lymphocyte count, D-Dimer and fibrinogen values, which was statistically significant ($p<0.05$). When IL-6 values before treatment and on the 3rd day of treatment were compared, it was observed that there was a statistically significant decrease ($p<0.001$). A statistically significant improvement in lung damage was found when the average percentage of injured lung area calculated from chest CT taken at hospitalization and 1 month after discharge was compared ($p<0.001$).

Conclusion: IVIG can be considered as an effective adjuvant therapy because it causes improvement in oxygenation, clinical symptoms and hyperinflammatory response. It should not be forgotten that it also provides improvement in the damaged lung areas in the late period.

Keywords: COVID-19, immunoglobulin, IVIG, pneumonia

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can be asymptomatic depending on the host's immune response and comorbidities, or it can cause life-threatening multi-organ dysfunction. In severe cases with refractory hypoxemia and developing acute respiratory distress syndrome (ARDS), mortality is determined by factors such as age, hypertension and chronic obstructive pulmonary disease, while the limited number of devices and intensive care beds in the health care system cannot meet the needs (1). In order to reduce the pressure of the disease on the health system, national and global vaccination programs have been initiated. Vaccination aims to reduce the severity of the disease and decrease mortality rates. Despite the rapid increase in vaccination rates all over the world, the emerging COVID-19 variants and the lack of effective treatment show that the pandemic remains serious. Today, while efficacy studies on vaccines continue, the role of adjuvant therapies such as convalescent plasma treatment, immunoglobulins, inflammatory modulators and stem cell therapies used against COVID-19 in the treatment is still being investigated (2).

Intravenous immunoglobulin (IVIG) has the ability to provide passive immunity against various pathogens and also can reduce the uncontrolled hyperinflammatory response against SARS-CoV-2 and inflammation-related lung damage by creating immunomodulation

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in severely and critically ill COVID-19 patients (3). However, the routine use of IVIG in the treatment of COVID-19 is controversial due to the lack of adequate clinical studies.

In this study, we aimed to present the effect of IVIG therapy used in critically ill COVID-19 patients in the intensive care unit (ICU) on symptoms, laboratory findings and percentage of injured lung area (ILA).

MATERIAL AND METHOD

After obtaining approval from the Ethical Committee for Clinical Researches of the Muğla Sıtkı Koçman University (Date: 03/02/2021, Decision No: 3/V), patients who were followed up in the Intensive Care Unit due to COVID-19-related acute respiratory failure (ARF) and were treated with IVIG at a dose of 0,4 mg/kg/day for 5 days in the years 2020 and 2021 were included in the study. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Medical records of 31 patients were reviewed retrospectively. Patients younger than 18 years of age, incomplete medical data and patients for whom 5-day IVIG treatment could not be completed were excluded (**Figure 1**).

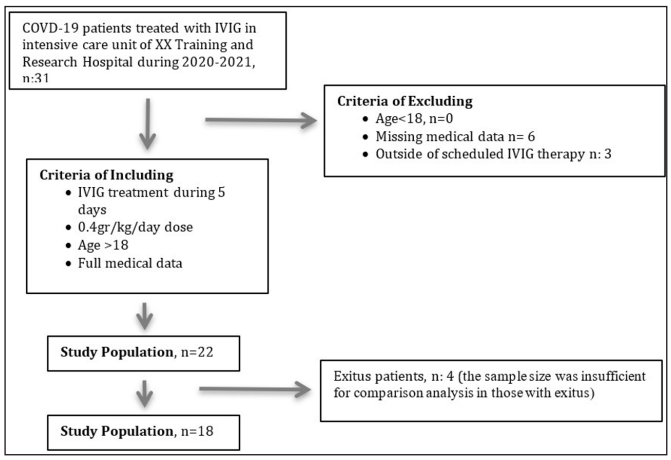

Figure 1. Flow chart displaying selective and exclusive process of patients with COVID-19 in the current study

Age, gender, APACHE II score, comorbidities, steroid use, presence of intubation, the day of initiation of IVIG treatment, number of days of hospitalization in the ICU and discharge status of the 22 patients included in the study were recorded. Before IVIG treatment, on the 1st, 3rd, and 5th days of the treatment, Sequential Organ Failure Assessment (SOFA) Score, the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FIO_2), respiratory rate (RR), white blood cell (WBC), lymphocyte, Lactate dehydrogenase (LDH), C-Reactive protein (CRP), D-Dimer, fibrinogen values were recorded. Interleukin-6 Cytokine (IL-6) values were noted before and on the 3rd day of treatment. All these data were considered as early results.

For late-term results, ILA percentages were calculated from thorax computed tomography taken at the time of admission to the ICU and 1 month after discharge. The percentage of ILA was calculated using chest CT taken during diagnosis of patients. Chest CT shots were obtained without contrast agent injection, during deep inspiration, in the supine position. The images were evaluated on a high-resolution medical screen. Three lobes on the right lung and 2 lobes on the left lung were examined separately. Each lobe was accepted by 20% and lobe volume was measured. The areas in the view of the consolidated and ground-glass area were calculated by volumetric voxel and calculated on the computer through the program. Their percentages were calculated over the total volume. The percentage values of all lobes were collected and total loss of lung aeration was found.

Statistical Analyses

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the study data, the distribution of the data was evaluated with the Shapiro-Wilk Test as well as the descriptive statistical methods (Mean, Standard Deviation, Median, Frequency, Ratio, Minimum, Maximum).

A total of 22 patients met the enrolment criteria of the study but the sample size was insufficient for comparison analysis in those with exitus. For this reason, comparison analyzes were made on living cases. The Friedman test was used for comparisons of quantitative data over three periods or more that did not show normal distribution. Wilcoxon test was used for comparison of the quantitative data between two periods that did not show normal distribution. Significance was evaluated at the $p<0.05$ level.

RESULTS

A total of 22 patients met the enrolment criteria of the study. The overall mortality was 18.18% (4/22) so 18 patients was evaluated after excluded exitus patients. The mean age of the patients was 53.64±11.75. Three (16.7 %) were females and 15 (83.3%) were males. Eight of the patients (38,9%) had no comorbidities. While 3 (16.7 %) of the patients were intubated, non-invasive mechanical ventilation, oxygen mask and/or high-flow oxygen therapy were used in 15 (83.3%) patients. (**Table 1**). The mean of APACHE II was calculated as 10.55±3.19 in the ICU hospitalizations of the patients (**Table 2**).

It was determined that steroids were used together with IVIG in the treatment of all patients. Dexamethasone was used in the treatment of 9 (40.9%) patients, and methylprednisolone was used in 13 (59.1%) patients. And patients received steroids for at least the duration of

IVIG therapy. Patients were treated with IVIG on average 8.95±3.39 days after the onset of the first COVID-19-related symptom. The duration of stay in the ICU was 14.9±10.85 days, and the duration of hospitalization was 26.62±20.74 days (**Table 2**).

Table 3 includes the data of the patients on the 1st, 3rd and 5th days of the treatment, and before IVIG treatment was started. While RR, SOFA, CRP values decreased with IVIG treatment, an increase was observed in PaO_2/FIO_2, white blood cell, lymphocyte count, D-Dimer and fibrinogen values, which was statistically significant ($p<0.05$). There was no statistical difference in lactate dehydrogenase and alanine aminotransferase values ($p>0.05$). While the mean of Interleukin 6 (IL-6) was 225.15±590.97 before IVIG treatment, it was calculated as 14,50±13,85 on the 3rd day of treatment, and this decrease in IL-6 was statistically significant ($p<0.001$) (**Table 4**). There were no complications associated with IVIG therapy in the patients.

When the average of the percentage of ILA calculated from the chest CT taken at the hospitalization and 1 month after the discharge was compared; It was determined that it regressed from 63.22 ±13.76% to 14.50 ±13.85%, and this improvement was found to be statistically significant ($p<0.001$) (**Table 5**).

Table 1. Demographic parameters of Patient (1)

	N (18)	%
Sex		
Female	3	16.7
Male	15	83.3
Comorbidity		
No comorbidity	7	38.9
Diabetes mellitus	2	11.1
Hypertension	4	22.2
Asthma	3	16.7
CAD	1	5.6
COPD	1	5.6
Steroids		
Dexamethasone	9	50.0
Methylprednisolone	9	50.0
Intubation		
No	15	83.3
Yes	3	16.7

CAD; coronary artery disease, COPD; Chronic obstructive pulmonary disease

Table 2. Demographic parameters of Patients (2)

	Mean ±Sd	Min-Max (Median)
Age	53.64±11.75	36-76 (52.5)
APACHE II	10.55±3.19	6-17 (9.5)
IVIG starting day	8.95±3.39	4-14 (8.5)
Length of stay in ICU	14.90±10.85	3-35 (11)
Length of stay in hospital	26.62±20.74	9-109 (22)

APACHE II; Acute Physiology and Chronic Health Evaluation II score, ICU; Intensive care unite

Table 3. Comparisons of SOFA and blood samples before IVIG treatment, 1st 3rd and 5th days of IVIG treatment

		Before IVIG	DAY 1	DAY 3	DAY 5	p-value
SOFA	Mean ±Sd Min-Max (Median)	4.33±0.77 4-6 (4)	3.94±0.73 2-6 (4)	2.89±1.57 1-8 (2)	1.83±1.1 1-4 (1.50)	0.001**
PaO_2/FIO_2	Mean ±Sd Min-Max (Median)	83.50±22.56 60-140 (77.50)	101±29.05 69-189 (94)	112.61±35.57 76-192 (107.50)	135.56±34.51 75-190 (140)	0.001**
RR	Mean ±Sd Min-Max (Median)	31.89±3.14 25-38 (32.50)	29.11±4.06 24-38 (28)	23.06±5.61 14-35 (22)	18.53±2.56 16-25 (18)	0.001**
WBC	Mean ±Sd Min-Max (Median)	11.56±4.41 4.72-22.12 (10.32)	11.67±3.99 4.12-21.9 (11.43)	10.82±3.42 6.83-23 (10.55)	9.27±3.51 5.55-21.9 (8.85)	0.029*
Lymp	Mean ±Sd Min-Max (Median)	0.64±0.22 0.29-0.95 (0.65)	0.66±0.27 0.21-1.41 (0.61)	0.9±0.38 0.48-1.98 (0.79)	1.02±0.36 0.37-2.06 (0.99)	0.001**
LDH	Mean ±Sd Min-Max (Median)	403.28±182.54 167-906 (363.50)	427.78±199.53 227-924 (374.50)	458.5±288.03 227 1403 (359.50)	490.39±370.28 190- 1661 (398.50)	0.721
ALT	Mean ±Sd Min-Max (Median)	52.72±44.54 13-198 (34.50)	52.56±52.06 17-246 (40)	55.50±47.65 19-197 (38)	59.22±42.91 24-170 (42)	0.777
CRP	Mean ±Sd Min-Max (Median)	107±80.39 20-327 (87)	92.87±67.12 9.6-246 (74)	63.43±40.82 8.78-143 (67)	47.33±58.35 4-257 (37.50)	0.001**
D-Dimer	Mean ±Sd Min-Max (Median)	732.78±570.54 144-1900 (683.50)	1106.10±1061.90 150-4073 (690)	1358.50±1093.7 269-4444 (880.50)	1434.94±1647.5 259-7412 (862)	0.012*
Fibrinogen	Mean ±Sd Min-Max (Median)	493.50±140.97 267-740 (528.50)	549.17±179.63 217-894 (550)	533.10±162.8 188 790 (521.50)	530.50±146.23 339-835 (500)	0.173

SOFA; Sequential Organ Failure Assessment Score, PaO_2/FIO_2; Pa02 to FIO_2 Ratio, RR; Respiratory Rate, WBC; White blood cell, Lymp; lymphocyte count, LDH; Lactate dehydrogenase, ALT; alanine aminotranspherase, CRP; C-reactive protein, * P-value<0.005, ** P-value<0.001

Table 4. Comparison of IL-6 value before and at the 3rd day of IVIG treatment

		Day 0	Day 3	p-value
IL-6	Mean ±Sd Min-Max (Median)	225.15±590.97 4.48-2503 (58.50)	156.44±295.3 1.36-1111 (31)	0.028*

IL-6; Interleukin 6, *P-value<0.005

Table 5. Comparison of ILA percentage in chest CT at the hospitalization to ICU and discharge from hospital

		Day 0 in ICU	Discharge Day	p-value
Percantege of ILA	Mean ±Sd Min-Max (Median)	63.22±13.76 40-80 (62)	14.5±13.85 0-40 (16)	0.001**

Percentage of ILA; percentage of injured lung area, ** P-value<0.001

DISCUSSION

The most effective treatment method has not been found for COVID-19, which has been affecting the whole world and increasing its pressure on health systems since December 2019. The failure of antiviral agents used in treatment has directed clinicians to use potential adjuvant therapies. IVIG is one of them and has been used in the treatment of COVID-19 due to its anti-inflammatory and immunomodulatory effects. There are different hypotheses regarding these immunomodulatory effect mechanisms (3). These are pathogenic antigen neutralization by divalent antibody fragments (F(ab)'2)-mediated mechanisms, immunomodulatory effects on endothelial cells and adaptive immune cells by fragment crystallisable (Fc)-mediated mechanisms, and immunomodulatory effects on other innate immune cells by Fc-mediated mechanisms (3). ((A) Neutralization of pathogenic antigens through the F(ab)'2-mediated mechanisms; (B) The immunomodulatory effects on endothelial cells through the Fc-mediated mechanisms; (C) The immunomodulatory effects on other innate immune cells through the Fc -mediated mechanisms; (D) The immunomodulatory effects on adaptive immune cells through the Fc-mediated mechanisms.) Although the molecular mechanisms for IVIG have been partially clarified, its role in the treatment of COVID-19 remains unclear. In this study, we evaluated in detail of the early clinical and laboratory data obtained during the treatment of COVID-19 patients receiving IVIG therapy.

It was observed that the PaO_2/FIO_2 ratios increased, RR decreased, and WBC, lymphocyte and CRP values decreased with high-dose IVIG treatment in our study. All values were analyzed before IVIG and on the 1st, 3rd and 5th days of treatment to emphasize the early results of IVIG treatment. In a prospective randomized trial, it was shown that there was an increase in PaO_2/FIO_2 and a decrease in the progression of mechanical ventilation requiring respiratory failure with IVIG treatment, and this was statistically significant in patients with an A-a gradient >200 mmHg (4). In this study, PaO_2/FIO_2 was evaluated on day 7. Herth et al. (5) emphasized that symptoms and laboratory findings improved with treatment in 12 cases they evaluated. This retrospective case series supports our study because it evaluated oxygenation simultaneously with treatment. Raman et al. (6) reported normalization in respiratory rate and oxygenation with IVIG. There are case reports in the literature in which IVIG was used in COVID-19 pneumonia and clinical improvement and improvement in oxygenation were observed (7-9). Although the results are promising, no data supporting the beneficial effect of IVIG use in COVID-19 could be obtained in the randomized controlled study of Tabarsi et al. (10). In the study, laboratory data were evaluated on days 0, 7 and 14, and no data on oxygenation was shared except for the need for mechanical ventilation. Secondary conditions occurring within the 14-day period may affect the laboratory data and cause different interpretation of the results (10). In our study, it was determined that the patients who received IVIG treatment had an improvement in oxygenation and respiratory rate. This situation can be considered as clinical improvement.

Theoretically, IVIG-related immunomodulatory effect occurs with high doses (3). Different doses of IVIG are used in studies in the treatment of COVID-19. In a multicentre retrospective cohort study conducted by Shao et al. (11), 28 and 60-day mortality was found to be lower in those who received IVIG higher than 15 g per day. However, high or low dose IVIG treatment had no effect on the number of days of hospitalization. In another study, it was reported that 20 g/day IVIG administration for 3 consecutive days may be effective and safe (12). In our study, high dose (0.4kg/kg/day) IVIG was administered to all patients for 5 days. In the study by Shao et al. (11), it was shown that the 60-day mortality decreased in those who started IVIG treatment within the first 7 days after hospital admission. There are also opinions that patients will not benefit much from IVIG treatment when systemic damage develops (13). In our study, the onset time of IVIG was 8.95±3.39 days after the onset of the first COVID-19-related symptom, and it can be said that IVIG treatment was started early.

The hyperinflammatory state resulting from the overproduction of proinflammatory cytokines such as IL-6 changes the prognosis of the disease (14). In our study, the IL-6 levels of the patients before IVIG treatment and on the 3rd day of treatment were compared and a statistically significant decrease was observed. According to our study results, it can be said that the hyperinflammatory state will decrease on the 3rd day of the treatment, and this may lead to positive results in the prognosis of the disease. Similar to our results, in a study comparing standard treatment and two groups using IVIG in addition to standard treatment, IL-6 levels were found to be lower in the group using IVIG (4).

In addition to the data evaluated during the treatment, the effect of IVIG use on late lung damage was also examined. A significant improvement was observed in the ILA percentages calculated from chest CT 1 month after discharge. Tabarsi et al. (10) evaluated 50% improvement in tomography on the 14th day of IVIG treatment, but they could not find a statistical difference. It is known that residual lung injury in post-COVID-19 syndrome continues even 4 months after clinical recovery (15). In this case, it may not be correct to associate the lack of improvement in the lung at the end of 14 days with IVIG treatment.

Our study has potential limitations. One limitation is the retrospective design of this study and the other limitation is the use of a single group and single ICU data. Despite the emphasis on the reduction in 28 and 60-day mortality with high-dose and early IVIG use (11), the relationship between IVIG and mortality was not evaluated in our study to avoid misinterpretation due to the low number of patients who died. This can be considered as a limitation.

CONCLUSION

As a result, IVIG can be considered as an effective adjuvant therapy in the COVID-19 pandemic, which could not be terminated despite the vaccines produced in a short time, because it causes improvement in oxygenation, clinical symptoms and hyperinflammatory response with high doses and early use. It should not be forgotten that it also provides improvement in the damaged lung areas in the late period.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Muğla Sıtkı Koçman University, Clinical Researches Ethics Committee (Date: 03/02/2021, Decision No: 3/V).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Predictors and outcome of hyponatremia in patients with COVID 19: a single-center experience

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ABSTRACT

Aim: Hyponatremia (serum sodium <135 mEq/L) portends a worse prognosis in patients with community-acquired pneumonia. Data regarding the outcome of hyponatremia in hospitalized COVID-19 patients are insufficient and controversial. The present study aimed to identify the predictors of hyponatremia and its impact on clinical outcome measures in hospitalized COVID-19 patients.

Material and Method: We conducted a retrospective study on 787 adult patients with SARS-CoV-2 infection admitted to a university hospital between March 10, 2020, and December 15, 2020, February. Demographic and laboratory features, comorbid diseases, medications, radiology results, and clinical outcome measures of the patients were obtained retrospectively from their medical records.

Findings: One hundred fifty-nine (20.2%) patients out of 787 had hyponatremia. Hyponatremia was mild (sodium: 130 -134 mEq/L) in majority of cases (n=124). The severity of pneumonia (p=0.013) and having diabetes (p < 0.001) were the independent predictors of hyponatremia at the time of admission. The median length of hospital stay (LOS) was longer in patients with hyponatremia than patients with normonatremia (10 days vs. 8 days, p < 0.001). In multivariate analysis, hyponatremia was significantly associated with ICU admission or the need for mechanical ventilation (adjusted OR, 1.72; 95% confidence interval [95% CI], 1.03 to 2.85; p=0.036). The severity of pneumonia, hemoglobin and lactate dehydrogenase levels, neutrophil-to-lymphocyte ratio (NLR), and body temperature were also associated with ICU admission or the need for mechanical ventilation. The oxygen saturation, male sex, serum albumin, NLR, and the ICU admission but not the hyponatremia on admission were significantly related to mortality.

Conclusion: Hyponatremia on admission predicts ICU admission or mechanic ventilation need but not mortality in COVID-19 patients, and it should be considered in risk stratification.

Keywords: Hyponatremia, COVID-19, outcomes

INTRODUCTION

Since it has been officially declared by World Health Organization (WHO) at the beginning of 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has had a significant impact on health and economy worldwide (1-5). Hyponatremia (serum sodium <135 mEq/L), even when mild, was found to be associated with mortality in a diverse spectrum of diseases, including cancer (6-9). Hyponatremia was linked to increased mortality and length of hospital stay in patients with community-acquired pneumonia (10-12). Hyponatremia was related to intensive care unit (ICU) admission and death in patients with SARS-CoV-1 (13). Older age, obesity, comorbid diseases, immunosuppressive use, and some laboratory variables (Lymphocytes count, C-reactive protein, lactate dehydrogenases, ferritin, vs.) have been associated with mortality and a worse outcome in patients with coronavirus disease 2019 (COVID-19) (4,5,14). Studies

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dealing with the impact of hyponatremia on outcomes and mortality in COVID-19 patients revealed conflicting results. The risk stratification is of supreme importance during pandemic given the limited hospital resources; whether hyponatremia at admission should be added to risk stratification tools is unknown. In the present study, conducted in a relatively large cohort of patients hospitalized for COVID-19, we aimed to investigate the association of hyponatremia with in-hospital mortality, length of hospital stay, ICU admission, and the need for mechanical ventilation. We also aimed to examine the risk factors for the development of hyponatremia in COVID-19 patients.

MATERIAL AND METHOD

The present study was designed retrospectively. This study was approved by Ondokuz Mayıs University Clinical Researches Ethics Committee (Date: 26.08.2021, Decision No: 2021/408). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients with real-time reverse transcription-polymerase chain reaction (RT-PCR) confirmed SARS-CoV-2 infection who were hospitalized at Ondokuz Mayıs University Hospital between March 10, 2020, and December 15, 2020, were enrolled in this study. Patients under the age of 18 were excluded. Normonatremic (serum sodium level 135-145 mEq/L) and hyponatremic (serum sodium level < 135 mEq/L) patients were included in the study. Hyponatremia was classified as mild (Na 130-134 mEq/L) and moderate-severe (Na < 130 mEq/L). Since hypernatremia could affect mortality, patients with hypernatremia (Na ≥ 146 mEq/L) were excluded. Patients with pseudo-hyponatremia due to elevated serum glucose, triglycerides, or osmotic agents were excluded from the statistical analysis.

The demographic (age, sex) and laboratory features (including serum sodium, potassium, blood urea nitrogen (BUN), creatinine, glucose, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), troponin I, D-dimer, albumin, hemoglobin, white blood cell and lymphocyte counts, mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), platelet counts, C-reactive protein (CRP), procalcitonin); clinical findings (volume status, fever, oxygen saturation) comorbid diseases (diabetes mellitus (DM), hypertension, previous or current malignancy, coronary artery disease (CAD), heart failure, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), any chronic liver disease), all medications (including renin-angiotensin-aldosterone system blockers and diuretics), radiology results, and clinical outcome measures of the patients (in-hospital mortality, length of hospital stay, ICU admission, or ventilator need) were obtained from medical records of the patients. The presence and extent of pneumonia were assessed by thoracic computerized tomography (CT), which was performed at the time of admission in all patients. The severity of pneumonia was classified as none, unilateral or bilateral pneumonia. All patients were treated according to the protocols released by the Turkish Ministry of Health.

Statistical Analysis

Data were analysed by using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). Kolmogorov-Smirnov test was used to determine whether the variables showed a normal distribution or not. Normally distributed numerical variables were specified as mean±standard deviation, and the numerical variables not showing normal distribution were expressed as median (Interquartile range, IQR). Categorical data were expressed as numbers and percentages (%). Student's t-test or Mann-Whitney U-test was used to compare between-group differences in numerical variables depending upon normal distribution. The Chi-square test or Fisher's Exact test was performed to assess categorical variables. Multivariate logistic regression models were used to explore the risk factors associated with mortality and the need for ICU or mechanical ventilation. A p-value of less than 0.05 was accepted as statistically significant.

RESULTS

A total of 787 patients were included in the study. One hundred fifty-nine (20.2%) patients had hyponatremia. One hundred twenty-four of hyponatremic individuals (78%) had a mild hyponatremia. The median age was 61 (22) years and 427 patients (54.3%) were female. Hypertension was the most common comorbid disease observed in 309 (39.3%) patients. **Table 1** lists the demographic and clinical features of the patients with hyponatremia and normonatremia. In multivariable analysis, the severity of pneumonia (adjusted OR, 1.66; 95% confidence interval (95% CI), 1.11 to 2.48; p < 0.013) and having diabetes (adjusted OR, 2.40; 95% confidence interval (95% CI), 1.49 to 3.86; p < 0.001) were significantly associated with hyponatremia (**Table 2**). The median length of hospital stay (LOS) was significantly longer in patients with hyponatremia (10 days vs. 8 days, p < 0.001) than patients with normonatremia (**Table 3**). Two hundred twelve (26.9%) patients required either ICU admission or mechanical ventilation. During follow-up, the need for mechanical ventilation was more common in patients with hyponatremia than patients with normonatremia (45.3% vs. 22.3%; p < 0.001). In multivariate analysis, hyponatremia was significantly associated with ICU admission or the need for mechanical ventilation (adjusted OR, 1.72; 95% confidence interval (95% CI),

1.03 to 2.85; p=0.036). The median length of hospital stay, the need for mechanical ventilation or ICU admission, and the mortality rate did not differ significantly between patients with mild hyponatremia and moderate to severe hyponatremia (serum Na < 130). Other important factors independently associated with ICU admission or need for mechanical ventilation were severity of pneumonia, hemoglobin and LDH levels, neutrophil-to-lymphocyte ratio (NLR), and body temperature (**Table 4**). Overall, 102 of 787 (13%) patients died in hospital. The patients with hyponatremia had a higher mortality rate compared to patients with normonatremia (24.5% vs. 10%; p < 0.001). Nevertheless, in multivariate analyses, oxygen saturation, male sex, serum albumin, NLR, and the ICU admission but not the hyponatremia were significantly associated with mortality (**Table 5**).

Table 2. Univariate and multivariate analysis of factors associated with hyponatremia

	Univariate		Multivariate	
Variable	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Age (years)	1.02 (1.01-1.04)	<0.001	1.01 (0.99-1.02)	0.589
Sex (male)	1.61 (1.13-2.31)	0.009	1.32 (0.81-2.13)	0.263
Diabetes mellitus (yes)	2.60 (1.79-3.79)	<0.001	2.40 (1.49-3.86)	<0.001*
CAD (yes)	1.80 (1.10-2.93)	0.018	1.07 (0.55-2.07)	0.840
CKD (yes)	1.91 (1.15-3.17)	0.013	1.24 (0.64-2.41)	0.532
Fever (yes)	1.44 (1.09-2.06)	0.045	1.29 (0.81-2.06)	0.283
Oxygen saturation	0.96 (0.94-0.98)	<0.001	0.99 (0.96-1.02)	0.598
Pneumonia severity classified as none, unilateral, bilateral	1.77 (1.33-2.37)	<0.001	1.66 (1.11-2.48)	0.013*
Albumin	0.44 (0.31-0.61)	<0.001	0.76 (0.47-1.23)	0.256
Hemoglobin	0.94 (0.86-1.01)	0.106	1.06 (0.94-1.20)	0.340
NLR	1.03 (1.02-1.05)	<0.001	1.01 (0.99-1.03)	0.265
CRP	1.01 (1.00-1.01)	<0.001	1.00 (1.00-1.01)	0.209
Procalcitonin	1.06 (1.02-1.11)	0.005	1.01 (0.97-1.05)	0.555
LDH	1.00 (1.00-1.00)	0.003	1.00 (0.99-1.00)	0.752
D-dimer	1.25 (1.00-1.00)	0.248	1.00 (1.00-1.00)	0.166

CAD: Coronary artery disease; CKD: Chronic kidney disease; NLR: Neutrophil to lymphocyte ratio; CRP: C-reactive protein; LDH: Lactate dehydrogenase; OR: Odds ratio; (95% CI): 95% Confidential interval; *p<0.05: Statistically significant parameters

Table 4. Risk factors for intensive care unit admission or mechanical ventilation need in COVID-19 patients

	ICU admission or mechanical ventilation need			
	Univariate		Multivariate	
Variable	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Hyponatremia	2.80 (1.90-4.13)	<0.001	1.72 (1.04-2.86)	0.036*
Procalcitonin	1.08 (1.03-1.13)	0.003	0.98 (0.94-1.01)	0.134
CRP	1.01 (1.01-1.01)	<0.001	1.00 (1.00-1.01)	0.051*
NLR	1.07 (1.05-1.09)	<0.001	1.03 (1.00-1.05)	0.035*
Pneumonia severity	3.80 (2.31-6.26)	<0.001	1.66 (1.09-2.45)	0.018*
Body temperature	2.03 (1.40-2.89)	<0.001	1.55 (1.17-2.06)	0.002*
LDH	1.00 (1.00-1.00)	<0.001	1. 00 (1.00-1.00)	0.006*
Hemoglobin	0.76 (0.70-0.83)	<0.001	0.84 (0.75-0.95)	0.005*

ICU: Intensive care unit; CRP: C-reactive protein; NLR: Neutrophil to lymphocyte ratio; LDH: Lactate dehydrogenase; OR: Odds ratio; (95% CI): 95% Confidential interval. *p<0.05: Statistically significant parameters

Table 1. Comorbidities, demographic and clinical characteristics of hypo- and normonatremic patients

	Hyponatremia (n=159)	Normonatremia (n=628)	p
	n (%)		
Sex (male)	101 (63.5)	326 (76.3)	0.009*
Diabetes mellitus	61 (38.4)	121 (19.3)	<0.001*
Hypertension	69 (43.4)	240 (38.2)	0.232
Malignancy	19 (11.9)	49 (7.8)	0.096
CAD	27 (17)	64 (10.2)	0.017*
Heart failure	12 (7.5)	26 (4.1)	0.073
CKD	25 (15.7)	56 (8.9)	0.012*
COPD	13 (8.2)	58 (9.2)	0.677
RAAS blocker	51 (32.1)	170 (27.0)	0.210
Diuretic use	47 (29.6)	141 (22.4)	0.098
Thiazide	27 (17)	93 (14.8)	
Furosemide	22 (13.8)	61 (9.7)	
Spironolactone	6 (3.8)	12 (1.9)	
Pneumonia on CT			<0.001*
None	14 (8.8)	137 (21.8)	
Unilateral	4 (2.5)	35 (5.6)	
Bilateral	141 (88.7)	457 (72.7)	
	Median (IQR)		
Age, years	60 (22)	64 (21)	<0.001*
Fever‡ (%)	66 (41.5)	208 (33.1)	0.047*
Hemoglobin, gr/dL	12 (2.6)	12.7 (2.9)	0.029*
White blood cell, 10^3/mm³	7.2 (5.5)	6.3 (3.7)	0.001*
Lymphocyte, 10^3/mm³	0.98 (0.87)	1.20 (0.82)	0.007*
MPV, fL	10.1 (1.4)	10.2 (1.1)	0.919
NLR	6.73 (10.18)	3.59 (5.74)	<0.001*
Platelet, 10^3/mm³	225 (110)	204 (111)	0.397
Oxygene saturation, %	94 (8)	96 (5.0)	<0.001*
CRP, mg/dL	89.1 (117.3)	34.8 (89.2)	<0.001*
Procalcitonin, ng/mL	0.22 (0.35)	0.08 (0.11)	<0.001*
LDH, IU/L	347 (233)	275 (170)	<0.001*
D-dimer, ng/mL	1109 (1917)	568 (869)	<0.001*
Troponin I, ng/mL	0.1 (0.0)	0.1 (0.0)	0.301
Glucose, mg/dL	128 (81)	123 (68)	0.371
BUN, mg/dL	17.7 (16.2)	16.9 (14.7)	0.090
Creatinine, mg/dL	0.95 (0.55)	0.97 (0.48)	0.629
Sodium, mEq/L	132 (3)	139 (4)	<0.001*
Potassium, mEq/L	4.3 (0.8)	4.3 (0.7)	0.676
ALT, IU/L	25 (26)	22 (25)	0.053
	Mean±SD		
Albumin, gr/dL	3.3±0.6	3.6±0.6	<0.001*

IQR: Interquartile range; SD: Standard deviation, CAD: Coronary artery disease; CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease; RAAS: Renin-angiotensin-aldosterone system; MPV: Mean platelet volume; NLR: Neutrophil to lymphocyte ratio; LDH: Lactate dehydrogenase; CRP: C-reactive protein; BUN: Blood urea nitrogen; ALT: Alanine aminotransferase; Fever‡>37.5 °C; CT: Computerized tomography; *p<0.05: Statistically significant parameters

Table 3. Outcome stratified by serum sodium level

	Hyponatremia (n=159)	Normonatremia (n=628)	p
Length of hospital stay, day	10 (9)	8 (8)	<0.001*
ICU admission or ventilator need, n (%)	72 (45.3)	140 (22.3)	<0.001*
Death, n (%)	39 (24.5)	63 (10.0)	<0.001*

ICU: Intensive care unit; *p<0.05: Statistically significant parameters

Table 5. Risk factors for mortality in COVID-19 patients

Variable	Mortality			
	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Sex (male)	1.36 (0.89-2.08)	0.157	2.30 (1.07-4.96)	0.034*
Hyponatremia	2.92 (1.87-4.55)	<0.001	1.45 (0.75-2.80)	0.268
Procalcitonin	1.11 (1.05-1.18)	<0.001	1.05 (0.99-1.12)	0.084
CRP	1.01 (1.01-1.01)	<0.001	1.00 (1.00-1.01)	0.135
NLR	1.06 (1.04-1.09)	<0.001	1.03 (1.00-1.06)	0.035*
Albumin	0.15 (0.10-0.24)	<0.001	0.34 (0.17-0.71)	0.004*
Oxygen saturation	0.90 (0.87-0.92)	<0.001	0.95 (0.91-0.99)	0.017*
LDH	1.00 (1.00-1.00)	<0.001	1.00 (1.00-1.00)	0.637
Hemoglobin	0.79 (0.72-0.87)	<0.001	0.88 (0.76-1.03)	0.122
ICU admission	14.88 (9.24-23.85)	<0.001	8.08 (3.65-17.87)	<0.001*

CRP: C-reactive protein; NLR: Neutrophil to lymphocyte ratio; LDH: Lactate dehydrogenase; ICU: Intensive care unit; OR: Odds ratio; (95% CI): 95% Confidential interval. *p<0.05: Statistically significant parameters

DISCUSSION

In the present study, we examined the predictors of hyponatremia on admission and its outcome in patients hospitalized for COVID-19. Our findings showed that diabetes mellitus and the severity of pneumonia were the independent predictors of the development of hyponatremia at the time of admission. Additionally, although we have not found a correlation between increased mortality and hyponatremia, we have documented that hyponatremia was associated with longer in-hospital stay and a higher incidence of ICU admission or ventilator need.

Hyponatremia is the most common electrolyte abnormality in hospitalized patients. It is identified in 30% of patients with community-acquired pneumonia (11,15). Although previous smaller studies had reported a higher incidence of hyponatremia in patients with COVID-19, more recent and more extensive studies had revealed a lower incidence that ranges from 20% to 45% (16-21). Leong et al. (13) have documented a similar incidence of hyponatremia (29%) in previous SARS epidemic in Hong Kong. We have found hyponatremia in 159 patients (20.2%), most of whom had mild hyponatremia in line with the previous reports.

The pathogenic mechanisms for the development of hyponatremia in patients with community-acquired pneumonia remain uncertain. Inappropriate ADH secretion stimulated by pain, hypovolemia, nausea, and certain medications has been postulated causative factors. The International Health Outcome Predictive Evaluation for COVID-19 (HOPE-COVID-19) registry found that chronic kidney disease, bilateral pneumonia, tachypnea, male sex, and at age ≥ 70 years were associated with hyponatremia (17). Similar to our findings, a French study revealed that pulmonary lesions on the thoracic CT-scan performed during admission were significantly more extensive in the hyponatremic patients with COVID-19 compared to the normonatremic group (22). Inflammation and increased IL-6 levels have been linked with non-osmotic ADH secretion (23). Bernie et al. (24,25) documented an inverse relationship between IL-6 levels and serum Na. IL-6 levels were available only for a limited number of patients in our study, so we did not include them in statistical analysis. Although we have found significantly higher serum levels of inflammatory markers including CRP, procalcitonin, and NLR in patients with hyponatremia than the normonatremic group, these markers were not associated with hyponatremia in multivariate analysis.

In the present study, we noted that the patient group with hyponatremia had a longer length of hospital stay than the normonatremic group. The former group needed ICU admission and mechanical ventilation more frequently. In a longitudinal retrospective cohort study, Tzoulis et al. (18) documented that hyponatremia at admission was linked with a 2.2-fold increase in the likelihood of needing ventilator support but not associated with length of hospital stay in patients with COVID-19. Similar to our findings, Atilla et al. (19) found that hyponatremia was associated with length of hospital stay, need for mechanical ventilation, and ICU admission in COVID-19 patients. In a retrospective, multicenter, observational cohort study of 4645 COVID-19 patients from New York, investigators concluded that hyponatremia was associated with increased risk of encephalopathy and mechanical ventilation. Comorbid diseases, serum creatinine, D-dimer, procalcitonin, ferritin, IL-6 levels, lymphocyte, and neutrophil numbers have been linked with the severity and the prognosis of COVID-19 infection (3,26-30). We believe that risk stratification is essential in the efficient use of hospital resources during the pandemic. We suggest that hyponatremia at the time of admission could be added to risk stratification tools for predicting clinical outcomes in COVID-19 patients.

We have found a relatively high in-hospital mortality (13%) rate for the entire cohort. The data considering the association of increased mortality and hyponatremia in COVID-19 patients are controversial (17-21). Our results revealed a higher mortality rate in patients with hyponatremia than patients with normonatremia. However, hyponatremia was not found to be an independent predictor of mortality in multivariate analysis. Nevertheless, most of our patients had mild hyponatremia, and our results regarding mortality and hyponatremia relationship in COVID-19 patients should be interpreted in the appropriate context.

Although we included a relatively large number of patients from a single center in our study and analyzed the effect of multiple factors besides hyponatremia

on clinical outcome measures, several limitations of our study deserve mention. First, the study design was retrospective. Second, we did not include the patients in the hyponatremia group if they were normonatremic at admission but developed hyponatremia during hospitalization. Third, we could only document volume status and the exact cause of hyponatremia in a minority of cases, given the study's retrospective nature and the lack of urine studies and serum osmolality. Finally, follow-up serum Na levels were not included in the analysis.

CONCLUSION

Our results indicated that hyponatremia is quite common among hospitalized patients with COVID-19. We have shown that hyponatremia was associated with more extended in-hospital stay and a higher incidence of ICU admission or ventilator need. Diabetes and the severity of pneumonia were found to be the independent predictors of hyponatremia in COVID-19 patients. We suggest that hyponatremia at the time of admission could be added to risk stratification tools in patients hospitalized for COVID-19.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Ondokuz Mayıs University Clinical Researches Ethics Committee (Date: 26.08.2021, Decision No: 2021/408).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article

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The most typical mistakes made during pelvic X-ray in pediatric patients typical mistakes made during pelvic X-ray

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ABSTRACT

Aim: This study aimed to identify the most common mistakes made during pelvic radiography in pediatric patients and to give advice to physicians and technicians on how to avoid these mistakes.

Material and Method:Between 2016 and 2020, 1150 pelvic radiographs (in pediatric patients for any reason) were analyzed by two independent orthopaedic surgeons. Radiographs taken in trauma patients were not included in the study due to the patient's discomfort and agitation. Interobserver and intraobserver reliability for radiographic measurements were determined using intra-class correlation coefficients (ICC) obtained from three replicate sets of measurements on a sample of 1150 radiographs recorded by each observer at least one week apart.

Results: The mean age of the patients included in this study was 4.28±3.86 (range: 0-14) years. Of the 1150 patients, 935 (81.30%) were female and 215 (18.69%) were male. When pediatric pelvic radiographs were evaluated, the most common mistake was made during gonad protection. It was found that 71.22% of the patients' gonads could not be protected. While this rate was 82.24% in female patients, this rate was 23.34% in male patients. There was a statistically significant difference when compared by gender (p= 0.015). Giving the malposition was the second most common mistake.

Conclusion: The most common errors in pediatric pelvic radiographs are failure to provide gonad protection and malposition of the patient. To minimize these errors, it is necessary to inform both the doctor and the technician.

Keywords: Gonad protection, malposition, pediatric patients, pelvic radiography

INTRODUCTION

Children are more susceptible to radiation exposure than adults due to an abundance of rapidly proliferating cells in the bodies (1-3). Additionally, pediatric patients' longer life expectancy significantly increases their risk of developing cytochemia as a result of radiation dosages when compared to adult patients (3). Due to the significant differences between pediatric imaging algorithms and those used in the adult population, it has been established that treating radiography errors differently in children versus adults is critical (4,5).

Menashe et al. focused their research on radiographic errors in pediatric chest and abdomen radiography. They raised awareness regarding radiographic errors through the cases presented in their study (5,6). Another critical issue with pediatric patients is the preservation of the gonads on pelvic radiographs (7-9).

We were unable to locate any study investigating the frequency of radiographic errors made during pelvic radiography in pediatric patients when we searched the literature. The purpose of this study was to determine the most frequently committed errors during pelvic radiography in pediatric patients and to advise physicians and technicians on how to avoid them.

MATERIAL AND METHOD

The study was approved by the SBÜ Haseki Training and Research Hospital Ethics Committee (Date: 24.11.2021, Decision No: KAEK- 2021-232). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Between 2016 and 2020, 1150 pelvic radiographs (in pediatric patients for any reason) were analyzed by two

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independent orthopedic surgeons using data received from the institution's database. Due to the patient's pain and agitation, radiographs obtained in trauma patients were excluded from the study. Independent analysis of the compared images was performed to ascertain the data's consistency. Interobserver and intraobserver reliability for radiographic measurements were determined using intra-class correlation coefficients (ICC) obtained from three replicate sets of measurements on a subset of 1150 radiographs taken at least one week apart by each observer. The following scores were used: ICC >0.80 indicates excellent; 0.70-0.80 indicates very good; 0.60-0.70 indicates good; 0.40-0.60 indicates fair; and 0.40 indicates poor.

The top five mistakes made during pelvic X-rays in pediatric patients were identified.

1. Positioning
2. Gonad protection
3. Radiation dose
4. Labels or markers
5. Movement

Positioning: X-ray of the pelvis, both hips visible, the obturator foramen should be in its optimal symmetrical position (10).

Radiation dose: The optimal radiation dose should be between 3.06 and 4.57 μSv (11).

Gonad protection: Shields should be placed around the testicles of males and the ovaries of females to protect them (12).

Labels or markers: While marking the radiographic, it was found essential to enter the patient's name, age, and the radiographic's direction.

Movement: The patient should not move during the X-ray.

For descriptive statistics, categorical variables were presented as numbers and percentages, and continuous variables were presented as mean, standard deviation, minimum and maximum values.

RESULT

The mean age of the patients included in this study was 4.28±3.86 years (range, 0-14 years). 935 (81.30%) of the 1150 patients were female, while 215 (18.69%) were male.

When pediatric pelvic radiographs were evaluated, the most common mistake was made during gonad protection. It was shown that 71.22% of patients' gonads were unable to be protected (**Figure 1**) (**Table 1**). While this rate was 82.24% for female patients, it was just 23.34% for male patients. When gender was compared, there was a statistically significant difference (p= 0.015) (**Table 2**). Giving the incorrect position was the second most frequent mistake (**Figure 2**). It was discovered that 27.34% of patients were unable to be properly positioned. In 5.65% of patients, a movement was identified during radiography. Radiation doses were within the acceptable level in all cases. 2.42% of patients had radiographs with incorrect labels or markers. There was no statistically significant correlation between the patients' ages and the mistakes made (p<0.001)

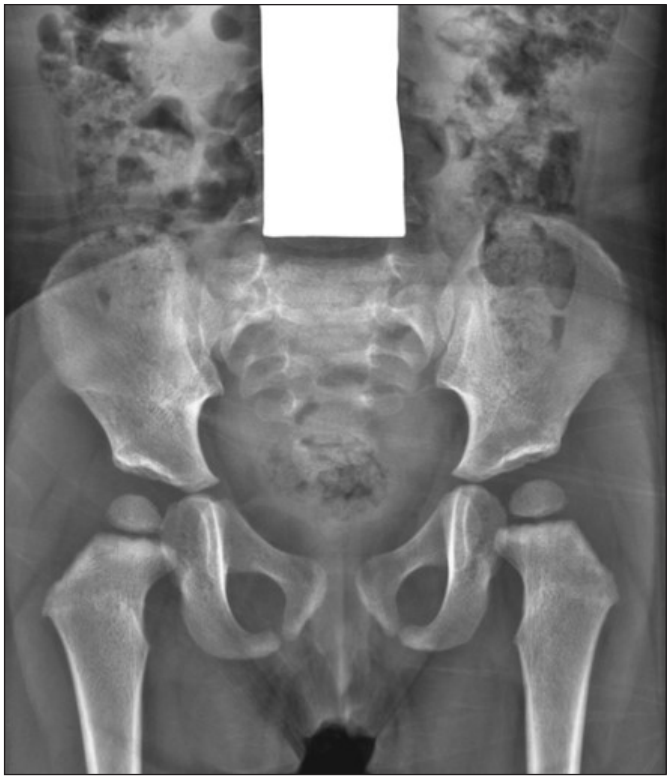

Figure 1. X-ray of the pelvis with incorrect application of the gonad protector

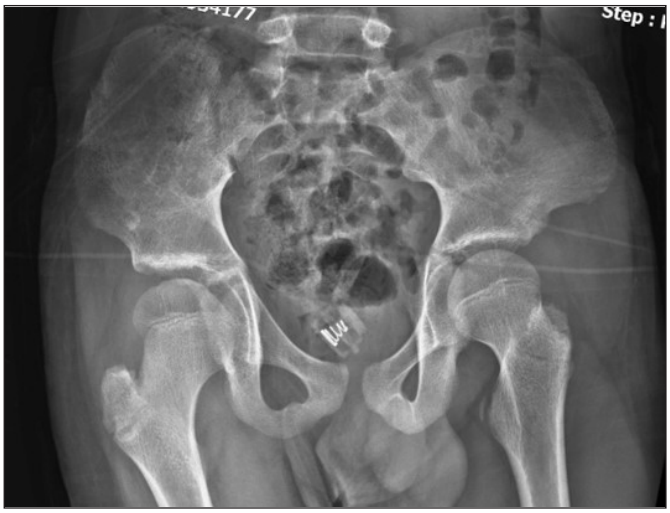

Figure 2. X-ray of the pelvis of a patient who has been given a malposition

When we analyzed intraobserver and interobserver correlations, we discovered that angle measurements had an almost perfect interobserver agreement (ICC, 0.97; confidence interval [CI], 0.96-0.99) and intraobserver agreement (ICC, 0.97; CI, 0.96-0.99) (ICC, 0.94; CI, 0.89-0.96).

Tablo 1. Percentage of mistakes

Mistakes	percent
Gonad protection	71.22%
Positioning	27.34%
Movement	5.65%
Labels or markers	2.42%
Radiation dose	0.0%

Table 2. Comparison of gonad protection mistakes by gender

Gender	Number	Percent	p-value
Female	935	81.30%	0.015
Male	215	18.69%	

DISCUSSION

The study revealed that the most frequently made error in pediatric patients was a failure to protect the gonads. We discovered that this error was made in 71.22% of cases. In comparison to other types of mistakes, this rate was relatively high. When we compared the findings to the existing literature, they were found to be consistent.

According to Liokas et al. (8), present shielding procedures did not adequately protect the ovaries from radiation exposure during pelvic radiography. They concluded that we did not preserve the gonads on prepubertal female pelvic X-rays. Kaplan et al. (13) stated in their study that the gonads could not be adequately protected in females and that the radiation dose absorbed by the gonads increased as a result of the protectors' incorrect positioning. They advised against its use. Kumar et al. (14) likened the gonadal shield to an Albatross hanging around the neck of developmental hip dysplasia. They reported that when gonad protectors are utilized, they degrade the image and may result in data loss. We believe that gonad protectors are unnecessary, as girls' gonads cannot be preserved. However, we advocate that male patients receive gonadal protection because it is simple and effective.

Malpositioning was the second most frequently occurring error in pediatric patients, occurring at a rate of 27.34%. Malpositioning might result in incorrect radiographic interpretation and misdiagnosis. According to Li et al. (15), malpositioning impairs the assessment of the lateral and anterior central margin angles. This study highlights the crucial importance of validating the radiographic quality to guide hip pathology treatment. Brockmeyer et al. (16) examined the use of basic devices to assure proper placement. The study's findings, however, indicated that simple positioning devices do not yield standardized anteroposterior pelvic radiographs. We recommend that technicians be taught how to properly position the graph to avoid positioning issues.

Another error that occurs during pediatric pelvic X-rays is the mismarking of the X-ray. The most critical information to note on the X-ray is the patient's name and age for accurate diagnosis and treatment. When we analyzed the radiographs, it was revealed that the most significant error made when marking was the lack of the direction sign. Given the critical nature of determining the correct orientation during surgical procedures, it is critical to eliminate this error.

Hip developmental dysplasia is the most prevalent reason for pelvic X-rays in children. From this vantage point, the fact that the same patient is repeatedly exposed to radiation during the controls is worrisome. Vogel et al. (16) assessed the radiation risk associated with children's X-ray exposure and identified the lifetime risk of malignancy in individuals undergoing roentgenography for hip developmental dysplasia as very low risk. Additionally, they stated that this information might be utilized to reassure parents who are concerned.

The limitations of this study were single-centered and the requirement of radiography was not evaluated.

CONCLUSION

The most frequently made errors in pediatric pelvic radiographs are failing to protect the gonads and patient malposition. To minimize these mistakes, the doctor should first be informed in detail about the issue, followed by the technician, who should be informed based on the frequency of the errors.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the SBÜ Haseki Training and Research Hospital Ethics Committee (Date: 24.11.2021, Decision No: KAEK-2021-232).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Evaluation of the need for hospital-based pediatric palliative care in a single center

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ABSTRACT

Introduction/Aim: Cancer is one of the leading causes of death for children . Evidence suggests that these children experience substantial suffering from physical and emotional symptoms. Over the past two decades, paediatric palliative care has emerged as an approach that aims to ease suffering for children and their families coping with any life-threatening illness. Our aim is to encourage the expansion of palliative care centers for children with cancer and integration of these into healthcare services to assist clinicians and policy makers in developing services that address these needs.

Material and Method: Our study is a single-center retrospective cohort study. The records of 39 patients who were followed up in the pediatric oncology clinic between 2010-2021 and died were reviewed retrospectively.

Results: The age of diagnosis of patients was the most in the 6-12 range (n=16; 41%) and the least in the adolescent (n=11; 28.2%) age range. The cause of death was related to a disease in 79.5%. Treatment-related deaths were seen in eight patients, four of which were chemotherapy toxicity, two were engraftment failure in autologous hematopoietic stem cell transplantation, and two were post-transplant GVHD. 76.9% of the patients died in the intensive care unit.

Conclusion: In our study, patients with cancer and those who lost their lives were evaluated retrospectively in terms of symptom load, invasive procedures, and psychosocial needs and the need for end-of-life palliative care. Many studies have confirmed that the timely integration of palliative care into routine oncological care has many advantages, such as improvements in physical and psychological symptoms, quality of life and prognosis, as well as reducing costs. In Turkey, palliative treatment is tried to be provided to late stage pediatric cancer patients by pediatric intensive care units and pediatric oncologists. This both increases the workload of physicians and causes intensive care bed occupation. The development and expansion of palliative care on late stage pediatric cancer patients will contribute significantly to the quality of life of both children and their families.

Keywords: Palliative care, children, cancer

INTRODUCTION

Cancer is the leading cause of death for children, especially in high-income countries (1). Despite important medical developments in recent years, approximately 20% of children diagnosed with cancer die (2). Children with cancer in low- and middle-income countries are four times more likely to die from the disease than children in high-income countries (1). It is estimated that 2500-3000 new pediatric cases will be seen in Turkey every year (3).

Palliative care is an approach that improves the quality of life of patients and their families facing a life-threatening disease-related problem through early detection, accurate assessment and treatment of pain and other problems (4). Palliative care is a multidisciplinary approach and is provided by a team that can handle the child's physical symptoms as well as the spiritual and psychosocial needs of children and their family (5). The World Health Organization evaluates cancer patients' access to pain relief and palliative care within global health inequalities and accepts palliative care as a human right (4).

There are limited number of studies on the palliative care needs of pediatric oncology patients in Turkey. In our study, patients with cancer and those who lost their lives were evaluated retrospectively in terms of symptom load, invasive procedures, and psychosocial needs, and the need for end-of-life palliative care. The necessity of end-of-life care, and palliative care units for pediatric cancer patients was discussed.

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MATERIAL AND METHOD

The study was carried out with the permission of Health Sciences University Non-interventional Clinical Researches Ethics Committee (Date: 08.01.2019, Decision No: 18/364). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Our study is a single-center retrospective cohort study. The records of 39 patients who were followed up in the pediatric oncology clinic between 2010-2021 and died were reviewed retrospectively. Patients' age, diagnosis, diagnosis category (leukemia/lymphoma, brain and other solid tumor), admission complaints, psychological support status, length of stay in hospital and intensive care unit, frequency and duration of intensive care hospitalization, invasive procedures, invasive supportive treatments, hematopoietic cell transplantation information, pain treatment and types, cardiopulmonary resuscitation, cause of death, and place of death were recorded. Ethics committee and patient and parents consent were obtained before the study was initiated. Inclusion criteria of the patients were determined as being in the pediatric age group, having received treatment in the oncology clinic, and being the patient who died.

Statistical Analysis

Statistical analyses were performed using SPSS version 15. Normality was checked by Shapiro Wilk and single-sample Kolmogorov Smirnov tests, histogram, and drawing QQ plot and box plot graphs. Data were given as mean, s.deviation, median, minimum, maximum, frequency, and percentage. Variables between two groups were analyzed with the Mann Whitney U test. Nominal variables were evaluated with Chi square test with Yates correction and Fisher's exact probability tests. The level of significance was taken as $p<0.05$ and bidirectional.

RESULTS

The mean age of the patients was 8.51±4.59 years (1-17), 26 (66.7) were male and 13 (33.3) were female. The most common type of malignancy was solid tumors (n=22, 56.4%), followed by brain tumors with 30.8% (n=12). Five patients (12.8%) had leukemia/lymphoma. The age of diagnosis of patients was the most in the 6-12 range (n=22; 56.4%) and the least in the adolescent (n=11; 28.2%) age range. Demographic data of the patients are shown in **Table 1**. The cause of death was related to a disease in 79.5%. Treatment-related deaths were seen in 8 patients, four of which were chemotherapy toxicity, 2 were engraftment failure in autologous hematopoietic stem cell transplantation, and 2 were post-transplant GVHD. 76.9% of the patients died in the intensive care unit. For the last three months, pain was the most common complaint

Table 1. Sociodemographic characteristics of the patients

	Mean±SD	Range
Age of diagnosis, years	8.51±4.59	1-17
Age of death, year	10.59±4.84	2-18
	n	**%**
Gender		
Male	26	66.7
Female	13	33.3
Age of Diagnosis (years)		
1-5	12	30.8
6-12	16	41
>13	11	28.2
Leukemia/lymphoma (n=5;12.8)		
Hodgkin's Lymphoma	1	2.56
Non-Hodgkin's lymphoma	2	5.13
Leukemia	2	5.13
Glioma (n=12;30.8)		
Anaplastic Astrocytoma	3	7.69
Diffuse Astrocytoma	1	2.56
Glioblastoma	3	7.69
Gliomatosis Cerebri	1	2.56
Medulloblastoma	4	10.25
Solid Tumor (n=22;56.4)		
Adrenocortical Carcinoma/ Rhabdomyosarcoma	1	2.56
Ewing Sarcoma	5	12.82
Hepatocellular Carcinoma	1	2.56
Neuroblastoma	11	28.21
Norofibroma/ Angiosarcoma	3	7.69
Retinoblastoma/ Osteosarcoma	1	2.56
Mix-Germ Cell Tumor	1	2.56
Cause of Death		
Treatment related	8	20.5
Disease related	31	79.5
Place of death		
Service	6	15.4
Intensive care	30	76.9
Home	3	7.7
Patient provided with spiritual care	0	0
Child patient receiving psychological support	22	56.41
Social Service Support	1	

Table 2. General complaints of patients with cancer diagnosis

Application complaint	n	%
Pain	19	48.7
Fatigue	13	33.3
Nausea-vomiting	3	7.7
Dyspnoea	1	2.6
Fever	1	2.6
Paroxysm	1	2.6
Blackout	1	2.6

(n=19; 48.7%). Pain was significantly higher especially in children with solid tumors (n=14; 63.6%) (**Table 2**). There was no statistical difference between the number of symptoms and age groups (p=0.13). It was observed that the number of symptoms was higher in children age groups. Fifteen patients (38.4%) underwent an invasive procedure in end-of-life care. The most common invasive procedure applied to the patients was chest tube and tracheostomy (n=4; 8.52%), while the invasive method was ventilator (n=28; 72.8). Ventilator support was provided to 28 patients (71.8%) and dialysis support was provided to 5 (12.8%). Late stage supportive treatments that the patients received are shown in **Table 3**. A total of 19 patients (48.7%) received pain treatment in their late stages. The most common treatment was Morphine by IV ROA (94.7%). Two of the patients with tracheostomy had brain tumors and two were Graft Versus Host Patient (GVHD) with lung involvement. Two of our patients who had a chest tube had neuroblastoma with a thoracic mass, one had Ewing's sarcoma originating from the ribs and causing compression in the lungs, and one had anaplastic ependymoma who was kept on the ventilator for a long time and developed pneumonia. Approximately one third of the patients (n=11, 28%) underwent HSCT (Hematopoietic Stem Cell Transplantation) at least once. Transplantation was performed after ten patients were in remission after chemotherapy, and one patient was transplanted while in partial remission. Of these patients, 5 had lymphoma/leukemia, 4 had neuroblastoma, and 2 had Ewing sarcoma. 71.8% (n:28) of the patients needed intensive care from the moment they were diagnosed. 95 percent of them were treated in the intensive care unit in the last month. The rate of those who were treated in the intensive care unit three times or more was approximately 15%. The mean duration of stay of the patients in the ICU was determined as 11.67± 3.2 days. Psychiatric support was provided to more than half of the patients (n=22; 56.4%). (**Table 4**) Their complaints before death were respiratory failure in 31 patients, bleeding in 7 patients, and 1 patient died due to liver failure. CPR was performed on every patient died in the hospital, but not on those who died at home.

Table 3. Patients' complaints and psychological support status according to their cancer types

	Application complaint		Psychological assistance	
	n	%	n	%
Leukemia/ lymphoma				
Pain	2	40	2	5.1
Fatigue	3	60		
Brain			7	17.9
Pain	3	25		
Fatigue	3	25		
Dyspnoea	1	8.3		
Nausea- vomiting	2	16.7		
Fever	1	8.3		
Paroxysm	1	8.3		
Blackout	1	8.3		
Solid tumor			15	38.5
Pain	14	63.6		
Fatigue	7	31.8		
Nausea-vomiting	1	4.5		

Table 4. Distribution of supportive treatments applied to patients

	Mean±SD	Range
The length of hospital stay, days	109±25	2-357
ICU length of stay, days	11.6±3.2	1-120
	n	%
Hospitalization for >3 weeks without clinical improvement		
Yes	22	62.86
No	13	37.14
Invasive procedures applied		
Liver biopsy	1	2.13
Paracentesis	2	4.26
Peritoneal catheter	1	2.13
Thoracentesis	3	6.38
Chest tube	4	8.51
Tracheotomy	4	8.51
Invasive support		
Ventilator	28	71.8
Dialysis	5	12.8
Stem cell transplantation		
Yes	11	28.2
No	28	71.8
Number of transplants		
0	28	71.8
1	8	20.5
2	2	5.1
3	1	2.6
Pain Treatment		
Yes	19	48.7
No	20	51.3
Which pain treatment?		
Morphine	7	36.84
Fentanyl, morphine	5	26.32
Fentanyl,morphine, tramadol	5	26.32
Morphine,tramadol, pethidine	1	5.26
Tramadol, fentanyl	1	5.26
Intensive care need		
Yes	28	71.8
No	11	28.2
Frequency of i ntensive care hospitalization		
1 hospitalization	18	64.29
2 hospitalizations	6	21.43
3 or more hospitalizations	4	14.29
CPR *		
Yes	36	92.3
No	3	7.7

*Three patients died at home. Two families were hesitant about CPR, yet later asked for it

DISCUSSION

Cancer is the leading cause of childhood death in Turkey and 30% of children diagnosed with cancer succumb to their disease. Child mortality from cancer ranks fourth in Turkey after infectious diseases, heart diseases, and accidents (4). Children in the terminal stage have to cope with symptoms such as pain, dyspnea, nausea-vomiting, which will necessitate palliative care in the last stages of their lives (5). Many studies have confirmed that the timely integration of palliative care into routine oncological care has many advantages, such as improvements in physical and psychological symptoms, quality of life and prognosis, as well as reducing costs. Since pediatric palliative clinics have not become widespread in Turkey yet, this service is provided by oncologists. Physicians working in oncology centers in Turkey stated that there is a lack of personnel, training, and physical space for pediatric palliative services, and that they do not feel qualified for this service (6). In order to contribute to the organization of the palliative care needs and scope of pediatric oncology patients, in this study, patients with palliative care needs were evaluated retrospectively.

Some of the late stage cancer patients need invasive procedures such as intensive care treatment, tracheostomy, and thoracic tube. In the study conducted by Corkum et al. (7) on advanced cancer patients , the rate of patients who underwent tracheostomy was found to be 30% (n=62), and the rate of patients who had a thoracic tube inserted was 14% (n=345). In our study, the rate of patients who underwent tracheostomy was 8.51% (n=4), and the rate of patients who had a thoracic tube inserted was 8.51% (n=4). Two of our patients with tracheostomy had brain tumors and two had GVHD with lung involvement. Two of our patients who had a chest tube had neuroblastoma with a thoracic mass, one had Ewing's sarcoma originating from the ribs and causing compression in the lungs, and one had anaplastic ependymoma who was kept on the ventilator for a long time and developed pneumonia. Invasive procedures may be required for masses or infections involving the thoracic region.

Stem cell transplantation, which is performed by applying high-dose chemotherapy and/or radiotherapy and can sometimes cause early and late side effects, is used as a treatment method in children with relapsed or advanced cancer and contributes to the decrease in mortality rates (5,8). One third of the patients had HSCT for at least once in the study. Of these patients, 5 had lymphoma/leukemia, 4 had neuroblastoma, and 2 had Ewing sarcoma. Palliative care, which requires patient-specific care, family support and multidisciplinary teamwork, has an important place in the management of symptoms, which emerge on patients with advanced cancer after performing HSCT, such as pain, dyspnea, nausea-vomiting. Especially in the parents of the patients who underwent HSCT and died, depression, anxiety and deterioration in quality of life were observed more than the parents of those who did not get HSCT treatment (8). Palliative care is extremely important for these families and patients.

Children with cancer included in our study and their families expressed pain as their most common symptom. Wolfe et al. (9) in their study, showed that pain in children with cancer at late stage increased heavily in the last 12-week period of their lives. It has been found that children who receive palliative care support in the last period of life manage pain better than those who do not (10). In our study, approximately half of the patients used morphine and similar opioids. Within the scope of the Pallia-Turk project, which was initiated in Turkey in 2010, it was aimed to increase the availability of opioids and to put the community-based palliative care model into practice (11). The use of opioid analgesics in Turkey has increased over the years, but has not reached the desired level (12). The amount of opioids per individual was 14.6 mg in Turkey in 2010. Considering that the average of the first twenty countries with the highest amount of opioid use is 216.7 mg, this is quite low (13). Access to opioid drugs in Turkey has become much more possible with the increase in adult palliative care. However, there are several barriers in opioid use. In a study conducted in Turkey, the obstacles for the pain treatment of pediatric patients were investigated, and these were determined as the pain was not evaluated regularly and permanently by physicians, the nurses did not fill the pain scales, and the absence of psychosocial support units in hospitals (14). Another study showed that mothers of children cancer patients who were treated at home avoided the use of analgesics because of insufficient education and concerns about side effects (15).

Three fourth of the patient group examined in this study received intensive care treatment. Studies have shown that one out of every three to four children with cancer receives intensive care treatment and the mortality of these patients is four to five times higher than the mortality of patients hospitalized in the pediatric intensive care unit (PICU) for non-cancer reasons (16,17). Pediatric intensive care units, which are limited in number in Turkey, are used by palliative patients (18). In the literature, it has been shown that pediatric oncology patients with early integration into pediatric palliative have lower intensive care unit admission and intervention rates, and have lower costs (19-22). We think that, with the spread of palliative care centers, pediatric intensive care units will be used less and invasive interventions will decrease in late stage patients.

In our study, most of the patients died in the hospital, mostly in the intensive care unit. It was determined that children who died in a hospital environment suffered more and their relatives experienced more depression, anxiety, stress, and a difficult mourning period than those of the children who died outside the hospital (23). It has been shown that the mortality rate of children receiving palliative care in the terminal stage in the intensive care unit and hospital decreases (10). The fact that the majority of patients in our study died in hospitals and intensive care units may be due to the lack of a palliative center in our hospital.

Life-threatening diseases confront individuals with the reality and imminence of death, and spirituality becomes more important to end-of-life patients. Spiritual care has been practiced in a modern setting for over a hundred years in Europe and the United States. The American Society of Clinical Oncology (ASCO) and the American Academy of Hospice and Palliative Medicine defined spiritual care for oncology patients as one of the criteria for quality palliative care (24). The European Association of Palliative Care (EAPC) also prepared guidelines on spiritual care in 2014 (25). However, spiritual care is a newly developing concept in Turkey (26). In our study, there was no record of whether the patients in the terminal stage received spiritual care or not.

In our study, only 12.5% of patient relatives did not want resuscitation (DNR). There are publications showing that pediatric patients who do not receive palliative care are almost eight times more likely to be resuscitated at the time of death than patients who do receive palliative care (10). There is no legal basis for limiting end-of-life support (EOL) in Turkey. There are not enough studies on end-of-life decisions and DNR among pediatricians in Turkey.

Studies have shown that the treatments applied on cancer patients, the side effects of the treatment, the fear of death, the uncertainty about life and treatment cause more emotional and behavioral problems in these children (27,20). Palliative care centers provide psychosocial support for both children and families. As in many hospitals in Turkey, psychosocial services are not a part of routine patient care in the hospital where we conducted the study. In cases in which the patient is thought to be in need, psychological support and social service support can be provided upon the request of the relevant physician. Psychiatric support was provided to more than half of the children in our study. Many studies have confirmed that the timely integration of palliative care into routine oncological care has many advantages, such as improvements in physical and psychological symptoms, quality of life and prognosis, as well as reducing costs.

CONCLUSION

In Turkey, palliative treatment is tried to be provided to late stage pediatric cancer patients by pediatric intensive care units and pediatric oncologists. This both increases the workload of physicians and causes intensive care bed occupation. In Turkey, the development and expansion of palliative care on late stage pediatric cancer patients will contribute significantly to the quality of life of both children and their families.

Our recommendation is to encourage the expansion of palliative care centers for children with cancer and the integration of these into healthcare services.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Health Sciences University Non-interventional Clinical Researches Ethics Committee (Date: 08.01.2019, Decision No: 18/364).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Original Article

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Evaluation of anatomical variations with morphological measurements and their relationship with rotator cuff tear and acromion types

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ABSTRACT

Introduction: There many more anatomical measurements such as the acromiohumeral distance and lateral acromion angle and acromial configuration was reported as might be associated with rotator cuff tear. In our study, we aimed to reveal the effect of acromion index, acromiohumeral distance, lateral acromion angle, critical shoulder angle values and the acromion type in the development of rotator cuff tear.

Material and Method: In our retrospective study, 58 patients and 29 asymptomatic volunteers who underwent shoulder magnetic resonance imaging examinations were examined. acromion index, acromion humaral distance, lateral acromion angle and critical shoulder angle were measured and their relationship with rotator cuff tear and acromion types were evaluated.

Results: Type III (hooked) of acromial shapes, higher acromion index and critical shoulder angle values, lower acromiohumeral distance and lateral acromion angle values are more frequently seen in rotator cuff tear patients, in our study.

Conclusion: Thus, we revealed anatomical malformations that predispose to rotator cuff tear concerning the shoulder joint.

Keywords: Shoulder joint, acromion types, rotator cuff tears

INTRODUCTION

The acromion is an important anatomical structure that exists in various configurations and some of these variations may lead to rotator cuff tear (RCT) (1). RCT is a frequently seen disease of shoulder, Almost 35% of patients undergoing magnetic resonance imaging (MRI) with shoulder pain have RCT. This rate reaches 50% in patients over the age of 66 (2). MRI enables a more comprehensive assessment of the glenohumeral joint by revealing the articular cartilage and labroligamentous structures in detail (3). Acromiohumeral distance (AHD) is measured as interval between the humeral head and the acromion (4). On MRI a narrowed AHD may cause decreased in lowering function of infraspinatus muscle and humeral head rise. Recently, narrowed AHD is found to be associated subacromial impingement and rotator cuff tear (4). Recently an relationship between the critical shoulder angle (CSA) and acromion index (AI) with RCTs were described (5). A high CSA (>35) was related to increased risk of RCT. Thus, shear forces apply an increasing force to the origin of the glenoid and rotator cuffs (5). As the lateral projection of the acromion increases, higher AI is measuered (6). For this reason, increased AI is associated with RCT. There many more anatomical measurements such as the AHD and lateral acromion angle (LAA) and acromial configuration was reported as might be associated with RCT (1). In our study, we aimed to reveal the effect of AI, AHD, LAA, CSA values and the acromion type in the development of RCT.

MATERIAL AND METHOD

The study was carried out with the permission of Tokat Gaziosmanpasa University Hospital, Non-invasive Clinical Researches Ethics Committee (Date: 20.01.2022, Decision No: 2022/02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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General Data

In our retrospective study, 68 patients and 29 asymptomatic volunteers who underwent shoulder MRI examinations between 2019 and 2021 were examined. A total of 10 patients who have previous surgery, prosthesis, fractures or solid lesions involving shoulder joint, cases with acromial spurs or severe osteoarthritis, history of chemoradiotherapy, and known systemic disease were excluded from our study. Since our measurements were highly affected by shoulder and arm position, many patients without appropriate positioning on MRI were excluded from the study. During shoulder MRI, the patient is placed in supine position and the arm adducted with a mild external rotation.Fifty-eight patients with RCT and 29 asymptomatic volunteers were enrolled in this study. Patients are divided into two groups partial-thickness tear and full thickness tear on MRI. The supraspinatus, infraspinatus, subscapularis and teres minor muscles are evaluated as rotator cuff muscles. Full-thickness RCTs are accepted as the tears extends from the bursal surface to the articular surface. Partial-thickness RCTs are accepted as the tears lack of full transmural extension from the articular to the bursal surfaces. On fat-saturated PD or T2W images, tear is detected as fluid signal intensity within the tendon. Types of the acromion were evaluated on the T1W sagittal oblique images. The acromion has group into four type according to its shape (7). Type 1 acromion is described as flat. Type 2 is accepted as curved with a concave surface. Type 3 has a hooked shape in the most anterior portion of acromion. Type 4 was described as convex shaped acromion (**Figure 1**). All measurements are performed by a radiologist (S.Ö).

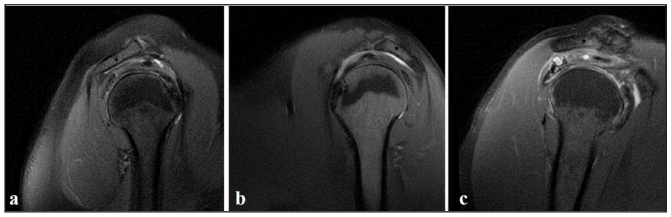


Figure 1. a) Type 1 acromion is described as flat. **b)** Type 2 is accepted as curved with a concave surface. **c)** Type 3 has a hooked shape in the most anterior portion of acromion (black star: acromion)

Measurements

In the coronal plane AI, AHD, LAA and CSA were measured (**Figure 2**). Measurements were obtained from the image where the lateral end of the glenoid and acromion could be seen simultaneously in the coronal section. The ratio between the distance from the glenoid cavity to the lateral edge of the humerus and the distance from the glenoid cavity to the lateral edge of the acromion is calculated as AI (8). To measure AHD two parallel auxiliary lines were drawn. First line placed on the lower edge of the acromion and second line is placed on the the osseous humeral head. The middle image of all images that represent the glenoid is chosen, on which the glenoid is shown larger (9). LAA was measured as the angle between the line passing through the middle of the joint and the line passing through the inferior surface of the acromion in the coronal plane where the acromioclavicular joint is best seen. CSA is measured as the angle between the superior and inferior bone margins of the glenoid and the most lateral border of the acromion (10). MRI image protocol was shown in **Table 1**.

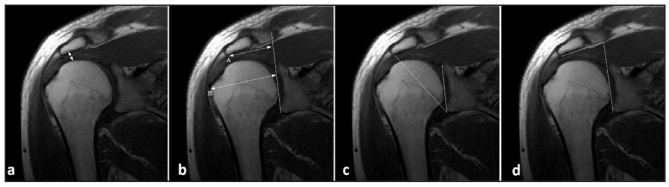


Figure 2. a.) Acromiohumeral distance (AHD) is measured as interval between the humeral head and the acromion b.) Acromion index (AI) is calculated as the ratio between the distance from the glenoid cavity to the lateral edge of the humerus and the distance from the glenoid cavity to the lateral edge of the acromion. c.) Critical shoulder angle (CSA) is measured as the angle between the superior and inferior bone margins of the glenoid and the most lateral border of the acromion d.) Lateral acromial angle (LAA) was measured as the angle between the line passing through the middle of the joint and the line passing through the inferior surface of the acromion

Table 1. Pulse sequence

	FOV (cm)	Slice thickness (mm)	Matrix	TR/TE (ms)
Axial PD fat suppression	20x20	4	512×512	3000/77
Coronal oblique T1	20x20	4.5	512×512	700/9
Coronal oblique PD with fat suppression	20x20	4.5	512×512	2350/62
Sagittal oblique PD with fat suppression	20x20	4.5	512×512	2350/62

FOV: field of view, TE: echo time, TR: repetition time, cm: centimeter, mm: millimeter, ms: millisecond, PD: Proton density

Statistical Analysis

All statistical analysis was performed using R version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org). Shapiro-Wilk's normality test and Q-Q plots were used to assess the normality of the data, and Levene's test was used to check the homogeneity of the variances. Numerical variables were expressed as mean±standard deviation, and estimated mean with 95% confidence intervals (CI) in case controlling for age. Categorical variables were described as count (n) and percentage (%). One-Way ANOVA (analysis of variances) and Welch's F test, and also independent sample t-test were used to compare the age, AI, AHM, LAA, and CSA values between the groups. The differences between the groups were investigated with Tukey HSD test after the ANOVA, and Games-

Howell multiple comparison test after the Welch's F test. Moreover, the analysis of covariance (ANCOVA) was run to determine the AI, AHM, LAA and CSA of study groups by controlling for age, since the age distribution of the groups were different. Multiple comparisons were performed with Bonferroni correction. Besides, Pearson chi-square test following by two proportion Z-test was applied to examine the association between study groups and gender distribution, and acromial shapes. The effect of the study groups on the acromial shapes was analyzed by multinomial logistic regression analysis by controlling for age. The receiver operating characteristic (ROC) curve analysis were conducted for AI, AHM, LAA, and CSA as a marker in diagnosing the RCT, and the area under the curve (AUC) values were calculated with 95% confidence intervals. An optimal cut-off values were determined using the Youden J index. The sensitivity, specificity, negative and positive predictive values were calculated with 95% confidence intervals for determined cut-off values. A p-value less than 5% was considered as statistically significant.

RESULTS

Fifty-eight patients with RCT were enrolled in this study. They were 31 (49.4%) females and 27 (50.6%) males. Their ages ranged from 23 to 81 years with a mean of 57.59±12.11 years. A control group of 29 asymptomatic volunteers without RCT was added. They were 12 females and 17 males. Their ages ranged from 21 to 68 years with a mean of 44.14±13.11 years. The mean age was 57.59±12.11 (ranged, 23-81) in RCT patients were higher than the healthy controls (44.14±13.11, ranged: 21-68, p<.001). However, the gender distribution of the groups was similar (p=.240). There was no statistically significant association between study groups and acromial shapes (p=.051). As a result of both ANOVA and age-adjusted ANCOVA analysis, AI and CSA values were significantly higher in patients with RCT compared to healthy controls, while AHM and LAA values were significantly lower (all p-values <.001, **Table 2**).

There was a significantly association between study groups and Type III (hooked) of acromial shapes, Type III proportion of acromial shapes was significantly higher in RCT patients than in healthy controls (n=14/58 [24.1%] vs. n=1/29 [3.4%], p=.031, **Table 3**). However, this association was not statistically significant when controlling for age (OR=3.68, 95% CI: 0.28-48.65, p=.323).

The mean and adjusted mean of the AI, AHM, LAA, and CSA values according to study groups were given in **Table 4**. A one-way ANOVA and Welch's F test were run to determine whether there was a statistically significant difference in AI, AHM, LAA and CSA values between study groups. There was a statistically significant difference in AI, AHM, LAA and CSA values between study groups (all p<.001). But, since the age distributions between the groups were different, a one-way covariance (ANCOVA) analysis was conducted to compare the AI, AHM, LAA and CSA of study groups by controlling for age. There was a significant difference in AI values between the study groups ($F_{2,83}=22.86$, p<.001, $\eta^2=0.36$). Post-hoc analysis, which is performed with a Bonferroni adjustment, showed that the AI values in patients with full thickness tear was (0.69, 95% CI:0.66-0.71) higher than patients with partial thickness tear (0.63, 95% CI: 0.61-0.66, adj. p=.005) and healthy controls (0.57, 95% CI: 0.54-0.59, adj. p<.001). In addition, the AI values were significantly higher in patients with

Table 2. Demographical characteristics and acromial measurements of the study groups

	Healthy controls (n=29)	RCT patients (n=58)	p-value
Age (years)	44.14±13.11 (21-68)	57.59±12.11 (23-81)	<.001[1]
Gender (Female/Male)	12/17	31/27	.240[2]
Acromial shapes			.051[2]
Type I (flat)	9 (31)	16 (27.6)	
Type II (curved)	19 (65.5)	28 (48.3)	
Type III (hooked)	1 (3.4)	14 (24.1)	
AI			
Mean±SD	0.57±0.05	0.66±0.06	<.001[1]
Estimated mean (95% CI)	0.57 (0.55-0.60)	0.66 (0.64-0.67)	<.001[3]
AHM (mm)			
Mean±SD	8.45±1.13	6.32±1.38	<.001[1]
Estimated mean (95% CI)	8.23 (7.72-8.74)	6.43 (6.08-6.77)	<.001[3]
LAA°			
Mean±SD	77.07±4.20	69.64±5.25	<.001[1]
Estimated mean (95% CI)	76.99 (75.01-78.98)	69.68 (68.32-71.03)	<.001[3]
CSA°			
Mean±SD	31.25±1.88	34.38±2.27	<.001[1]
Estimated mean (95% CI)	31.55 (30.70-32.40)	34.68 (34.10-35.26)	<.001[3]

Data were presented as mean±standard deviation (range) and estimated mean with 95% confidence intervals, and also were described as count (n) and percentage (%). Bold values denote that statistically significant difference between the groups. 1 Independent samples t-test 2 Pearson chi-square 3 ANCOVA Abbreviations: SD; standard deviations, AI; acromial index, AHM;, LAA; lateral acromion angle, CSA; critical shoulder angle, 95% CI; 95% confidence interval, RCT; rotator cuff tear

partial thickness tear compared to healthy controls (adj. p<.001) (**Graphic 1-A**). There was a significant difference in AHM values between the study groups (F2,83=41.71, p<.001, η2=0.50). Post-hoc analysis, which is performed with a Bonferroni adjustment, showed that the AHM values in patients with full thickness tear was (5.33, 95% CI:4.87-5.78) lower than patients with partial thickness tear (7.27, 95% CI: 6.88-7.66, adj. p<.001) and healthy controls (8.49, 95% CI: 8.06-8.92, adj. p<.001). In addition, the AHM values were significantly lower in patients with partial thickness tear compared to healthy controls (adj. p<.001) (**Graphic 1-B**). There was a significant difference in LAA values between the study groups (F2,83=17.53, p<.001, η2=0.30). Post-hoc analysis, which is performed with a Bonferroni adjustment, showed that the LAA values in patients with full thickness tear (68.65, 95% CI:66.53-70.77) and in patients with partial thickness tear were (70.46, 95% CI: 68.62-72.30) lower than healthy controls (77.24, 95% CI: 75.22-79.25, all adj. p<.001). However, the LAA values were similar in patients with full thickness tear and partial thickness tear (adj. p=.653) (**Graphic 1-C**). There was a significant difference in CSA values between the study groups (F2,83=22.44, p<.001, η2=0.35). Post-hoc analysis, which is performed with a Bonferroni adjustment, showed that the CSA values in patients with full thickness tear was (35.68, 95% CI:34.81-36.55) higher than patients with partial thickness tear (33.91, 95% CI: 33.16-34.67, adj. p=.012) and healthy controls (31.31, 95% CI: 30.48-32.14, adj. p<.001). In addition, the CSA values were significantly higher in patients with partial thickness tear compared to healthy controls (adj. p<.001) (**Graphic 1-D**).

The diagnostic performance of AI, CSA, AHM, and LAA to differentiate RCT patients from healthy controls were given in **Table 4** and **Graphic 2**. The cut-off values for discriminating the RCT and healthy controls were as follows: AI>0.61, AHM≤7.1, LAA≤72 and CSA>32.7. The area under the curve (AUC) was 0.885 (95% CI, 0.799-0.944) with 81% of sensitivity, 83% of specificity, 90% of PPV, and 69% of NPV for the AI, 0.894 (95% CI, 0.809-0.950) with 74% of sensitivity, 93% of specificity, 96% of PPV, and 64% of NPV for the AHM, 0.857 (95% CI, 0.765-0.923) with 71% of sensitivity, 90% of specificity, 93% of PPV, and 61% of NPV for the LAA, and 0.888 (95% CI, 0.802-0.945) with 85% of sensitivity, 80% of specificity, 89% of PPV, and 72% of NPV for the CSA.

Table 3. The comparisons of demographical characteristics and acromial measurements in healthy controls and RCT patient groups

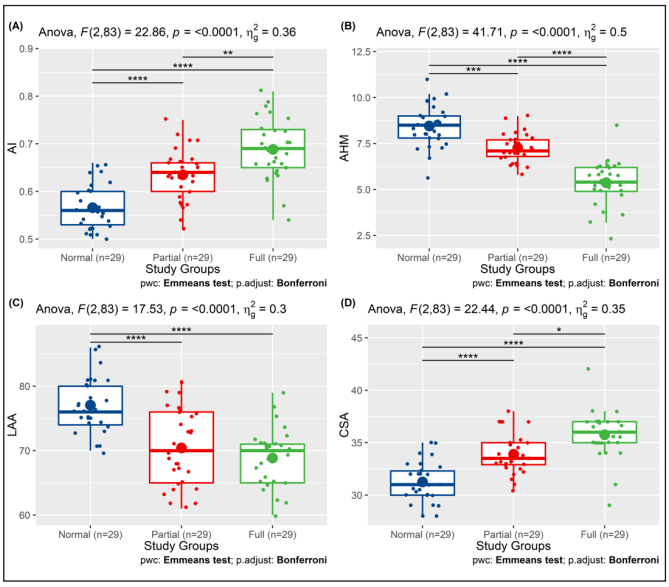
		RCT patients		
	Heathy controls (n=29)	**Partial thickness (n=29)**	**Full thickness (n=29)**	**p value**
Age (years)	44.14±13.11[a]	50.79±10.45[a]	64.38±9.68[b]	<.001[1]
Gender (F/M)	12/17	13/16	18/11	.240[2]
Acromial shapes				.031[2]
Type I (flat)	9 (31)	10 (34.5)	6 (20.7)	
Type II (curved)	19 (65.5)	15 (51.7)	13 (44.8)	
Type III (hooked)	1 (3.4)[a]	4 (13.8)	10 (34.5)[b]	
AI				
Mean±SD	0.57±0.05[a]	0.63±0.05[b]	0.69±0.06[c]	<.001[1]
Est. mean (95% CI)	0.57 (0.54-0.59)[a]	0.63 (0.61-0.66)[b]	0.69 (0.66-0.71)[c]	<.001[4]
AHM (mm)				
Mean±SD	8.45±1.13[a]	7.26±0.78[b]	5.37±1.19[c]	<.001[1]
Est. mean (95% CI)	8.49 (8.06-8.92)[a]	7.27 (6.88-7.66)[b]	5.33 (4.87-5.78)[c]	<.001[4]
LAA°				
Mean±SD	77.07±4.20[a]	70.41±5.88[b]	68.86±4.50[b]	<.001[3]
Est. mean (95% CI)	77.24 (75.22-79.25)[a]	70.46 (68.62-72.30[b]	68.65 (66.53-70.77)[b]	<.001[4]
CSA°				
Mean±SD	31.25±1.88[a]	33.90±1.92[b]	35.76±2.25[c]	<.001[1]
Est. mean (95% CI)	31.31 (30.48-32.14)[a]	33.91 (33.16-34.67)[b]	35.68 (34.81-36.55)[c]	<.001[4]

Data were presented as mean±standard deviation (range) and estimated mean with 95% confidence intervals, and also were described as count (n) and percentage (%). Bold values denote that statistically significant difference between the groups. Different superscript small letters in each row shows statistically significant difference between groups after pairwise comparison tests. 1 One-Way ANOVA 2 Pearson chi-square 3 Welch's F test 4 ANCOVA Abbreviations: SD; standard deviations, AI; acromial index, AHM;, LAA; lateral acromion angle, CSA; critical shoulder angle, 95% CI; 95% confidence interval, RCT; rotator cuff tear, Est. mean; estimated mean, F; female, M; male

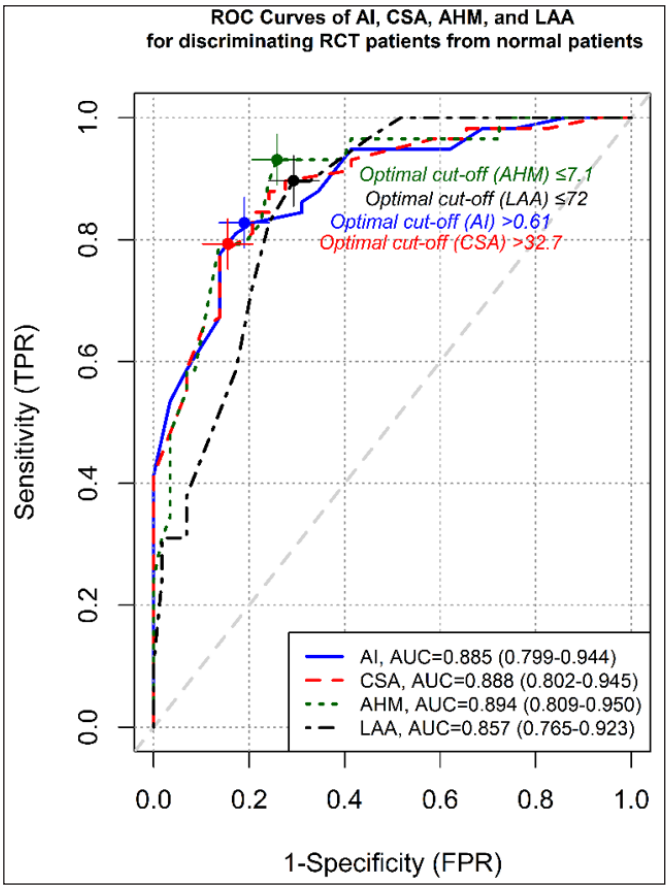
Table 4. Diagnostic performance of the AI, AHM, LAA, and CSA in diagnosis of RCT

	ROC curve analysis				**Statistical diagnostic measures, (95% CI)**			
	AUC (95% CI)	**p-value**	**Cut-off**	**J**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
AI	0.885 (0.799-0.944)	<.001	>0.61	0.638	81 (69-90)	83 (64-94)	90 (81-96)	69 (56-79)
AHM	0.894 (0.809-0.950)	<.001	≤7.1	0.672	74 (61-85)	93 (77-99)	96 (85-99)	64 (54-74)
LAA	0.857 (0.765-0.923)	<.001	≤72	0.603	71 (57-82)	90 (73-98)	93 (82-98)	61 (50-70)
CSA	0.888 (0.802-0.945)	<.001	>32.7	0.638	85 (73-93)	80 (60-92)	89 (80-94)	72 (58-83)

Abbreviations: AI; acromial index, AHM;, LAA; lateral acromion angle, CSA; critical shoulder angle, AUC (95% CI); area under the curve (95% confidence interval), ROC; receiver operating characteristic curve, J; Youden J index, PPV; positive predictive value, NPV; negative predictive value



Graphic 1. Box-plots of AI, AHM, LAA, and CSA in patients with RCT and healthy controls. (A) the level of AI in study groups



Graphic 2. Receiver operating characteristics (ROC) curve analysis of the diagnostic performance of AI, CSA, AHM, and LAA

DISCUSSION

Anatomical variations of the acromion may be a predisposing factor for RCT by causing changes in the resultant of vector forces. The extent to which the anatomical factors play a role in RCT is not fully known (1). MRI provide us to evaluated the status of the rotator cuff and the shape of the acromion. Hamid et al. reported that classification for acromial morphology is unreliable and operator dependent. AI is not significantly associated with RCTs but increased AI is related to shoulder pain (11). In another study, they argued that the reason why the relationship between acromion shape and RCT is not fully established in the literature is that it is affected too much by interobserver variability during the evaluation of acromine classification. And they argued from the same study that the shape of acromine should be considered 3-dimensional (12). Thus, it was revealed that bone spurs at the anterior and lateral edges of the acromion lead to RCT (12). In our study we found that there was a significantly association between study groups and Type III (hooked) of acromial shapes, Type III proportion of acromial shapes was significantly higher in RCT patients than in healthy controls. Hirano et al. reported that the size of rotator cuff tears in type III acromions was significantly larger (13), which was consistent with our results. In several studies, they found that the type III acromion was the most frequently seen in patients with RCT (12,14). Nicholson et al. (15) revealed that while one of the most important causes of RCTs is increased age, it is seen that the acromial shape is an age-independent anatomical structure. They also argued that changes such as bone spur within the acromion develop over time as a result of RCT rather than the cause of RCT. Unlike many studies, Almokhtar et al. (16) found that a flat acromion is the most frequently seen type among all patients with RCTs.

AI provides the evaluation of the morphology of the acromion and compared the lateral extension of the acromion. Many studies suggested that AI was significantly higher in patients with RCT and AI as a reliable predictor of a rotator cuff tear (17). Kum et al. (17) suggested that AI was an effective predictive factor for RCT in a Korean population while other researchers suggested that AI might be changed according to race. Our study showed that the AI values in patients with full thickness tear was higher than patients with partial thickness tear. In addition, the AI values were significantly higher in patients with partial thickness tear compared to healthy controls. In another study, reported that AI is a useful measurement in distinguishing a partial-thickness articular-side tear and a large-to-massive rotator cuff tear pre-operatively. But the AI can not estimate the tear size in full-thickness tear patients (18).

Another anatomical variation that leads to large and massive RCT is reduced AHD and increased CSA. The AHD <6 mm and CSA >35° is usefulin predicting RCT. However, the AHD <6 mm, an acquired pathology as a result of RCT. With weakening of the rotator cuff muscle, the humeral head subluxes superiorly, resulting in narrowing of the AHD (19). The joint shear and joint

compression forces are in the balanced within the joint. The enlarged CSA is deteriorate the compensatory effect of the supraspinatus tendon (20). In our study, AHD values in patients with full thickness tear was lower than patients with partial thickness tear and healthy controls. In addition, the AHM values were significantly lower in patients with partial thickness tear compared to healthy controls. While it is stated in the literature that AHD <7 mm may cause poor outcome after RCT repair, it has been stated that repair of the infraspinatus rupture is most likely not possible when AHD <4 mm. We found that the cut-off values for discriminating the RCT and healthy controls were as follows: AI >0.61, AHM ≤7.1, LAA ≤72 and CSA >32.7. Narrowed AHD lead to the fatty degeneration of the infraspinatus (21). Saupe et al. (22) suggested that AHD measurement is not affected from the inter and itraobservervariability, and they also suggested that AHD is an effective measurement in predicting RCT on both radiograph and MRI. However, in another study emphasize that there is a significant difference in AHD measurements between radiograph and MRI and should not be used interchangeably (23). Many of the studies suggested AHD is useful in predicting RCT, but literature still remain controversary on which imaging modality more reliable (21-23).

CSA is one of the important biomechanical measurement which is highly affected the shoulder abduction, glenoid compression, and joint shear forces. It is a delicate measurement and may interfere with different positioning of the scapula and acromion (24). Measured CSA on radiograph can estimate the necessity of preoperative MRI in the evaluation of the rotator cuff. As the CSA increased, number of tendons torn and anchors used for repair is increased (24). The CSA >35° may alter deltoid vectors and cause increased superior shear forces on the rotator cuff muscles. Increased shear forces may predispose for the RCT. The CSA <30° is related to osteo arthritis by the mechanism of the increased compressive forces across the glenohumeral joint (25). In our study CSA values in patients with full thickness tear was higher than patients with partial thickness tear and healthy controls. LAA reflect the inclination of the inferior surface of the acromion proportionate to the glenoid plane.of scapula (1). LAA is helpful measurement in understanding the shape of the acromion and its association with RCT. Decreased LAA might be related to RCT. Recently in a study found that acromial thickness, AHD, AI and LAA, were significantly different in patients with type-III acromial shape and RCT (1). Guishan et al. (25) defined these morphological variations of the acromion as "congenital and osteal etiological Factors" for RCT. We also found that LAA values in patients with full thickness tear and in patients with partial thickness tear were lower than healthy controls.

Our study has some limitations. First, the mean age of the patients with RCT and the control group were different from each other, and the different results that would arise from this were not taken into consideration. Another limitation of our study is that it was not conducted prospectively and only a small number of patients were examined.

CONCLUSION

Type III (hooked) of acromial shapes, higher AI and CSA values, lower AHD and LAA values are more frequently seen in RCT patients, in our study. Thus, we revealed anatomical malformations that predispose to RCT concerning the shoulder joint.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tokat Gaziosmanpaşa University Hospital, Non-invasive Clinical Researches Ethics Committee (Date: 20.01.2022, Decision No: 2022/02).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Original Article

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Investigation of the CHOKAI score used to predict ureteral stones in patients presenting to the emergency department with renal colic

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ABSTRACT

Aim: To investigate the adequacy of the CHOKAI score in the prediction of ureteral stones in patients presenting to the emergency department with renal colic.

Material and Method: The data of all patients aged over 18 years, who presented to the emergency department with the complaint of renal colic and were diagnosed with ureteral stones during the study period were retrospectively analyzed using the electronic-based hospital information system. The area under the receiver operating characteristic curve and the area under the curve were used to assess for each patient to determine the cut-off value of the CHOKAI score in the prediction of ureteral stones.

Results: The study was completed with 219 patients, of whom 146 were men, and the mean age was 39.4±16.1 years. When the cut-off value of the CHOKAI score was >6, its sensitivity was 84.1%, specificity was 96.7%, positive likelihood ratio was 25.2, negative likelihood ratio was 0.2, positive predictive value was 99.4%, and negative predictive value was 49.2%.

Conclusion: In this study, it was concluded that the CHOKAI score had high accuracy in terms of diagnostic power in detecting ureteral stones. However, further studies are needed to demonstrate the broader applicability of the score.

Keywords: CHOKAI score, diagnosis, emergency medicine, ureter

INTRODUCTION

While the prevalence of urolithiasis was one in 20 people in 1994, it increased to one in 14 people in 2010. The general prevalence of urinary system stone disease in Turkey has been reported as 14.8%. In Turkey, the incidence of this disease in men is three times higher compared to women. There is a recurrence rate of approximately 50% within five years of stone formation. In developed countries, it is seen at a rate of 1-5%. The average lifetime prevalence of symptomatic urolithiasis is 13% in men and 7% in women. In recent years, the prevalence of stone disease has been increasing due to dietary habits and dietary changes in Western societies. Twenty-five percent of patients with urolithiasis have a family history of the disease. The incidence of urolithiasis is also higher in Caucasians and in cold climates. In Turkey, the disease is more commonly seen in the Mediterranean, Black Sea and Southeastern Anatolia regions (1-5).

Scoring systems are widely used in emergency practice. Using scoring systems, early diagnosis and rapid intervention are possible in many critical diseases, such as acute abdomen and gastrointestinal bleeding (6-9). Scoring systems have also been developed for early diagnosis in patients with renal colic, which is one of the most common reasons for presentation to the emergency department. The CHOKAI score was developed by Fukuhara et al. in 2017 to predict the presence of ureteral stones. This score consists of seven parameters including nausea or vomiting, hydronephrosis, hematuria, stone history, gender, age, and pain duration, each scored from 0 to 4, and the total score varies between 0 and 13. While 0-5 points represent low risk for ureteral stones, 6-13 points indicate high risk (10).

The aim of this study was to investigate the adequacy of the CHOKAI score in the prediction of ureteral stones in patients presenting to the emergency department with renal colic.

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MATERIAL AND METHOD

This single-center, retrospective, and observational study was carried out in the emergency department of a tertiary teaching hospital between July 1, 2021, and January 1, 2022. The research was approved by the Kartal Dr. Lütfi Kırdar City Hospical Clinical Researches Ethics Committee (Date: 30.03.2022, Decision No: 2022/514/222/20). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

During the period determined for the study, all the patients aged over 18 years, who presented to the emergency department with the complaint of renal colic and were diagnosed with ureteral stones using computed tomography (CT) constituted the population of the study. Patients with abnormal vital signs (high fever and hypotension), active cancer, and leukocytes in urinalysis and C-reactive protein concentration of ≥6 mg/L, those who did not undergo ultrasound (USG)-CT or complete urinalysis, and those whose CHOKAI score could not be calculated were excluded. After recording the data of each patient included in the study, their CHOKAI scores were separately calculated. The accuracy of the CHOKAI score was determined with reference to CT, which is the gold standard in the diagnosis of ureteral stones.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 and MedCalc Statistical Software version 19.0.6. Descriptive criteria were presented as median, minimum-maximum, and percentage values. The conformity of the data to the normal distribution was checked with the Kolmogorov-Smirnov test. The receiver operating characteristic (ROC) analysis was used to determine the cut-off value of the CHOKAI score in detecting ureteral stones. The ROC analysis was performed using the DeLong method. The area under the curve (AUC), sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), positive predictive value (PPV), negative predictive value (NPV), and Youden's J index (YJI) were calculated to evaluate the performance of the CHOKAI score in predicting ureteral stones. The statistical significance level was taken as $p < 0.05$.

RESULTS

This study was completed with a total of 219 patients with a mean age of 39.4±16.1 years. Seventy-three patients were female and 146 were male. Various characteristics of the individuals participating in the study are presented in **Table 1**.

Table 1. General characteristics of the study population

	Min	Max	Mean Number	SDPercentage
Age	16.0	92.0	39.4	16.1
Gender				
Female			73	33.3
Male			146	66.6
Nausea			77	35.2
Vomiting			20	9.1
Onset of pain				
<6 hours			86	39.3
6-24 hours			64	29.2
>24 hours			69	31.5
Stone			189	86.3
Stone side				
Right			97	47.3
Left			108	52.7
Stone localization				
Proximal			32	16.9
Middle			21	11.1
Distal			136	72.0
Hematuria			139	63.5
Stone history			88	40.2
Hydronephrosis			186	84.9
Stone size	3.0	20.0	6.2	2.5
CHOKAI score	1.0	13.0	8.4	2.9

SD, standard deviation

As a result of the ROC analysis of the CHOAKI score in the prediction of urinary stones, the AUC value was calculated as 0.959 (95% confidence interval: 0.923-0.981), and YJI was 0.808. According to the statistical analysis, the CHOAKI score was statistically significant in predicting the presence of stones. When the cut-off value of the CHOKAI score was taken as >6, it had a sensitivity of 84.1%, specificity of 96.7%, LR+ value of 25.2, LR- value of 0.2, PPV of 99.4%, and NPV of 49.2% (**Table 2**, **Figure 1**).

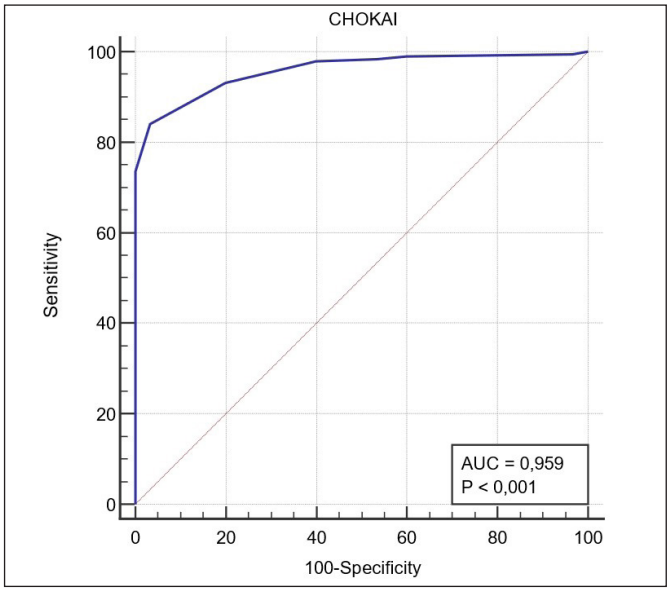

Figure 1. Receiver operating characteristic curve of the CHOKAI score (AUC, area under the curve)

Table 2. Results of the ROC analysis of the CHOKAI score for the diagnosis of ureteral stones

	AUC	Cut-off	Sensitivity	Specificity	LR+	LR-	PPV	NPV	Youden's index
CHOKAI score	0.959 (0.923-0.981)	>6	84.1(77.5-88.3)	96.7(82.7-99.4)	25.2	0.2	99.4	49.2	0.808

AUC, area under the curve; LR+, positive likelihood ratio; LR-, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value

DISCUSSION

Renal colic is one of the common reasons for presentation to the emergency department. Generally, these patients have very severe pain complaints and require early diagnosis and pain palliation. The current study aimed to validate the CHOKAI score in the prediction of ureteral stones in patients presentation to the emergency department with renal colic. It was concluded that the CHOKAI score had high diagnostic accuracy with an AUC value of 0.95.

In 2017, Fukuhara et al. (10) defined the CHOKAI score in a study conducted with 96 patients of Japanese origin, and reported the AUC value of this score to be 0.97 at a cut-off value of 6. The authors reported the LR+ and LR- values of the score as 15.49 and 0.094, respectively. It is generally accepted that LR+ >10 and LR- <0.1 provide strong evidence to rule out or not exclude a diagnosis (11). In the current study, we determined that at a cut-off value of >6, the CHOKAI score had an LR+ value of 25.2 and LR- value of 0.2.

When used together with other parameters in the diagnosis of urolithiasis, in addition to its diagnostic advantage, ultrasonography (USG) has been reported to provide a significant reduction in the cumulative radiation exposure caused by repeated CT imaging and the duration of stay in the emergency department (12,13). In cases where USG will not be used as a diagnostic imaging method, in order to reduce radiation exposure, the American Association of Urology (AUA) and the European Association of Urology (EAU) recommend using low-dose CT, which has almost the same sensitivity and specificity as non-contrast CT, in the detection of ureteral stones (14). The CHOKAI score also involves checking whether there is hydronephrosis on USG for the prediction of ureteral stones, and the presence of hydronephrosis is scored 4 points.

When studies on the CHOKAI score in the literature are examined, it is observed that this score was mostly compared with the STONE scoring system. The STONE score, which was defined by Moore et al. (15) consists of five variables (gender, duration of pain, race, nausea-vomiting, and hematuria), each scored between 0 and 13. In calculating the probability of kidney stones, 0-5, 6-9, and 10-13 points represent low, moderate and high probability, respectively. In a multicenter prospective validation of the CHOKAI score, it was found to have an AUC value of 0.95, sensitivity of 0.93, specificity of 0.90, LR+ of 9.3, and LR- of 0.079. In the same study, it was reported that at an AUC value of 0.88, the STONE score had a sensitivity of 0.68, specificity of 0.90, LR+ of 6.8, and LR- of 0.36 (16). In another study comparing the two scores, the sensitivity of the CHOKAI score in detecting ureteral stones was 83% and its specificity was 94.87%, while the cut-off value was calculated to be >6 (AUC=0.945). The STONE score was found to have a sensitivity of 79.50% and specificity of 84.62% in detecting ureteral stones, and its cut-off value was found to be >6 (AUC=0.860) (17). In a study conducted in Turkey, the external validation of the STONE and CHOKAI scores was performed, and the specificity and sensitivity values in the diagnosis of ureteral stones were determined to be 64.71% and 71.70%, respectively for the STONE score and 66.67 and 90.57, respectively for the CHOKAI score (18). The results of our study were similar to previous studies in the literature. When the cut-off value of the CHOKAI score was taken as >6 in determining the presence of stones, the sensitivity was calculated as 84.1%, specificity as 96.7%, LR+ as 25.2, LR- as 0.2, PPV as 99.4%, and NPV as 49.2.

Limitations

The main limitation of our study is its single-center design and retrospective design. Since CT is the gold standard in the diagnosis of ureteral stones and USG was used in the CHOKAI scoring system, we excluded patients who did not undergo CT and USG, which resulted in a smaller sample size.

CONCLUSION

In this study, it was determined that the CHOKAI score had high accuracy in terms of diagnostic power in detecting ureteral stones. However, further studies are needed to demonstrate the wider applicability of this scoring system.

ETHICAL DECLARATIONS

Ethics Committee Approval: The research was approved by the Kartal Dr. Lütfi Kırdar City Hospical Clinical Researches Ethics Committee. (Date: 30.03.2022, Decision No: 2022/514/222/20).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Measurement of 0 to 2 age normal eyeball volume by the use of multidetector computed tomography

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ABSTRACT

Aim: To diagnose microphthalmia or macrophthalmia, it is necessary to know the normal reference values of the eyeball volumes. However, we do not have a table of normal reference values to evaluate normal eyeball volume at 0-2 years of age. To compensate for this deficiency, we aimed to detect normal eyeball volumes in the age group of 0-2 years with multidetector computed tomography (MDCT).

Material and Method: A total of 90 patients who underwent MDCT with a prior diagnosis of head trauma but without traumatic pathology were included in the study. Patients were divided into age groups of 0-1 months, 2-6 months, 7-12 months, 13-24 months. The mean values of patients in each age group were examined with a 95% confidence interval. The correlation between bilateral eyeball volumes and age was calculated using the Pearson correlation test. The relationship between the age groups and the volume of the eyeball was studied using one-way ANOVA test. The relationship between eyeball volumes and sex was evaluated using the Mann Whitney U test.

Results: Mean eyeball volume was measured as 3.91±0.54 cm^3 for 0-1 months, 4.44 ±0.66 cm^3 for 2-6 months, 5.81 ±0.68 cm^3 for 7-12 months, and 5.83±1.09 cm^3 for 13-24 months. A strong positive correlation was observed between eyeball volume and age ($p < 0.001$). There was no statistically significant relationship between eyeball volume and sex ($p > 0.05$). No statistically significant difference was observed between right eyeball volume and left eyeball volume ($p> 0.05$).

Conclusions: The increase in eyeball volume is most rapid in the first year of life. In this study, the reference eyeball volumes were determined in order to properly assess this rapid increase.

Keywords: Eye, eye length, buphtalmos, microphtalmos

INTRODUCTION

The eye is a visual receptor organ that contains dense nerve endings in the posterior part and is filled with humor in the central part (1). Eyeball volume can change in many diseases. Trauma, benign or malignant neoplasms, glaucoma, myopia etc. Normal values are required to diagnose microphthalmos, buphthalmos or macrophthalmos and to consider appropriate treatment options. In addition, it is important to know the eyeball volume for craniofacial surgery (2-5).

In the literature, there are publications using ultrasonography, computed tomography or magnetic resonance for measurement of eyeball volume or other structures (6-12). There are volumetric eyeball measurements for various races, but the majority of them are for adults. There have been studies on the diameters of orbital structures in pediatric age groups (13-15). With the help of computer programs, it is possible to obtain volume measurements that are as close to the truth as possible in tomography images, reducing the margin of error. In comparison to a single diameter comparison, two-dimensional images produce more accurate results.

The aim of this study was to determine the normal value range of eyeball volume in the pediatric patient population aged 0-2 years, and to evaluate its relationship with gender and age in multi-detector computed tomography (MDCT) images.

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MATERIAL METHOD

The study was approved by the Gazi University Non-Interventional Studies Ethics Committee (Date: 29.1.2021, Decision Number:140). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The informed consent was waived due to the retrospective nature of the study and the assessment utilized anonymous research findings. The study included 107 cranial MDCT scans. All cases consisted of patients who were referred to the emergency radiology department due to traumatic reasons such as falling from a height and an in-vehicle traffic accident. Cases with fractures involving the orbital walls and images that were not clear, due to motion artifacts were excluded from the study. The final group consisted of 90 patients (47 males, 43 females). This study was conducted over a nine-month period from March 2021 to December 2021.

All of the MDCT studies were performed using a multidetector 192 row helical CT scanner (Somatom Force, Siemens Healthineers). The following parameters for scanning were applied: tube voltage 120 Kv, tube current 300 mA, beam collimation 1 mm×16, field of view 240 mm, gantry rotation time was 0.75 s. Thin-section CT data were reconstructed at a slice thickness of 1 mm with 0.8-mm intervals. Image matrix of 512 x 512. Intravenous contrast medium was not administered.

Volume of the eyeball were analyzed using a workstation Syngo.via (Siemens Healthineers) by only one radiologist (M.K.) with 15 years experienced in Head - Neck Imaging. The eyeball contour was manually evaluate by the examiner.

Via a closed polygon tool, the contours of the eyeball were defined by two dimensional segmentation. The volumes were measured by manually segmenting the region of interest in each CT slice using Syngo. via (Siemens Healthineers) postprocessing imaging software after drawing the outlines of the eyeball in all slices. Eventually, the volume was measured by the software and three-dimensional reconstruction of the organ was produced (**Figure 1A,1B,1C**). The eyeball volume was measured as cm^3.

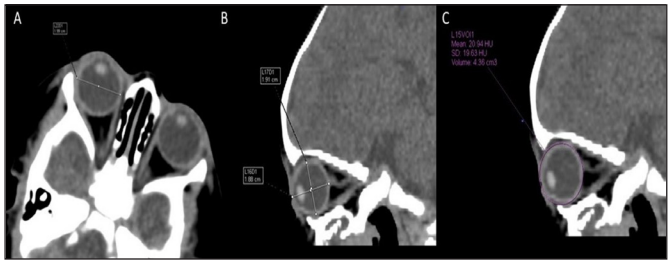

Figure 1A,B,C. Orbital diameter and volume measurements

The bilateral eyeball was re-evaluated 1 week after the initial evaluation to assess the reproducibility of the same observer for 20 patients.

Statistical Analysis

Statistical analyzes were performed via the SPSS v.22 package program (IBM SPSS Statistics, Chicago, IL, USA). Participant age and gender were recorded. Descriptive statistics are given with mean, standard deviation, median, minimum, maximum values for categorical variables. Kolmogorov–Smirnov test was used as a test of normality. Patients were divided into age groups of 0-1 months, 2-6 months, 7-12 months, 13-24 months. Minimum, maximum, mean and standard deviations have been calculated for all age groups and all case groups. The relationship between eyeball volume and gender, and the relationship between right eyeball volume and left eyeball volume were evaluated using the Mann Whitney U test. In addition, one way ANOVA tests were used to assess the relationship between age groups and eyeball volumes. The correlation between the bilateral eyeball volumes and age was calculated with the Pearson test. P-value of less than 0.05 was considered statistically significant. The intraclass correlation coefficient (ICC) test was used to analyze intraobserver reliability for repeated measurements with a 95% confidence interval. ICC was interpreted as follows: below 0.50: poor, between 0.50 and 0.75: moderate between 0.75 and 0.90: good, above 0.90: excellent.

RESULTS

A total of 107 participants were included in the study. The clinical characteristic of the participants are presented in **Table 1**. In 17 patients, the measurement could not be obtained due to the technical reasons, such as the motion artifact.

Table 1. The clinical characteristic of the participants

Clinical characteristic	Total (n=90)
Age (month)	7.27±6.28 (1-24)
Gender (M/F)	47/43

-Data are expressed as n (number) or the mean± standard deviation (range). F: Female M: Male

The ICC values for all diameter measurements and volumes were>0.80-0.90, indicating good and excellent agreement.

There was no statistically significant relationship between eyeball diameters,volumes and sex (p>0.05). No significant difference was observed between the right and left eyeballs in terms of diameters and volumes (P>0.05).

Pearson correlation analysis was performed for comparison of eyeball diameters,volumes and age. It was observed that age had a strong positive correlation with eyeball diameters and eyeball volumes (p<0.001) (**Table 2**).

Because the Levene test did not show a significant difference between all age groups, the datashowed a homogeneous distribution (P>0.05). Mean eyeball measurements with upper and lower 95% confidence intervals for right and left eyeball according to age groups are listed in **Table 3**. In REAPD, although a significant relationship was found between the 1st and 2nd age groups, no statistically significant relationship was found in other measurements. In addition, no significant difference was found in measurements of eyeball diameter and volume in the 3rd and 4th groups in all age groups (p> 0.05).

Table 2. Correlation Test Between month and eyeball diameters and volumes

Parameters	REAPD	RETRD	RECCD	REV	LEAPD	LETRD	LECCD	LEV
r	.729**	.740**	.750**	.783**	.759**	.731**	.757**	.792**
Pa	.000	.000	.000	.000	.000	.000	.000	.000

[a]Pearson correlation test. Statistically significant at P<.05. REAPD: Right eyeball anterior posterior diameter. LEAPD: Left eyeball anterior posterior diameter. RETRD: Right eyeball transverse diameter. LETRD: Left eyeball transverse diameter. RECCD: Right eyeball craniocaudal diameter. LECCD: Left eyeball craniocaudal diameter. REV: Right eyeball volume. LEV: Left eyeball volume

Table 3. Eyeball diameters and volumes according to age groups (Months)

		N	Mean	Std. Deviation	95% Confidence Interval for Mean		F	Pa	Difference
					Lower Bound	Upper Bound			
REAPD	1 (0 to 1)	13	18.92	.862	18.40	19.44	20.048	0.029	1 to 2
	2 (2 to 6)	35	20.06	1.103	19.68	20.44		.000	1 to 3
	3 (7 to 12)	18	21.58	.845	21.16	22.00		.000	1 to 4
	4 (13 to 24)	24	21.61	1.672	20.89	22.33		.000	2 to 3
	Total	90	20.60	1.551	20.27	20.93		.000 1.000	2 to 4 3 to 4
LEAPD	1 (0 to 1)	13	18.77	.832	18.27	19.27	30.460	0.076	1 to 2
	2 (2 to 6)	35	19.74	1.067	19.38	20.11		.000	1 to 3
	3 (7 to 12)	18	21.89	.832	21.47	22.30		.000	1 to 4
	4 (13 to 24)	24	21.70	1.636	20.99	22.40		.000	2 to 3
	Total	90	20.54	1.666	20.19	20.89		.000 1.000	2 to 4 3 to 4
RETRD	1 (0 to 1)	13	19.85	.899	19.30	20.39	23.400	.913	1 to 2
	2 (2 to 6)	35	20.43	1.139	20.04	20.82		.000	1 to 3
	3 (7 to 12)	18	22.61	.916	22.16	23.07		.000	1 to 4
	4 (13 to 24)	24	22.30	1.690	21.57	23.04		.000	2 to 3
	Total	90	21.27	1.648	20.92	21.62		.000 1.000	2 to 4 3 to 4
LETRD	1 (0 to 1)	13	19.69	.947	19.12	20.26	21.710	.181	1 to 2
	2 (2 to 6)	35	20.56	1.143	20.16	20.95		.000	1 to 3
	3 (7 to 12)	18	22.33	1.085	21.79	22.87		.000	1 to 4
	4 (13 to 24)	24	22.30	1.490	21.66	22.95		.000	2 to 3
	Total	90	21.24	1.578	20.91	21.57		.000 1.000	2 to 4 3 to 4
RECCD	1 (0 to 1)	13	19.85	1.345	19.03	20.66	23.928	.195	1 to 2
	2 (2 to 6)	35	20.76	1.114	20.37	21.14		.000	1 to 3
	3 (7 to 12)	18	22.72	1.074	22.19	23.26		.000	1 to 4
	4 (13 to 24)	24	22.78	1.622	22.08	23.48		.000	2 to 3
	Total	90	21.54	1.722	21.18	21.91		.000 1.000	2 to 4 3 to 4
LECCD	1 (0 to 1)	13	20.08	1.115	19.40	20.75	23.891	1.000	1 to 2
	2 (2 to 6)	35	20.59	1.154	20.19	20.98		.000	1 to 3
	3 (7 to 12)	18	22.56	1.042	22.04	23.07		.000	1 to 4
	4 (13 to 24)	24	22.78	1.594	22.09	23.47		.000	2 to 3
	Total	90	21.48	1.679	21.12	21.83		.000 1.000	2 to 4 3 to 4
REV	1 (0 to 1)	13	3.9231	.55701	3.5865	4.2597	27.216	.193	1 to 2
	2 (2 to 6)	35	4.4886	.67247	4.2576	4.7196		.000	1 to 3
	3 (7 to 12)	18	5.8278	.66049	5.4993	6.1562		.000	1 to 4
	4 (13 to 24)	24	5.8261	1.12501	5.3396	6.3126		.000	2 to 3
	Total	90	5.0225	1.10049	4.7907	5.2543		.000 1.000	2 to 4 3 to 4
LEV	1 (0 to 1)	13	3.9000	.53229	3.5783	4.2217	30.911	.285	1 to 2
	2 (2 to 6)	35	4.4057	.64941	4.1826	4.6288		.000	1 to 3
	3 (7 to 12)	18	5.7944	.70333	5.4447	6.1442		.000	1 to 4
	4 (13 to 24)	24	5.8391	1.06248	5.3797	6.2986		.000	2 to 3
	Total	90	4.9831	1.10064	4.7513	5.2150		.000 1.000	2 to 4 3 to 4

ANOVA=analysis of variance. a One-way ANOVA. Statistically significant at P < .05. REAPD: Right eyeball anterior posterior diameter, LEAPD: Left eyeball anterior posterior diameter, RETRD: Right eyeball transverse diameter, LETRD: Left eyeball transverse diameter, RECCD: Right eyeball craniocaudal diameter, LECCD: Left eyeball craniocaudal diameter, REV: Right eyeball volume, LEV: Left eyeball volume, *Unit of length: mm, *Unit of volume: cm^3

DISCUSSION

Understanding the normal value ranges for the eyeball volume is critical for diagnosis, treatment and, evaluation prior to surgical procedures (16-19). Although MDCT is more successful than other in vivo imaging modalities for assessing eyeball volume, it is not preferred for routine examination because it contains ionizing radiation (7). However, as demonstrated in our study, volume calculations can be performed retrospectively on MDCTs obtained for any reason (e.g., trauma).

In our study, mean eyeball volume was measured as 3.91±0.54 cm^3 for 0-1 months, 4.44 ±0.66 cm^3 for 2-6 months, 5.81 ±0.68 cm^3 for 7-12 months, and 5.83±1.09 cm^3 for 13-24 months. The volume of the eyeball increased with age.

In their study of 198 participants (83 females, 115 males) aged 5 to 74 years, Ozer et al. (16) found that right eyeball volume was 6.50±0.80 ml and left eyeball volume was 6.46±0.76 ml in the entire study group. In the same study, no statistically significant relationship was found between eyeball volumes and gender. Furthermore, in the same study, no significant relationship was found between age and eyeball volumes in the entire study group. Our study differs from the study of Özer et al. in terms of age group. In our study, pediatric age groups were defined as follows: 0-1 month, 2-6 months, 7-12 months, 13-24 months. Eyeball volume increased with age in both boys and girls. In addition, a positive correlation between age and eyeball volume was found in the entire study group.

In their study of 200 participants (122 males, 78 females) aged 3 to 84 years, Igbinedion et al. (7) reported both eyeball volumes as 5282.23 mm^3±1755.13 mm^3 (mean±2SD) The right eyeball volume was 5264.26 mm^3 ±1781.12 mm^3, and the left eyeball volume was 5300.20 mm^3 ±1771.57 mm^3. In the same study, they found a positive correlation between the ages of the patients and their eyeball volumes. Chau et al. (20) found no significant relationship between gender and the volume of both eyeballs in a magnetic resonance study of 33 adult patients with varying degrees of ametropia. In this study, the mean eyeball volume was reported as 6.70 cm^3 (5.11-8.83). Hahn et al. (6) found a rapid rise in eyeball volume from birth to two years of age, followed by a relative increase until the age of thirty in their CT-based investigation with 100 participants. In our study, we found that the increase in eye volume increased rapidly from birth to the first year of life, but there was no significant increase in eye volume between the ages of 1 and 2 years.

Our study had two major limitations. First of all,our study includes only a limited number of patients and is single centered. Future multi-center studies with higher patient numbers will provide more accurate information. Secondly, ethnicity may alter the normal values obtained, our study reflects only the normal values in the Turkish population. We think that our study will serve as a modal for larger series and multicenter studies conducted in other ethnic groups in the pediatric age group.

CONCLUSION

It is vital to understand the variations in eyeball volumes with age in order to diagnose macrophthalmos or microphthalmos. To the best of our knowledge, our study is the most comprehensive MDCT study showing normal eyeball volumes in the age group 0-2 years.

ETHICAL DECLARATIONS

Ethical Committee Approval: The study was approved by the Gazi University Non-Interventional Studies Ethics Committee (Date: 29.1.2021, Decision Number:140).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of interest: The authors declare that they have no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Original Article

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Mediastinal lymphnode positivity clinical scoring system for lung adenocarsinoma-mediastinal lymph node evaluation and staging

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ABSTRACT

Aim: The study-cohort aims to assess PET-CT's correlation with adenocarcinomas' subtypes and propose a scoring system for mediastinal lymph nodes staging.

Material and Method: The patient cohort is a multicenter, retrospective analysis of 268 patient that underwent surgery for NSCLC adenocarcinoma. Preoperative PET-CT results for mediastinal lymph node staging was pathologically confirmed on tissue specimens obtained at anatomical resection. Statistical evaluation of PET CT, radiological and pathological outcomes were performed on all subgroups.

Results: The low FDG affinity in the lepidic pattern was statistically significant in the study ($p < 0.001$). Among all cases, 65 had an increase in the disease stage to 3B. According to the multivariate logistic regression analysis, Stage 3 disease (OR=2.7), SUV max ≥6.85 (OR=2.1), lepidic type disease (OR=2.6), and tumor size ≥31 mm (OR=2.2) were found to be independently associated with post-op stage 3B disease. Based on their respective odds ratios, points were assigned to each item ranging from 2 to 3. AUC of the scoring system to diagnose post-op stage 3B disease was 0.753 (0.686-0.819), $p<0.001$. The optimal threshold was ≥4 points; this yielded a sensitivity of 80%, a specificity of 64%, a positive likelihood ratio of 2.2, and a diagnostic odds ratio of 7 (95% CI=3.5-13.6).

Conclusion: The lepidic subtype of adenocarcinomas could have a lower FDG affinity. The MLP scoring system based on SUVmax, tumor size, pathological subtype and cancer stage could help the clinicians to evaluate the mediastinal staging in NSCLC Adenocarcinomas accurately.

Keywords: Lung cancer, adenocarcinomas, mediastinal staging, pozitron emission tomography

INTRODUCTION

The most critical step in the surgical treatment of lung cancers is staging. Avoidance of unnecessary surgery depends on it. Furthermore, proper staging leads to improved preoperative planning. In the past, staging necessitated a combination of multiple invasive and non-invasive procedures. Nowadays,the positron emission tomography scan (PET-CT) has become the clinical standard (1). Mediastinal lymph node assessment is probably the most crucial part of the work-up. Computed tomography (CT) has both low sensitivity and specificity in the evaluation of lymph node metastasis (2). For lymph nodes under 1 centimeter, its value is even further reduced for CT (3).

One of the biggest challenges in staging non-small cell lung cancers (NSCLC) with PET-CT is the evaluation of adenocarcinomas and stage 3B cases that have not been detected preoperatively. Lung adenocarcinomas can show heterogeneous assessments in PET-CT Some parenchymal lesions that do not show malignant

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involvement in PET CT may actually be malignant lesions. (4,5). For this reason, invasive staging may be required for patients with preoperatively diagnosed adenocarcinoma.

In 2011, the pathological classification of lung adenocarcinomas was changed (5). Each subtype has the potential to express different metabolic activity at the cellular level, which may lead to varying FDG uptakes. Therefore, in our study, we aimed to evaluate the lung adenocarcinoma subgroups in terms of FDG uptake for diagnosis and mediastinal staging.

MATERIAL AND METHOD

The study was approved by the Ethical Committees of the İzmir Katip Çelebi University (Date: 2021, Decision No: 371). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All patients gave written informed consent.

This study is a retrospective analysis of patients that underwent surgery for lung adenocarcinoma. The patients cohort was derived from medical records of Yıldırım Beyazıt University, Ankara Chest Diseases Hospital, and Katip Çelebi University. In our study, in addition to evaluating the FDG affinity of the NSCLC adenocarcimoma subgroups, we aimed to compare the PET-CT mediastinal staging of the subgroups in the new pathological classification with the pathology mediastinal staging that we accept as gold-standard. So, all patients had a preoperative PET-CT and postoperative pathological subtyping results of the adenocarcinoma. The Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma (2011) was used for pathological evaluation (6).

The age and gender of the patients, as well as primary tumor diameter (longest axis that determines the stage), anatomical location of the tumors and the type of surgeries performed were recorded. The stages of the cases were determined with preoperative PET-CT, and standart up-take value (SUV-max) values were added to analysis.The International Cancer Control Association and the American Cancer Committee eighth TNM classification were used for staging (7). Tumoral involvement of the mediastinal lymph nodes and their specific location were derived from pathological records. Lymph node evaluation was done according to Mountain and Dresler's classification (8).

Exclusion criterias were: diabetes or hyperglycemia defined as plasma glucose >140 mg/dL before PET-CT, preoperative chemotherapy and/or radiotherapy, interstitial lung disease, and rheumatological connective tissue disorder. Patients with insufficient data and/or inadequate surgery were excluded. Patients diagnosed with metastatic cancer at sixth-month control were also excluded. (**Figure 1**). Naturally, cases having mediastinal and parenchymal lymph node involvement were not included in this study. In our study, we applied the exclusion criteria to minimize the effect of N1 disease and anatomical location, which we believe that there might be other effective factors to evaluate the distribution of NSCLC adenocarcimoma subgroups in skipped stage 3B cases.

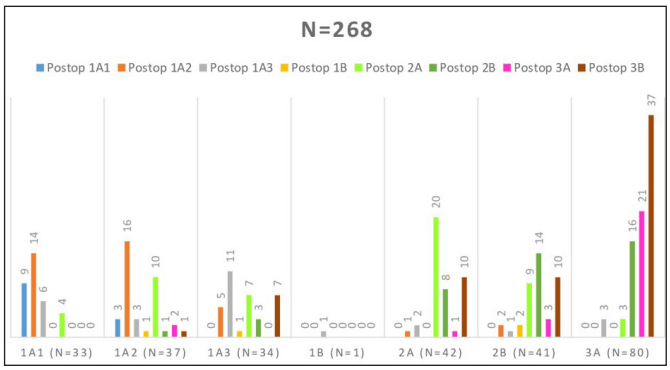

Figure 1. Distribution of PatientsAccordingtoPreoperativestage in thepostoperativeperiod

The surgical approach applied for all patients was anatomical resection and mediastinal lymph node dissection with posterolateral thoracotomy. Since there may not be standardization in mediastinal sampling in anatomical resections performed with thoracotomy and Video-Assisted Thoracoscopy Surgery (VATS), patients who underwent VATS were excluded from the study. Mediastinoscopy, mediastinostomy, and other invasive staging methods cases were not evaluated in this study since mediastinal lymph node stations were not fully exemplified.

All centers used the same device and protocol for PET-CT (ECAT model 951/31, Siemens/ CTI, Knoxville, Tenn.). FDG was synthesized according to the standard method by a high-performance liquid chromatography controlled synthesis modul (9). Patients were instructed to fast for six hours before the imaging and FDG (370 MBq) was administered intravenously. Data were reconstructed into coronal, sagittal, and transverse sections and a three-dimensional rotating projection. SUV-max 2.5 and above for mediastinal lymph nodes were accepted as the cut-off value for malignancy (10).

Statistical analyses were done using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as numbers and percentages for categorical variables and mean±standard deviation, median (IRQ) for continuous variables. Normal distribution for continuous variables

was assessed with visual (histograms and probability graphics) and analytic methods (Kolmogorov-Smirnov and Shapiro-Wilk's test). The data that does not fit the normal distribution, the Mann-Whitney U test was used for comparative analysis between the two independent groups and the independent sample t-test was used for the data that fit the normal distribution. Comparison analyses for categorical variables between separate groups were done by the chi-square test.

We used receiver operating charecteristic (ROC) analysis to determine cut-off SUV-max and tumor size values to predict Stage 3B disease. The area under the ROC curve (AUC) results were considered excellent for AUC values between 0.9-1, good for AUC values between 0.8-0.9, fair for AUC values between 0.7-0.8, poor for AUC values between 0.6-0.7 and failed for AUC values between 0.5-0.6 (11,12). Yound index was used to decide the cut-off point [the Youden Index is calculated as max (sensitivity + specificity – 1)] (13). Variables that may be independent risk factors for stage 3B in the post-op period were evaluated by multivariate logistic regression analysis. In order to predict the mediastinal involvement of the patients in the preop period (post op stage 3B), a scoring was developed by weighting with the odds ratio (OR) values of independent predictor factors (significant ones in multivariate analysis) (14).

RESULTS

Considering the basal demographic characteristics of the groups; there was no statistical difference between the groups in terms of age distribution (p=0.971), gender (p=0.097), surgical characteristics (p=0.825) and primary tumor size (p=0.493). While there was no significant difference in receiving radiotherapy after the postoperative staging (p=0.683), there was a significant difference was determined in the rate of receiving chemotherapy (p=0.001)(There was no significant difference who delivered radiotherapy after the postoperative staging (p=0.683) whereas there was a significant difference who received chemotherapy (p=0.001) (**Figure 1**)(**Table 1**).

When we examined the pathological subtypes according to SUV-max, the values were; 7.6 (4-12.3) for aciner, 7.8 (5.4-10.4) for micropapillary, 5.1 (2.2-8.1) for lepidic, 7 (3.8-10) for papillary and 9.4 (5.2-13.8) for solid subtypes. The low SUV-max in lepidic pattern was statistically significant (p <0.001).

Postoperative pathological stages were different than pre-operative PET-based staging, as expected. Number of patients that had an upgrade in disease stage to 3B were as follows: nill for 1A1 group, 1(2.7%) for 1A2, 7 (20.6%) for 1A3, nill for 1B, 10 (23.8%) for 2A, 10 (24.4%) for 2B and 37 (56.9%) for3A patients. In total 65 patients were up-staged.

The percentage of patients that was diagnosed postoperatively with stage 3B had no statistically difference according to adenocancer subtype (p=0.198) (**Figure 2**) (**Table 1**).

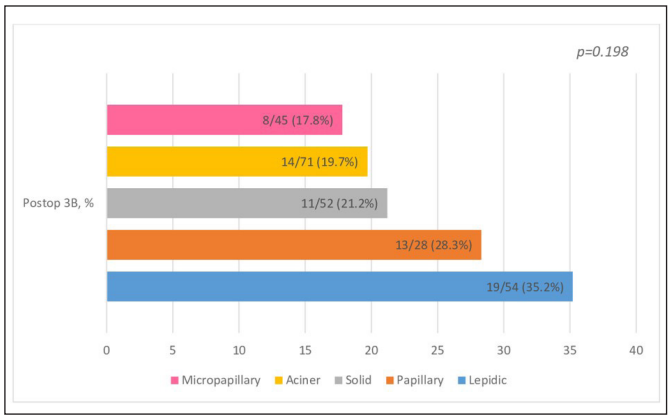

Figure 2. Transition rates to post-operative stage 3B according to histological types

Patients with lepidic subtype cancer tended to have malignant disease within the lesions with SUV-max lesser than 2.5 (p=0.010). The difference was not significant between patients with other subtypes. In addition, Stage 1 disease was marginally associated with lesions with SUV-max less than 2.5 (p=0.049).

The comparison results of the patient groups diagnosed at stage 3B and other stages in the postop period are presented in **Table 2**.The age and gender distributions of both groups were similar. SUV-max and tumor size measurements of the post-op Stage 3B group were significantly higher than the other groups (p <0.001 and p <0.001, respectively). It is noteworthy that 56.9% of pre-operative 3A cases had been diagnosed as 3B disease postoperatively. This is the highest percentage when all cases are considered (p<0.001). The cut-off values that could predict Stage 3B in the postop period for SUV-max and tumor size were examined by ROC analysis (**Table 3**, **Figure-3a**).

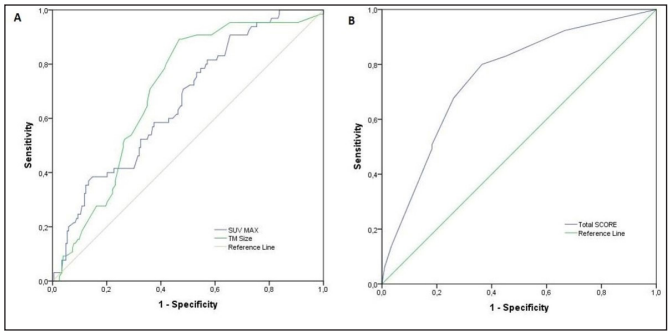

Figure 3.ROC Curves for Postoperative Stage of 3B. The Mediastinal lap Positive Score (MLP-Score) including the Four Markers (Pre-operative Stage, Suv-Max, Histopathology and Radiological Tumor Size) for Predicting the Diagnosis of Postoperative Stage 3B

Table 1. Baseline data of patients

Characteristics N=268	Aciner N=71	Micropapillary N=45	Lepidic N=54	Papillary N=46	Solid N=52	p
Age (year) Mean±sd	59.4±8.4	58.4±7.6	59.0±8.8	59.1±6.2	58.9±6.6	0.971*
Sex, n(%)						0.097#
Female	14 (19.7)	10 (22.2)	16 (29.6)	19 (41.3)	12 (23.1)	
Male	57 (80.3)	35 (77.8)	38 (70.4)	27 (58.7)	40 (76.9)	
KT, n(%)						0.001#
No	31 (43.7)	6 (13.3)	22 (40.7)	8 (17.4)	13 (25)	
Yes	40 (56.3)	39 (86.7)	32 (59.3)	38 (82.6)	39 (75)	
RT, n(%)						0.683#
No	57 (80.3)	38 (84.4)	45 (83.3)	34 (73.9)	40 (76.9)	
Yes	14 (19.7)	7 (15.6)	9 (16.7)	12 (26.1)	12 (23.1)	
Surgery Type, n (%)						0.825#
LLL	13 (18.3)	10 (22.2)	13 (24.1)	12 (26.1)	14 (26.9)	
LUL	20 (28.2)	7 (15.6)	12 (22.2)	7 (15.2)	8 (15.4)	
RBI Lobectomy	1 (1.4)	1 (2.2)	2 (3.7)	2 (4.3)	4 (7.7)	
RLL	14 (19.7)	15 (33.3)	13 (24.1)	12 (26.1)	9 (17.3)	
RUL	19 (26.8)	10 (22.2)	13 (24.1)	12 (26.1)	15 (28.8)	
Segmentectomy	4 (5.6)	2 (4.4)	1 (1.9)	1 (2.2)	2 (3.8)	
Primer Tm size Median (IRQ)	25 (15-45)	28 (15.5-43.5)	29.5 (17.8-40)	28 (14.8-48.5)	34 (21-48)	0.493**
Suv max Median (IRQ)	7.6 (4-12.3)	7.8 (5.4-10.4)	5.1 (2.2-8.1)	7 (3.8-10)	9.4 (5.2-13.8)	<0.001**
Suv max, n (%)						0.010#
<2.5	8 (11.3)	4 (8.9)	15 (27.8)	6 (13.0)	3 (5.8)	
≥2.5	63 (88.7)	41 (91.1)	39 (72.2)	40 (87.0)	49 (94.2)	
Postop Stage I						0.049#
Suv max, n (%)	n=22	n=13	n=18	n=12	n=16	
<2.5	8 (36.4)	2 (15.4)	10 (55.6)	5 (41.7)	2 (12.5)	
≥2.5	14 (63.6)	11 (84.6)	8 (44.4)	7 (58.3)	14 (87.5)	
Preop stage, n (%)						0.093#
1A1	5 (7)	7 (15.6)	8 (14.8)	9 (19.6)	4 (7.7)	
1A2	11 (15.5)	6 (13.3)	10 (18.5)	4 (8.7)	6 (11.5)	
1A3	19 (26.8)	3 (6.7)	3 (5.6)	2 (4.3)	7 (13.5)	
1B	0	0	0	0	1 (1.9)	
2A	8 (11.3)	8 (17.8)	8 (14.8)	8 (17.4)	10 (19.2)	
2B	9 (12.7)	10 (22.2)	8 (14.8)	8 (17.4)	6 (11.5)	
3A	19 (26.8)	11 (24.4)	17 (31.5)	15 (32.6)	18 (34.6)	
Postop stage, n (%)						0.258#
1A1	5 (7)	2 (4.4)	2 (3.7)	3 (6.5)	0	
1A2	9(12.7)	7 (15.6)	12 (22.2)	3 (6.5)	7 (13.5)	
1A3	7 (9.9)	2 (4.4)	3 (5.6)	6 (13)	9 (17.3)	
1B	1 (1.4)	2 (4.4)	1 (1.9)	0	0	
2A	14 (19.7)	10 (22.2)	9 (16.7)	10 (21.7)	10 (19.2)	
2B	11 (15.5)	12 (26.7)	4 (7.4)	6 (13)	9 (17.3)	
3A	10 (14.1)	2 (4.4)	4 (7.4)	5 (10.9)	6 (11.5)	
3B	14 (19.7)	8 (17.8)	19 (35.2)	13 (28.3)	11 (21.2)	

*Independent Student T-test, #Chi-Square Test, **Mann-Whitney U test

Variables that have independent risk factors for stage 3B in the postoperative period were evaluated by multivariate logistic regression analysis.Variables with p <0.2 as a result of univariate analyzes (**Table 2**) (stage in preop period, suv max, histological type and tm size) were included in the multivariate logistic regression model (**Table 4**).According to the results of the multivariate logistic regression analysis; stage 3 disease (OR=2.7), suv max ≥6.85 (OR=2.1), lepidic type disease (OR=2.6) and tumor size ≥31 mm (OR=2.2) predict postoperative stage 3B disease. According to the scoring system formed by weighting with the OR values from the multivariate logistic regression, the stage 3 disease in the preop period was assigned 3 points, suv max ≥6.85 got 2 points, lepidic type disease 3 points and tumors size ≥31mm as 2 points. The patients can get maximum 10 minimum from 0 points for this scoring. (**Table 4**).

In order to predict mediastinal LAP involvement (The Mediastinal Lymph Adeno-Pathy Positive Score), the optimal cut off obtained by ROC analysis (**Figure-3b**) was found to be 4. For this cut off, diagnostic Odds ratio (DOR), sensitivity and specificity were found 7 (95% CI=3.5-13.6), 80% and 64% respectively (**Table 3**).

Table 2. Evaluation according to the postoperative stage				
Characteristics N=268		**Stage 3A ve others N=203**	**Stage 3B N=65**	**p**
Age (year)				0.291*
Mean±sd		58.7±7.4	59.9±8.4	
Sex, n(%)				0.636#
Female		51 (25.1)	20 (30.8)	
Male		152 (74.9)	45 (69.2)	
Histopathology, (%)				0.198#
Aciner		57 (28.1)	14 (21.5)	
Micropapillary		37 (18.2)	8 (12.3)	
Lepidic		35 (17.2)	19 (29.3)	
Papillary		33 (16.3)	13 (20)	
Solid		41 (20.2)	11 (16.9)	
Suv max				
Median (IRQ)		6.8 (3.6-10)	9.2 (6.2-13.2)	<0.001**
Preop stage, (%)				<0.001#
Stage 1		97 (47.8)	8 (12.2)	
Stage 2		63 (31.0)	20 (30.8)	
Stage 3A		43 (21.2)	37 (56.9)	
Suv max, n (%)				0.009#
	<2.5	34 (16.7)	2 (3.1)	
	≥2.5	169 (83.3)	63 (96.9)	
Preop Stage 1A 1B 1C		n=97	n=8	0.264#
	<2.5	34 (35.1)	1 (12.5)	
	≥2.5	63 (64.9)	7 (87.5)	
Preop Stage 2A 2B		n=63	n=20	0.241#
	<2.5	0	1 (5)	
	≥2.5	63 (100)	19 (95)	
Preop Stage 3A		n=43	n=37	NA
	<2.5	0	0	
	≥2.5	43 (100)	37 (100)	
Primer Tm size	Median (IRQ)	25 (15-40)	39 (30-50)	<0.001**
Preop Stage 1A 1B 1		n=97	n=8	0.264#
	<2.5	34 (35.1)	1 (12.5)	
	≥2.5	63 (64.9)	7 (87.5)	
Preop Stage 2A 2B		n=63	n=20	0.241#
	<2.5	0	1 (5)	
	≥2.5	63 (100)	19 (95)	
Preop Stage 3A		n=43	n=37	NA
	<2.5	0	0	
	≥2.5	43 (100)	37 (100)	
Primer Tm size	Median (IRQ)	25 (15-40)	39 (30-50)	<0.001**
Preop Stage 1A 1B	Median (IRQ)	n=97 15 (12-20)	n=8 31 (21.3-38.8)	0.004**
Preop Stage 2A 2B	Median (IRQ)	n=63 30 (25-36)	n=20 31 (28-35)	0.514**
Preop Stage 3A	Median (IRQ)	n=43 59 (47-60)	n=37 46 (35.5-55)	0.122**

*Independent Student T test, #Chi-Square Test, **Mann-Whitney U test, NA: not available

Table 3. Statistical Parameters of Various Diagnostic Approaches for Post-operative 3B Stage									
	AUC (95% CI)	**P**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**+LHR**	**PPV (%)**	**NPV (%)**	**Max Youden index**
SUV MAX	0.662 (0.590-0.734)	<0.001	≥6.85	70.8	51.7	1.4	31.9	84.7	0.23
TM Size	0.700 (0.632-0.767)	<0.001	≥31	70.8	64.5	1.9	39	87.3	0.35
MLP SCORE (0-10)	0.753 (0.686-0.819)	<0.001	≥4	80	64	2.2	41.3	90.8	0.44

+LHR: Positive Likelihood Ratio, PPV: Positive Predictive Value, NPV: Negative Predictive Value, MLP score=The Mediastinal Lap Positive score

Table 4. Multivariate logistic regression analysis on risk factors for positive mediastinal lymphadenopathy and scoring system for the diagnosis of positive mediastinal lymphadenopathy

	Multivariate logistic regression analysis model*		
	Adjusted OR (95% CI)	P	MLP Score (points)
Preop Stage 3A (ref: stage I and II)	2.7 (1.3-5.6)	0.007	3
Histopathology (ref: aciner)			
Micropapillary	0.8 (0.3-2.2)	0.662	3
Lepidic	2.6 (1.1-6.4)	0.037	
Papillary	1.6 (0.6-4.1)	0.331	
Solid	0.8 (0.3-2.1)	0.662	
SUV Max, ≥6.85, (ref: <6.85)	2.1 (1.1-4.2)	0.046	2
TM size, ≥31 (ref: <31)	2.2 (1.1-4.6)	0.049	2

DISCUSSION

Developments in PET-CT technology have reduced diagnostic difficulties in lung cancer. PET-CT aids clinicians in diagnosis, staging and treatment follow-up Hochhegger et al. (1) and Groheux D et al. (15). Nevertheless, lung adenocarcinoma still has potential limitations that is partly because some adenocarcinomas may manifest as subsolid nodule with malignancies commonly have low levels of FDG afinity Erasmus JJ et al. (5). Evaluation of mediastinal lymph nodes and solitary pulmonary nodules is especially challenging Feng M et al. (16). This situation also limits the clinical benefits of PET-CT Song SH et al. (17). The heterogeneous nature of FDG uptake of lung adenocarcinomas requires detailed evaluation. In 2011, the pathological classification of lung adenocarcinomas was changed Erasmus JJ et al. (5). Therefore, evaluating the clinical correlation with PET-CT in newly defined subtypes of lung adenocarcinomas may provide valuable information in daily clinical routine practices.

In the study, SUV-max values were found to be significantly lower in some adenocarcinoma pathological subtypes, especially in lepidic pattern Xiaoliang S et al. (18) and Şanlı B. (19). This represents a different level of glucose metabolism at both the morphological and cellular levels. Early diagnosis and treatment of early stage tumors are crucial for correct intervention. Solitary pulmonary nodules due to adenocarcinomas have a higher potential for being misdiagnosis as benign Erasmus JJ et al. (5), Xiaoliang S et al. (18), Cruickshank A et al. (20). PET-CT is less reliable when the tumor size is less than 8-10 mm, however it yields much better results for tumors larger than 11 mm in size Tang K et al. (21). Also, our results indicate that false negative PET-CT results even for large tumors can be seen lepidic pattern. This finding implies low FDG affinity SPN's might be due to adenorcarcinoma with lepidic pattern. Some certain morphologic findings and anatomical location in chest CT's can be used in such cases. However, this approach is subjective and dependent on the clinician's experience Tang K et al. (21).

The most critical step in determining the correct treatment approach for lung cancers is the correct evaluation of the mediastinal lymph nodes. If N2 disease is not present, curative surgery may be possible Schmidt HM et al. Erasmus JJ et al. (3,5). PET-CT in evaluation of mediastinal lymph nodes have been extensively studided. In a review involving 45 study PET-CT's sensitivity and specificity estimates for the SUVmax ≥2.5 PET-CT positivity criterion were 81.3% (95% CI 70.2 to 88.9) and 79.4% (95% CI 70 to 86.5), respectively. The review has shown that accuracy of PET-CT is insufficient to allow management based on PET-CT alone. In fact, the risk of unforeseen nodal involvement in low-FDG avid tumors has been considered before in the 2014 Revised ESTS guidelines for preoperative mediastinal lymph node staging De-Leyn P et al. (22,23). However, this does not exactly represent the data we want to test or access.

In our study, we tried to reach data that could provide us with new information only in adenocarcinoma subgroups. Therefore, we aimed to minimize the impact of anatomic location and N1 disease in our results. It is a fact that we will have a very high reliability evaluation chance with the programs that can be created with the development of digital algorithms. Thus, it is to be able to see in mediastinal sampling whether the feature of adenocarcinoma subgroups can gain a place in the algorithm. Though this review is limited by heterogenity of the studies. Most of them involve all pathologic subtypes in non-squamous lung cancers Ambrasini et al. (24).

Conducting these studies separately in each pathological subtype such as isolated adenocarcinomas and evaluating the sub-pathological types of adenocarcinoma will provide much more valuable information. Our study is the only study conducted in this respect and our results contain very valuable data. Invasive mediastinal lymph node sampling is recommended for this patient group with insufficient evidence based data which results in unnecessary sampling with EBUS and EUS. Furthermore if sampling is not performed, the rate of unnecessary surgery and more invasive procedures increase Sivriköz CM et al. (25) and Thornblade LW et al. (26). In this dilemma, the desired results are not achieved in this patient group. For such reasons, much more objective quantitative approaches are needed in the evaluation of lung adenocarcinomas.

Patients with lepidic pattern cancers have more mediastinal lymph nodes which are negative preoperatively but postoperatively detected. However, the difference is not statistically significant. Perhaps statistically significant results can be reached in larger series. But the findings led us to steer to a scoring system, which includes tumor size, pre-op stage, SUV-max. That particular system provides better sensitivity and spesificity than PET CT. MLP score has a great negative predictive value when under 4. It is also efficient in predicting post-operative 3B disease risk. We believe MLP score will reduce the rate of unnecessary surgery. Our statistics prove that this scoring system is more valuable than PET CT for each of its components.

The approach defined by the European Society for Medical Oncology in 2013 has had general acceptance in clinical practice Eberhardt WE et al. (27). If the mediastinal lymph node is negative in PET-CT, invasive staging is not recommended. The exceptions to this are; centrally located tumors and N1 lymph node above the long axis of the tumor is >3cm, while Thorax Ct are cases with lymph nodes more than 1 cm in the short axis. Of course, there are also various suggestions from the literature Ambrasini V et al. (24) and Smith DE et al. (28). Tumor localization has an important place in some of the studies. Although it is very logical about lymphatic drainage, classification and evaluation can not be standardized.

Stage 3A is undoubtedly the most troublesome in terms of staging. Tumor size was seen to be significant when over 3 cm and our data is compatible Tang K et al. (21) and Eberhardt et al. (27). For the SUV-max value, higher metabolic levels have better results for positive predictive values Yalçınkaya E et al. (29). This is in line with the hypotheses, which suggest SUV-max cut-off values over 2.5 need to be evaluated for mediastinal lymph nodes Yalçınkaya E et al. (29).

Our scoring system defines a baseline systematic and naturally it has many constraints. Our study includes a retrospective evaluation and was conducted on a limited patient population. For this reason, our results needs testing with prospectively designed multicenter and multinational studies. Tumor volumes instead of the tumor size yield more meaningful results. Mediastinal lymph node localization needs international standardization, after which studies may focus on locations. N1 disease and anatomical location would be appropriate to be included in the evaluation criteria. Of course, standardization of the anatomical location should not depend on subjective criteria.

Finally, our scoring system has a great negative predictive value but an under desired positive predictive value. This may be due to our focus on the goal of reducing the number of cases with unnecessary surgery with correct staging. Despite these limitations, our study is the only study including a large number of patiens, focused on PET-CT with a new pathological classification of lung cancer adenocarcinoma. We believe it will contribute positively to the clinical decision-making process and the future studies in establishing a baseline systematic.

CONCLUSION

Up to date pathological subtypes of lung adenocarcinoma greatly correlate with PET-CT results and clinical features. The lepidic subtype has a lower FDG affinity. Negative PET scan results should be considered in solitary pulmonary nodules especially when lepidic subtype is present, which underlines the importance of surveillance in solitary nodules. Mediastinal staging for lung adenocarcinomas employing clinical scoring with subtype, tumor size, FDG uptake and PET scan stage has a high accuracy. MLP scoring may form the basis for new studies about mediastinal lymph node evaluation and will be able to provide more precise data by adding new criteria.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the İzmir Katip Çelebi University Hospital Ethics Committee (Date: 2021, Decision No: 371).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Integrity loss of glycosylated hemoglobin with deepening anemia

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ABSTRACT

Introduction: Iron deficiency anemia (IDA) has been shown to cause a false increase in glycosylated hemoglobin (HBA1c), but how much increase in hemoglobin (Hgb) causes a certain decrease in HBA1c remains unknown. Knowledge of this ratio will enable more accurate clinical diagnosis and follow-up of diabetes. This study aimed to investigate whether IDA causes a decrease in HBA1c and if it does, how much of a decrease it causes.

Material and Method: One hundred and twenty-two patients with IDA made up the study group and sixty-two health volunteers formed the control group. 270 mg ferrous sulphate (=80 mg elemental iron) were administered to the study group each day, orally for 3 months, and a control of age/sex matched healthy participants were monitored. Hgb, serum iron, serum iron binding capacity (SIBC), ferritin and HbA1c levels of all participants were measured and compared at baseline and at the third month of the study.

Results: There was a significant decrease in HbA1c and SIBC levels at baseline and 3 months in the study group ($p<0.001$, $p<0.001$, $p<0.001$ respectively) and a significant increase in serum iron, ferritin and Hgb ($p<0.001$, $p<0.001$, $p<0.001$ respectively). It was found that a 1 mg/dl increase in Hgb level resulted in a 0.113% decrease in HbA1c.

Conclusion: As anemia deepens, HBA1c loses its reliability for diagnosis and follow-up of DM. IDA should be considered before any diagnosis or treatment decisions are made according to HbA1c levels.

Keywords: anemia, diabetes, glycosylated hemoglobin

INTRODUCTION

Anemia is a worldwide public health issue affecting 1.62 billion people or a quarter of the world's population, both in the first world and developing countries. There are a range of anemia types but the most prevalent is IDA, which constitutes one third of all anemia cases in the world (1).

HbA1c is commonly used to diagnose as well as monitor DM (2). The main factor influencing HbA1c is blood glucose, however, circumstances such as IDA, hemolytic anemia, alcohol, pregnancy, blood loss and uremia are believed to alter HbA1c levels independent of glycemic state (3). Relying only on HbA1c measurements for patients with DM is controversial, and studies have shown IDA to cause false high HbA1c values (4-6), though the cause remains elusive (7). Glycosylation of hemoglobin is irreversible, therefore, HbA1c levels in RBC rise with cell age (8). IDA is correlated with longer RBC survival which leads to higher HbA1c levels. In addition, elevated malondialdehyde (MDA) levels in IDA increase Hgb glycosylation. It has been alleged that a combination of these two mechanisms may result in an erroneous increase in HbA1c levels in patients with IDA (9).

Both DM and IDA are very common around the world and as such, the possibility of these two diseases co-existing is also very high (3). Clinical consequences of any correlation between body iron and HBA1c will affect many patients with DM and IDA. Because false raised HBA1c will lead to an incorrect diagnosis of diabetes and inappropriate follow-up and treatment of diabetic patients. If the HBA1c level decreases after iron treatment in a patient with IDA, it is necessary to demonstrate how much increase in Hgb causes a decrease in HBA1c

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in order for the decrease in HBA1c to be useful in the diagnosis and follow-up of diabetic patients. There is only one study on this subject in the diabetic patient population without a control group (10), and further studies are needed. Our study was carried out in the non-diabetic patient group in comparison with the control group. Our study was conducted to investigate whether the HBA1c level decreases after iron deficiency anemia treatment and if it does, how much of an increase in Hgb causes a certain decrease in HBA1c.

MATERIAL AND METHOD

The clinical trial protocol was approved by the Ethics Committee of Kütahya Health Sciences University, Kütahya Evliya Çelebi Training and Research Hospital (Date: 08.07.2021 Decision No: 2021/12-04). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

This study was carried out between October 2020 and July 2021 in the Department of Endocrinology and Metabolism Diseases of Kütahya Health Sciences University, Kütahya Evliya Çelebi Training and Research Hospital. Patients who met the inclusion criteria were sequentially included in the study after written informed consent was obtained.

Patients with IDA included in the study group did not have any prior diagnosis of DM, were not on any anti-diabetic medication and had fasting plasma glucose (FPG) < 100 mg/dl, HbA1c < 6.5% female hemoglobin (Hgb) < 12 g/dl and male 13 g/dl, mean corpuscular volume (MCV) < 80 (fL), ferritin < 15 ng/ml. Since ferritin, which shows iron stores in the body, is also an acute phase reactant and may be influenced by any infectious circumstance, only patients with normal c-reactive protein (CRP) values were included in the study. Patients on any medication that may influence body weight were excluded from the study as these may alter HbA1c by affecting insulin resistance.

One hundred and thirty four patients of the study group and 66 healthy volunteers in the control group meeting the inclusion and exclusion criteria were included in the study. In the study group, 3 patients who did not comply with treatment and follow up, 6 patients who could not tolerate oral iron treatment and 3 patients whose Hgb failed to increase despite iron treatment were excluded from the study. Also, four patients who failed to comply with follow up were excluded in the control group. The study was completed with 122 patients in total in the study group and 62 healthy volunteers in the control group.

Eligibility

Inclusion criteria: (a) Male and female patients over age 18 (b) No previous diagnosis of DM and not on any anti-diabetic medication (c) For study group, a diagnosis of IDA (male Hgb <13 mg/dl, female Hb <12 mg/dL, MCV <80 (fL), ferritin <15 ng/dl), for control group male Hb >13 mg/dl, female Hb >12 mg/dL, MCV >80 (fL), ferritin >15 ng/dl) (d) CRP within normal range (e) Compliance with treatment and follow up (f) Acceptance of inclusion in the study.

Exclusion criteria: (a) Diagnosed with DM or use of any anti-diabetic medication (b) Diagnosis of anemia other than IDA (c) Patients whose Hgb failed to increase despite oral iron treatment (d) Patients with high CRP values (e) Patients on medications which aid weight loss such as a glucagon like peptid-1 (GLP1) analogue or orlistat (f) Patients on any medication known to affect body weight (g) Patients with abnormal thyroid function tests, on levothyroxine or anti-thyroid medication (h) Patients with a history of surgery for obesity (i) Patients with any endocrinopathy that may result in obesity (Cushing's syndrome, acromegaly, hypothyroidism, etc.) (j) Patients with acute coronary syndrome, heart failure, cerebrovascular disease, pregnancy, chronic liver disease, abnormal renal function tests and malignancy (k) Patients with a history of blood transfusion during the past year (l) Patients who failed to comply with monitoring and treatment (m) Patients who declined to be included in the study.

Treatment and Follow-up

Patients were administered 270 mg/day ferrous sulphate (=80 mg elemental iron) for three months for the treatment of IDA. Age/sex matched healthy participants were followed as the control group. Hb, MCV, hematocrit (Hct), red blood cells (RBC), platelets (PLT), white blood cells (WBC), serum iron, serum iron binding capacity (SIBK), serum ferritin, fasting plasma glucose (FPG), HbA1c, body mass index (BMI), CRP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and blood urea nitrogen (BUN) values of patients were measured at the start and following 3 months of treatment with iron and compared.

Biochemical Analysis

Blood samples were obtained in the morning at the start of the study and following 3 months of treatment with iron after at least 10 hours of fasting through the night. Venous fasting blood samples were obtained from one antecubital vein in 8 ml anticoagulant free tubes. Blood in the straight tubes was allowed to coagulate for 30 minutes and centrifuged at 3000 rpm at room temperature. Repeated freezing and defrosting was avoided. HbA1c was measured with high performance liquid chromatography (HPLC) using a TOSOH device, and serum ferritin was measured with electrochemiluminescence immunoassay

(ECLIA) using a Beckman Coulter UniCel Dxl 600 device. Hemogram was evaluated flow cytometrically with a Mindray BC-6800 device. Serum iron, SIBK, CRP, FPG, creatinine, AST, ALT, BUN values were measured with a Beckman Coulter Systems' au5800 series device using spectrophotometry.

Statistical Analysis

The normality of distribution was examined using the Kolmogorov-Smirnov test. Descriptive statistical methods including percentage and mean±standard deviation (SD) or median (min.- max.) were used to provide basic characteristics of the data. A Wilcoxon signed ranks test was used for non-normally distributed continuous variables for the study group (FPG, Hgb, Hct, RBC, MCV, WBC, serum iron, SIBC, ferritin, CRP) and the control group (FPG, Hgb, RBC, MCV, PLT, serum iron, ferritin, CRP). A paired samples t-test was used for normally distributed continuous variables of the study group (HBA1c, BMI, PLT) and control group (HBA1c, Hct, WBC, SIBC, BMI) Statistical analyses were carried out using SPSS23.0 version (IBM Corporation, Armonk, NY, US). When two-tailed p <0.05, differences were considered statistically significant. Regression models were used for increases and decreases in Hgb values. Results were obtained by using Hgb values at baseline and third month. A regression model analysis was carried out using Excel 16.0 (2016).

RESULTS

Baseline Characteristics

The total number of patients included 93 women (76.23%) and 29 men (23.7%) in the study group, and 47 women (75.81%) plus 15 men (24.19%) in the control group. The median age of women was 41.13±8.73 and men 44.21±8.42 in the study group, and 41.04±7.58 for women and men 44.93±4.34 in the control group (**Table 1**). When the baseline and 3 month data were compared, there was no significant difference between the study group and the control group in terms of BMI (p:0.332, p:0.399 respectively) (**Table 2-3**).

Blood Glucose Parameters

While the baseline HbA1c for the study group was 5.90 ±0.37 (%), it was 5.48±0.48 (%) at third month, which was significantly lower (p <0.001). Baseline HbA1c for the control group was 5.52±0.33 (%) and 5.53±0.30 (%) at the third month, showing no statistically significant difference (p:0.578). Baseline and third month FPG (mg/dL) levels for the study group were 94.00 (81-138) and 92.00 (80-142) respectively, and there were no statistically significant difference (p:0.256). Baseline and third month FPG (mg/dL) levels for the control group (mg/dL) were 94.50 (81-128) and 92.00 (80-138) respectively and also showed no statistically significant difference (p:0.071) (**Table 2-3**).

Table. 1 Demographic parameters

Characteristic	Study group (n=122)	Control group (n=62)
Mean age, years	Male : 44.21±8.42	Male: 44.93±4.34
	Female: 41.13±8.73	Female: 41.04±7.58
Sex male/female %	Male: 29 (23.77 %)	Male: 15 (24.19%)
	Female: 93 (76.23 %)	Female: 47 (75.81%)

Table 2. Comparison of baseline and 3rd month values of study group

	Baseline	3rd Month	p
HbA 1c, (%) †	5,90±0,37	5,48±0,48	<0.001
FPG (mg/dL) *	94.00 (81-138)	92.00 (80-142)	0.256
Hgb (g/dL) *	10.25 (5.7-11.8)	12.70 (11.6-15.0)	<0.001
Hct (%) *	32.95 (23.1-38,0)	40.25 (38.1-45.4)	<0.001
RBC (10^6/uL) *	4.40 (2.7-5.6)	4.80 (3.9-6.8)	<0.001
MCV (fL) *	73.00 (62.0-79.0)	82.90 (77.5-92.0)	<0.001
PLT (10^3/uL) †	335.17±77.43	287.52±64.70	<0.001
WBC (10^3/uL)*	7.40 (3.9-7.3)	7.50 (4.2-8.3)	0.190
Serum Iron (ug/dL) *	20.00 (2-57)	58.00 (18-272)	<0.001
SIBC (ug/dL) *	417.50 (273-584)	331.00 (131-481)	<0.001
Ferriti (ug/L) *	3.00 (1-29)	14.00 (3-208)	<0.001
CRP (mg/L) *	1.60 (0.10-4.70)	1.80 (0.20-4.60)	<0.001
BMI (kg/m²) †	26.94±3.32	26.97±3.38	0.332

HBA1c: glycosylated hemoglobin, FPG: fasting plasma glucose, Hgb: hemoglobin, Hct: hematocrit, RBC: red blood cell, MCV: mean corpuscular volume, PLT: thrombocyte, WBC: white blood cells, SIBC: serum iron binding capacity, CRP: c-reactive protein, BMI: body mass index
* Data are presented as median (minimum-maximum and compared by Wilcoxon signed ranks)
† Data are presented as mean±SD and compared by paired samples t test

Table 3. Comparison of baseline and 3rd month values of control group

	Baseline	3rd Month	p
HbA 1c, (%) †	5.52±0.33	5.53±0.30	0.578
FPG (mg/dL)*	94.50 (81-128)	92.00 (80-138)	0.071
Hgb (g/dL)*	13.00 (12.0-14.9)	13.10 (12.0-14.4)	0.441
Hct (%)†	41.37±1.68	40.92±1.32	0.017
RBC (10^6/uL)*	4.80 (4.1-5.8)	4.85 (4.2-6.8)	0.494
MCV (fL) *	84.67±2.81	84.89±2.84	0.558
PLT (10^3/uL)*	281.50 (184-442)	286.00 (175-442)	0.685
WBC (10^3/uL)†	7.10±1.74	7.38±1.75	0.067
Serum Iron (ug/dL)*	61.50 (12-266)	61.50 (20-185)	0.840
SIBC (ug/dL)†	314.08±62,10	308.03±71,05	0.537
Ferritin (ug/L)*	18.00 (6-96)	20.00 (5-113)	0.768
CRP (mg/L)*	1.60 (0.10-4.70)	1.80 (0.20-4.60)	0.592
BMI (kg/m²)†	27.07±3.09	27.11±3.17	0.399

HBA1c: glycosylated hemoglobin, FPG: fasting plasma glucose, Hgb: hemoglobin, Hct: hematocrit, RBC: red blood cell, MCV: mean corpuscular volume, PLT: thrombocyte, WBC: white blood cells, SIBC: serum iron binding capacity, CRP: c-reactive protein, BMI: body mass index
* Data are presented as median (minimum-maximum and compared by Wilcoxon signed ranks)
† Data are presented as mean±SD and compared by paired samples t test

Hematological Parameters

When baseline and third month WBC levels in the study group were compared, no significant differences were observed (p:0.190), however, SIBC and PLT showed significant decreases (p<0.001, p<0.001 respectively), and RBC, MCV, serum iron, ferritin and Hgb showed significant increases (p<0.001, p<0.001, p<0.001,

p<0.001, p<0.001 respectively) (**Table 2**). When baseline and third month serum iron, SIBC, ferritin, Hgb, MCV, RBC, WBC and PLT in control group values were compared, no statistically significant differences were observed (p:0.840, p:0.537, p:0.768, p:0.441, p:0.558, p:0.494, p:0.067, p:0.685 respectively) (**Table 3**).

Regression between Hgb and HbA1c

An increase of 1 mg/dL in Hgb was found to result in a 0.113784 % decrease in HbA1c. The table below shows the progression of the regression method when 1 was added to Hgb values of 3 randomly selected patients.

HbA1c values showed a 0.113784% decrease at the third month of study					
	Baseline HbA1c Study Group	**Baseline Hgb Study Group**	**Hgb at 3rd Month Study Group**	**HbA1C at 3rd Month Study Group**	**Difference**
Patient 1	7.1	11.2	12.9	6.452213505	0
Patient 1	7.1	11.2	13.9	6.338429777	0.113784
Patient 1	7.1	11.2	14.9	6.224646049	0.113784
Patient 2	5.7	10.7	12.1	5.427191807	0
Patient 2	5.7	10.7	13.1	5.313408079	0.113784
Patient 2	5.7	10.7	14.1	5.199624351	0.113784
Patient 3	6	11.1	13.8	5.479490192	0
Patient 3	6	11.1	14.8	5.365706464	0.113784
Patient 3	6	11.1	15.8	5.251922736	0.113784

DISCUSSION

In our study, IDA was found to be correlated with increased HbA1c concentrations with statistically significant decreases observed following iron treatment. The most important finding of our study was that a 1 mg/dL increase in Hgb level causes a 0.113% decrease in HBA1c. We believe this may be due to normalization of RBC survival which was prolonged and decrease in malondialdehyde levels following iron treatment.

The first research to study the influence of IDA on HbA1c levels was by Horton and Huisman (11), who showed HbA1c concentrations to be medium, 5.3%, in 14 healthy individuals and 4.9% in patients with IDA.

Studies were often performed on normoglycemic patient groups and the relationship between IDA and HbA1c evaluated. The baseline HbA1c of 50 patients with IDA without DM and 50 healthy volunteers without IDA or DM were compared and HbA1c was observed to be significantly higher in the group with IDA (12). Similarly, in a study by Son et al. (13), of 112 patients with IDA and 217 healthy individuals without IDA, the baseline HbA1c was proposed to have low specificity in the group with anemia, therefore, HbA1c was a limited parameter for diagnosis of DM. In studies with IDA patients without DM where HbA1c levels were evaluated following iron treatment, HbA1c showed significant decreases with iron and HbA1c was reported to result in the false diagnosis of DM in patients with IDA (4-6, 14-15).

In a review in 2017, it was concluded that IDA is correlated with increased HbA1c concentrations both in diabetic and non-diabetic individuals and iron treatment results in a decrease in HbA1c (7). The findings of our study are consistent with these studies. All data support the idea that iron deficiency should be corrected before making a diagnosis of pre-diabetes or diabetes.

Although the design and results of our study and the studies mentioned above differ, the rationale for research is similar. In short, there is an uncertainty and possibility of error in utilizing HbA1c in the diagnosis and monitoring of diabetes. The International Expert Committee warned clinicians to be wary of any conditions which may affect RBC turnover during the follow-up with diabetes patients (16). IDA is the most prevalent reason affecting RBC turnover. The interpretation of HbA1c based on information from a hematological examination and iron metabolism indices may help to prevent misdiagnosis or under-diagnosis, and HbA1c should be evaluated carefully as a parameter of glycemic control in patients with IDA (17).

The American Diabetes Association (ADA) suggested using only plasma glucose, not HbA1c as a diagnostic criterion for diabetes in patients with IDA (18). In accordance with the ADA, our study also supports not using HbA1c for the diagnosis and monitoring of patients with IDA.

Following the observation that HbA1c decreases with treatment of IDA, the first question is: how much HbA1c decrease is caused by the rise in Hgb. There is only one study on this subject in diabetic patients without a control group, and it was found that 2.2 mg/dL increase in Hgb value caused a 0.4% decrease in HBA1c level (10). In our study, it was determined that 1 mg/dL increase in Hgb value caused a 0.113% decrease in HBA1c value. This means that the deeper the IDA, the more the reliability of HBA1 is lost. This ratio should be supported with multi-centre studies and more patient participation. We believe our study is valuable to future research as it may be the beginning of a new ratio to be included in DM diagnosis and monitoring guidelines.

IDA is more common especially in young women of reproductive age (1). The majority of the patient

population in our study consisted of female patients at this age. The results of our study showed that it is of particular importance whether IDA is coexisting when diagnosing diabetes according to HBA1c in women in this group or when following a diabetic patient. If IDA coexists, it would be more appropriate to diagnose and follow-up diabetes based on the HBA1c level after eliminating the iron deficiency. Gestational diabetes mellitus (GDM), is one of the most widely encountered metabolic disturbances. Macrosomy, neonatal hypoglicemia, neonatal hypocalcemia, neonatal hypomagnesemia and potential respiratory problems of newborn are more prevalent in the newborn of women with GDM (19). For this reason, in a pregnant patient, examining HBA1c level after the iron deficiency is eliminated would enable a more accurate diagnosis of whether it is gestational DM or is it pregestational DM.

The limitations of our study are low patient numbers and study being performed in a single centre. Multi-centre studies with more participants are required.

CONCLUSION

In our study, we found that a 1 mg/dL increase in Hbg causes a 0.113% decrease in HBA1c. This shows that HBA1c loses its reliability in the diagnosis and follow-up of DM as the anemia deepens. Elimination of iron deficiency before any diagnosis or treatment decision is made based on the HbA1c level will prevent patients from being misdiagnosed with diabetes and prevent additional unnecessary intervention in diabetes treatment of diabetic patients. Nevertheless, implementing additional treatment to diabetic patients with IDA based on the high HBA1c will increase the risk of hypoglycemia in patients and will bring additional drug costs to the state economy. Early diagnosis and treatment of IDA in diabetic patients can improve glycemic control and prevent or delay complications.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Clinical Research Ethics Committee of Kütahya Health Sciences University, Kütahya Evliya Çelebi Training and Research Hospital (Date: 08.07.2021 Decision No: 2021/12-04).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The diagnostic value of calcium binding protein S100A8/A9 and S100A12 in acute pancreatitis

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ABSTRACT

Background: S100A8/A9 and S100A12 which are the major calcium-binding proinflammatory proteins secreted by granulocytes, has been proposed to be related to distinct disease states of inflammatory origin. This study aims to explore the circulating levels of S100A8/A9 and S100A12 in acute pancreatitis (AP) and reveal their relationship with conventional inflammatory markers.

Material and Method: Serum S100A8/A9 and S100A12 were determined in AP patients (male/female: 17/13) by using a specific enzyme-linked immunosorbent assay (ELISA) method at both onset and remission and in 30 healthy controls (male/female: 17/13).

Results: Significantly higher S100A8/A9 and S100A12 levels were found in AP patients compared to healthy controls ($p<0.001$). Circulating levels of S100A8/9, S100A12 and C-reactive protein (CRP) were found to be elevated in AP patients at disease onset compared with remission. The correlation analysis demonstrated a significant association between S100A8/A9 and S100A12 ($r=0.366$, $p=0.047$). The cut-off level for S100A8/A9 for detecting AP was ≥54.4 ng/ml with a sensitivity and specificity of 96.7% and 73.3% (AUC: 0.958). The optimum cut-off level for S100A12 for detecting AP was ≥350.25 ng/ml with a sensitivity and specificity of 73.3% and 76.7% (AUC: 0.752) respectively.

Conclusion: Circulating S100A8/A9 and S100A12 levels were found to be elevated in AP patients. Both of these markers might serve as an additional tool in the diagnostic workup in AP since S100A8/A9 and S100A12 were significantly correlated with CRP.

Keywords: Acute pancreatitis, S100A8/A9, S100A12, CRP, inflammation

INTRODUCTION

Acute pancreatitis (AP) is one of the most encountered gastrointestinal reason for emergency department (ED) admission and shows an annual incidence up to 12 to 45 per 100,000 population (1). An increase in serum amylase and lipase levels with an acute onset of abdominal pain continues to be the most widely used diagnostic criteria of AP. Although both of these enzymes are usually used for the diagnosis, their popularity does not appear to be justified because of their low specificity in which elevated levels could also be observed in perforated gastric or duodenal ulcers, kidney diseases, gastrointestinal obstruction, tubo-ovarian disease, and mesenteric infarction (2). Although there are several additional diagnostic assays including urinary trypsinogen-2 and trypsinogen activation peptide exist, they are unfortunately less widely available. Therefore there is an eager need for better noninvasive diagnosing markers that can help identify the exact diagnosis at an earlier time point, and evaluate the efficacy of medical treatment.

Amongst a number of calcium-binding proteins (CBP), S100 protein family consists the mostly encountered one. From this CBPs, S100A8, S100A9, and S100A12, are very well defined by a distinctive expression pattern,

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with strong prevalence in myeloid origin cells (3). In this context, the S100A8/A9 heterocomplex and the S100A12 are elements of the calgranulin family and released from inflammatory cells of the myeloid lineage. It has been suggested that increased circulating S100A8/A9 and S100A12 levels might be an indicator of a subclinical inflammation in which increased levels were reported in distinct disease states including inflammatory bowel disease, otitis media, alcoholic liver cirrhosis, Hodgkin lymphoma, and myocarditis (4-10). Unfortunately, there is no literature data that reveals the possible role of these CBPs in AP pathophysiology.

Based on the emerging roles of S100A8/A9 and S100A12 in inflammation-associated diseases, we designed the current trial to examine the possible involvement of S100A8/A9 and S100A12 in AP and to determine whether these two parameters are correlated with conventional inflammatory markers commonly used to evaluate inflammatory response in AP course.

MATERIAL AND METHOD

The study was approved by the Çanakkale Onsekiz Mart University Clinical Researches Ethics Committee (Date: 27.11.2019, Decision No: 2019-19). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Participants

Thirty patients with AP (M/F: 17/13) admitted to the ED of Çanakkale Onsekiz Mart University (COMU) and hospitalized whether to a regular ward or intensive care unit (ICU) were initially recruited for the study. After treatment, all patients achieved remission and were included in the main analysis. Thirty healthy subjects (M/F: 17/13) were recruited from healthy individuals seeking a routine health check-up who had no previous history of acute/chronic inflammatory disorders, or a history of any drug use. Exclusion criteria of the study included; patients referred from an outside hospital, patients with an inflammatory and malignant condition including inflammatory bowel disease, otitis media, alcoholic liver cirrhosis, Hodgkin lymphoma, chronic pancreatitis and myocarditis. Each of the 30 patients was diagnosed with AP within 12 h of admittance to ED and this assessment was based on the revised Atlanta classification that includes physical examination with the presence of abdominal pain, elevated levels of serum lipase and/or amylase (more than three times from upper normal limit), and characteristic computed tomography (CT) or magnetic resonance imaging (11). After hospitalization, each patient was closely followed up until discharge from the hospital. Remission of AP was defined as the disappearance of symptoms and radiologic imaging findings after the initial medical treatment.

Clinical and Laboratory Assessment

Clinical, demographic and biochemical parameters of each patient were recorded. The obtained data include; age, gender, and disease severity Routine hemogram and laboratory parameters including lipase, amylase, lipase, alanine aminotransferase, aspartate aminotransferase, albumin, creatinine, calcium, glucose, lactate dehydrogenase, C-reactive protein (CRP) and sedimentation rate (ESR) were recorded. Fasting serum samples were obtained from each study participant at both the onset and remission of the disease after overnight fasting without any anticoagulant use. Blood samples were left on the clot, and serum was separated from cellular elements by centrifugation (3,000 rounds per minute for 15 minutes) within two hours after blood sampling. All serum samples were stored at -80°C until the analysis was performed.

S100A 8/9 and S100A 12 Assay

S100A 8/9 levels were measured by a double antibody Enzyme-linked immunosorbent assay (ELISA) kit, from Bioassay Technology Laboratory made in Shangai China, Catalogue Number E4010Hu. S100A 12 levels were measured using commercially available ELISA kits from Bioassay Technology Laboratory made in Shangai China, Catalogue Number E3074Hu.

Statistical Analysis

Frequencies and percentages (%) were used to express categorical variables. Continuous variables were expressed median and interquartile range (IQR). The Shapiro–Wilk test was used to evaluate the normality assumption for the continuous variables. Mann–Whitney U test was performed for analyzing differences between two groups in conditions with the existence of non-normally distributed continuous variables. Categorical variables were evaluated using Pearson's chi-square test or Fisher's exact test. Spearman's correlation test was used to perform correlation analysis. ROC analysis was used to calculate the areas under the receiving operator curves (AUROC) with 95% confidence intervals for S100A8-9 and S100A12 to predict acute pancreatitis. Statistical evaluations were performed by using SPSS19.0 for Windows (IBM Corp., Armonk, NY, USA). P values below 0.05 were accepted as significant.

RESULTS

A total of 30 patients with AP and 30 control subjects were enrolled in this study. Thirteen (43.3%) AP patients and 13 (43.3%) of the healthy controls were female. Median age of AP patients was 67.0 (57.0-75.0) years and healthy controls had a median age of 63.5 (48.5-76.3) years. No significant differences were demonstrated between study groups in respect to age and sex. The median serum

S100A 8/9 levels in patients with AP was significantly elevated (p<0.001) compared with healthy control group [102.2(64.7-177.5)] (ng/mL) vs 51.2(43.9-55.4) (ng/mL)]. Similarly the median serum S100A12 levels in patients with AP was significantly elevated (p<0.001) compared with healthy control group [409.5(304.8-711.8) (ng/mL) vs 312.4(268.1-346.2) (ng/mL)]. **Table 1** demonstrates the clinical, demographic characteristics and biochemical values of the study groups. **Figure 1** demonstrates the median S100A 8/9 and S100A12 levels of the AP patients at the onset and remission compared with those of the healthy controls.

Table 1. Demographic characteristics and laboratory values of the acute pancreatitis patients and controls

	Acute pancreatitis (n=30)	Control group (n=30)	p
Age (years)	67.0 (57.0-75.0)	63.5 (48.5-76.3)	0.280
Sex (F/M)	17/13	17/13	1.00
Amilaz (U/L)	1123.0 (514.0-1922.0)	64.5 (42.0-82.3)	<0.001
Lipaz (U/L)	1908.0 (1178.0-3912.0)	37.0 (28.5-52.0)	<0.001
ALT (U/L)	35.0 (15.0-173.0)	16.0 (13.75-23.25)	0.004
AST (U/L)	40.0 (23.0-227.0)	21.0 (16.0-30.3)	<0.001
ALP (U/L)	114.0 (78.0-197.0)	76.5 (53.25-93.5)	<0.001
GGT (U/L)	142.0 (37.0-298.0)	27.0 (12.8-36.3)	<0.001
Total Bilirubin (mg/dl)	1.0 (0.6-3.0)	0.5 (0.33-0.73)	0.001
WBC (/mm³×10³)	11.4 (9.0-14.7)	6.7 (5.4-8.4)	<0.001
Hemoglobin (g/dL)	13.0 (11.5-14.5)	12.7 (11.6-13.6)	0.416
Platelet (/mm³×10³)	248.0 (208.0-310.0)	221.5 (169.0-280.0)	0.109
CRP (mg/L)	5.9 (2.7-13.6)	0.40 (0.2-0.7)	<0.001
Sedimentation (mm/h)	34.0 (17.0-60.0)	15.0 (11.0-20.3)	0.003
S100A8/A9 (ng/mL)	102.2 (64.7-177.5)	51.2 (43.9-55.4)	<0.001
S100A12 (ng/mL)	409.5 (304.8-711.8)	312.4 (268.1-346.2)	<0.001

ALT: Alanine aminotransferase, AST: Aspartate aminotrasferase, ALP: Alkaline Phosphatase, GGT: Gamma Glutamyl Transferase, WBC: White Blood Count, CRP: C-Reactive Protein

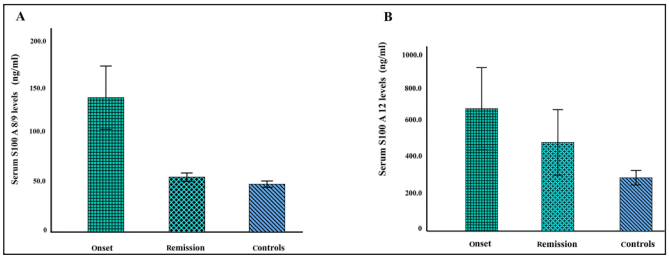

Figure 1. Bar plots and corresponding error bars of (A) median S100A 8/9 and S100A 12 levels of the acute pancreatitis patients and controls; (B) median S100A 8/9 and S100A 12 levels of the acute pancreatitis patients at onset and remission

The comparison of serum inflammation markers in conjunction with S100A 8/9 and S100A12 are given in **Table 2**. Both S100A 8/9 and S100A12 were found to be elevated at onset of the disease compared with remission. Likewise, white blood cell (WBC), CRP and ESR values were significantly elevated in AP patients at onset of the disease. Spearman correlation analysis revealed a significant correlation between CRP and S100A 8/9 (r=0.467, p=0.009) and S 100A 12 (r=0.555, p=0.001) (**Figure 2**). Moreover, both S100A 8/9 and S100A12 levels were found to be correlated at the onset of the disease (r=0.366, p=0.047) (**Table 3**).

Table 2. Comparison of serum S100A8/A9 and S100A12 levels and other markers of inflammation at onset and remission of acute pancreatitis

	Onset	Remission	p
WBC (/mm³×103)	11.4 (9.0-14.7)	6.8 (5.15-8.7)	<0.001
Neutrophil (/mm³×10³)	10.3 (6.9-13.1)	4.5 (2.9-5.7)	<0.001
Lymphocyte (/mm³×10³)	1.1 (0.7-1.4)	1.4 (1.1-1.9)	0.047
Platelet (/mm³×10³)	248.0 (208.0-310.0)	240.0 (188.5-350.0)	0.976
CRP (mg/dL)	5.9 (6.7-13.6)	0.8 (0.5-1.5)	<0.001
Sedimentation (mm/h)	34.0 (17.0-60.0)	17.0 (11.5-26.0)	0.007
S100A8/A9 (ng/mL)	102.2 (64.7-177.6)	54.2 (49.7-59.9)	<0.001
S100A12 (ng/mL)	409.5 (304.8-711.8)	282.9 (242.7-564.5)	0.015

WBC: White Blood Count, CRP: C-Reactive Protein

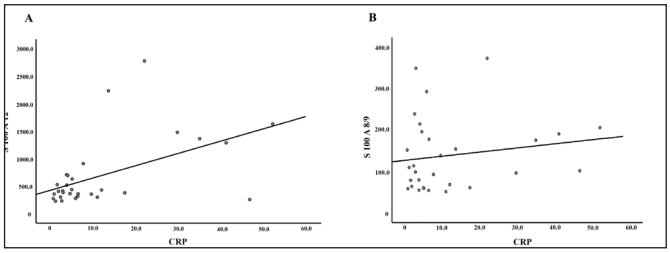

Figure 2. Correlations of (A) S100A 8/9 with CRP; (B) S100A 12 with CR

The Receiver Operating Characteristic (ROC) curve analysis revealed that the ideal S100 A8/9 level cut-off points for determining AP was ≥54.4 ng/ml with a sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of 96.7%, 73.3%, 95.7 and 78.4 respectively (AUC: 0.958). The optimum S100A12 level cut-off value for determining AP was ≥350.25 ng/ml with a sensitivity, specificity, NPV and PPV of 73.3, 76.7%, 74.2 and 75.9, respectively (AUC: 0.752) (**Table 4**). **Figure 3** demonstrates ROC curve analysis to predict acute pancreatitis.

Table 3. Correlation analysis of study variables at onset of acute pancreatitis

	S100A8/9		S100A 12		Sedimentation		CRP	
	r	p	r	p	r	p	r	p
WBC	0.036	0.851	0.118	0.535	0.487	0.010	0.258	0.169
CRP	0.467	0.009	0.555	0.001	0.309	0.117	-	-
Sedimentation	0.326	0.097	0.091	0.651	-	-	-	-
S100A12	0.366	0.047			-	-	-	-

WBC: White Blood Count, CRP: C-Reactive Protein

Table 4. ROC analyses of S100A8/A9 and S100A12 with other conventional inflammation markers to determine acute pancreatitis

Acute pancreatitis vs controls	AUC	Cut-Off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
WBC (/mm³×10³)	0.849	7.1	93.3	60.0	70.0	90.0
CRP (mg/dl)	0.959	1.0	93.3	83.3	84.8	92.6
Sedimentation (%)	0.731	16.5	70	63.3	65.6	67.9
S100 A8/9 (ng/ml)	0.958	54.4	96.7	73.3	78.4	95.7
S100 A12 (ng/ml)	0.752	350.25	73.3	76.7	75.9	74.2

WBC: White Blood Count, CRP: C-Reactive Protein, AUC: Area Under Curve, PPV: Positive Predictive Value, NPV: Negative Predictive Value, ROC: Reciever Operating Characteristics

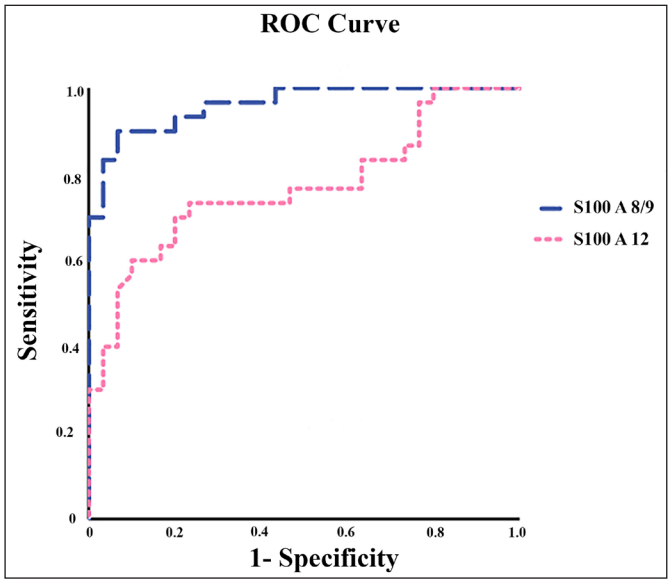

Figure 3. Receiver operating characteristic curves of study parameters to predict acute pancreatitis

DISCUSSION

This study demonstrated that S100A8/A9 and S100A12 levels are increased in AP compared to healthy controls. Moreover, in AP patients after remission was achieved, S100A8/A9 and S100A12 levels were returned to normal values compatible with healthy controls. In addition, circulating S100A8/A9 and S100A12 levels were found to have high specificity, sensitivity, and predictive values in patients with AP suggesting that both of these markers could be regarded as useful serum markers of inflammation in these patient group. Thus, our findings add new and relevant proof to the growing body of evidence on the role of proinflammatory S100 proteins in AP patients with a prospect for potential diagnostic and therapeutic strategies

At present, the severe form of AP is associated with an exceptionally high mortality rate rising to 30% due to rapid inflammatory development and complex pathophysiological conditions during the disease course (12,13). Therefore, there is an urgent and compelling need to determine the inflammatory status in AP patients especially in the very first days of hospital admission (14). In order to accomplish this goal and to determine the existence and the severity of pancreatic inflammation, clinical and biochemical evaluations are generally used in clinical practice (15). Unfortunately, the precise molecular mechanisms underlying pancreatic inflammation and destruction is still challenging (16). In this context, this study for the first time uncovered the potential role of two CBPs, S100A8/A9 and S100A12, in AP patients and investigated their roles in inflammation by analysing their correlation with conventional inflammation markers.

The S100 protein family involves over 20 members which are expressed entirely in vertebrates and exert intra- and extracellular regulatory properties (17). Of the S100 protein family, S100A8, S100A9 and 100A12 are strictly associated with inflammation (17-20). S100A8 and S100A9, both of which are different proteins, constitute the greater part of the calcium-binding capability in phagocytes and arise a heterocomplex to create the structure of calprotectin (21). S100A8/A9 heterocomplex increase the secretion of nuclear factor kappa B and proinflammatory mediators upon Toll-like receptor 4 (TLR4) stimulation. Furthermore, the S100A8/A9 is substantially responsive to oxidative stress and has a key function in the scavenging of the oxidants and the maintenance of tissue components and proteins (17,22) Although, there is no literature data revealing a possible role of S100A8/A9 heterodimer in pancreatitis, a recent paper by Gammal et al. (23) the role of S100A8/A9 protein in chronic pancreatitis, intraductal papillary mucinous tumour and adenocarcinoma of the pancreas

was investigated. Authors' reported that in all of these three diseases S100A8/A9 serum levels were increased and they suggested that S100A8/A9 heterodimer levels could help to distinguish patients with malignant and/ or inflammatory disease from normal and non-malignant pathological conditions. In this study, we also revealed that S100A8/A9 heterodimer is increased in AP and we think that this finding is important because it provides mechanism that centers S100 protein family in the pathophysiology of AP.

The other calcium binding protein that is investigated in the present study is S100A12 which is a protein that is specifically expressed in granulocytes and early differentiation stages of monocytes (24). S100A12 has been demonstrated to be present on circulating leukocytes and is considered a susceptible marker for the local inflammatory process related to oxidative stress. Similarly to S100A8/A9, S100A12 is considered phagocyte-specific, displays pro-inflammatory activities and has already been related to distinct diseases of inflammatory origin, including inflammatory bowel disease, otitis media, hepatitis B-related acute-on-chronic liver failure and juvenile idiopathic arthritis (4,5,25,26). Even though there is no data in humans on the relation between S100A12 and AP, there is one study in literature exploring a potential effect of S100A12 on severity evaluation and curative effect of severe AP in a mice model of AP (27). This experimental mice model shows huge promise that S100A12 can be used to monitor the development and prognosis of severe AP. Furthermore, the inhibition of S100A12 expression, the excessive activation of neutrophils demonstrated to be controlled, which will weaken the inflammatory reaction of severe AP by reducing the release of inflammatory mediators.

This study has several limitations that need to be addressed. First, we accept that the relatively low number of patients recruited for this study limits us to generalize our findings. Second, although we found that S100A8/A9 and S100A12 are sensitive markers of inflammation, our study does not clearly address whether these findings are specific for AP. Third, it would be noteworthy of examining the other factors such as oxidative stress marker expression, in order to fully understand the pathophysiological role of S100A protein family in AP associated inflammatory response.

CONCLUSION

It is apparent that the upregulation of S100A8/A9 heterocomplex and S100A12 is not limited to the boundaries of pancreas region, but is also reflected systemically and consequently observed in patient serum. Furthermore elevated levels of S100A8/A9 and s100A12 is related to AP development and significantly correlated with CRP. These findings suggest that S100A8/A9 heterocomplex and S100A12 in serum might be a potential biomarker of AP with providing significant information regarding ongoing inflammatory status in AP patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Çanakkale Onsekiz Mart University Clinical Researches Ethics Committee (Date: 27.11.2019, Decision No: 2019-19).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Evaluation of obstetric and neonatal outcomes and cesarean section rates of Syrian and Turkish adolescent pregnant women according to the Robson ten group classification system

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ABSTRACT

Aim: Our research has two purposes. To begin with, we sought to determine whether there were any differences in maternal and newborn outcomes between Syrian adolescent and adult pregnant women living in Turkey after the Syrian civil war and Turkish adolescent and adult pregnant women. Second, we wanted to examine and compare the rates of cesarean section (CS) and spontaneous vaginal delivery (SVD) in adolescent and adult pregnant women using the Robson ten group classification system (RTGCS).

Material and Method: Our study investigated data from a retrospective cross-sectional study of 1823 Turkish and Syrian pregnant women who gave birth between September 2020 and August 2021 in a tertiary reference hospital in Turkey's Mediterranean area. Our study enrolled 838 pregnant adolescent girls between the ages of 13 and 19 and 985 pregnant adult women between the ages of 20 and 47.

Results: The probability of adolescent pregnancy is 3.081 times greater among Syrian refugees than among Turkish natives ($p<0.001$, OR: 3.081, 95% CI: 2.544–3.731). Primary school graduates face a 2.757-fold greater risk of adolescent pregnancy than secondary school graduates ($p<0.001$, OR: 2.757, 95% CI: 2.15–3.536). Syrian nationality is 1.51 times more likely to be associated with late preterm birth in adolescent pregnancies than throughout the term gestational week ($p=0.033$, OR: 1.51 95% CI: 1.035–2.203). The probability of Syrian nationality in late preterm pregnancies is 1.51 times higher in adolescent women compared to term pregnancies. Ethnicity does not significantly affect the delivery week in adult pregnant women or the total ($p>0.050$). Among pregnant adolescents, newborns with a low birth weight (LBW) are 2.041 times more likely to be Syrian nationals than infants with ≥ 2500 g. Regardless of the gestational week, infants with LBW are 2.33 times more likely to be Syrian nationals than infants ≥ 2500 g.

Conclusion: Adolescent pregnancy is particularly prevalent among young females with poor levels of education and Syrian adolescent girls. Pregnant adolescent women face a greater risk of obstetric and neonatal problems than pregnant adult women of reproductive age. A country-based assessment of the RTGCS will aid in the development of effective strategies for achieving The World Health Organization (WHO)-recommended CS rates by identifying the factors that contribute to the rise in CS rates.

Keywords: Adolescent pregnancy, pregnancy outcomes, refugees, cesarean section, Robson classification

INTRODUCTION

Twenty-one million girls become pregnant in developing countries each year (1). Twelve million of these pregnancies between the ages of 15 and 19 result in birth (1). Around 17 thousand of these pregnant girls are expected to die because of complications associated with parturiency or childbirth problems (2). Pregnancies in adolescents can result in cephalopelvic disproportion (CPD), low birth weight, maternal anemia, preterm birth, acute fetal distress (AFD), perinatal fetal loss, pregnancy-induced hypertension (PIH), eclampsia, postpartum hemorrhage, and emergency cesarean delivery among other complications (3).

Robson's classification (4). RTGCS is a well-known and reliable method for determining CS rates (4). This classification system is supported by the WHO as a global standard for monitoring, assessing, and comparing CS

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rates at all levels (5). CS rates are an essential indicator of a country's access to and quality of maternal health services (5). According to a 1985 WHO report, mother and infant fatalities fell in a country with a CS incidence of 10-15% (6). In Turkey, CS rates included 45% to 53% of all live births (7). The reason for this difference in CS rates depends on several factors. Some of these include financial incentives, high compensation that midwives and physicians must pay due to malpractice law, and differences in professional training (7). The WHO advises using the RTGCS as the worldwide standard for comparing and evaluating CS rates in hospital settings (8). The RTGCS allows for a more reliable comparison and analysis of CS rates across hospitals, cities, regions, and countries (9) (**Table 1**).

Table 1. Robson ten group delivery classification system (RTGCS) (5)

	Robson classification groups description
Group 1	Nulliparous, singleton pregnancy, cephalic presentation, gestation period of ≥37 weeks (spontaneous labor).
Group 2	Nulliparous, singleton pregnancy, cephalic presentation, gestation period of ≥37 weeks (induced or CS before labor).
Group 3	Multiparous (without a preexisting uterine wound), singleton pregnancy, cephalic presentation, and gestation of ≥37 weeks (spontaneous labor).
Group 4	Multiparous (without a preexisting uterine wound), singleton pregnancy, cephalic presentation, and gestation of ≥37 weeks (induced or CS before labor).
Group 5	Multiparous, singleton pregnancy, prior CS, cephalic presentation, gestation of ≥37 weeks.
Group 6	Consists of nulliparous women with a single breech presentation.
Group 7	Consists of multiparous women with a single breech presentation (including women with previous CS).
Group 8	All pregnant women, including those who have had several pregnancies (including women with previous CS).
Group 9	All pregnant women with a singleton, transverse, or oblique lying (including women with previous CS).
Group 10	Includes all pregnant women with a singleton pregnancy, cephalic presentation, and a gestational age of <37 weeks (including women with previous CS).

We aim to examine the obstetric and neonatal outcomes of pregnant adult Turkish and Syrian refugee women and Turkish and Syrian adolescents. The results for which we sought answers in our study are 1) What are the differences between obstetric and neonatal outcomes among pregnant adolescents in Turkey and Syrian refugee adolescent pregnant women? 2) What distinctions exist between adolescent and adult pregnant women regarding obstetric and neonatal outcomes? 3) Are there differences in obstetric and neonatal outcomes between Syrian refugee pregnant women and Turkish-citizen pregnant women? In line with the results of our research, we will interpret the solutions considering the literature. In this way, our research aims to help policymakers, ministries, and non-governmental groups decide how to avoid obstetric and neonatal complications for pregnant adolescents and foreign nationals.

MATERIAL AND METHOD

The study was approved by the Health Sciences University Adana City Training and Research Hospital Clinical Researches Ethics Committee (Dated: 26.08.2020, Decision No: 1046). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki which is one of the largest regional hospitals in Turkey's southern region. Additionally, this study was approved by the Scientific Research Platform of the Turkish Ministry of Health, General Directorate of Health Services (2021-07-08T21_48_13). All patients hospitalized in our clinic consent to the use of patient data, procedures to be performed, and complications that may occur during hospitalization, provided that their data remains confidential. All medical documents of our patients can be retrieved from the central electronic data processing system archives with a unique login password defined for each doctor.

As shown in **Figure 1**, we identified 1923 patients as potentially eligible for the study.

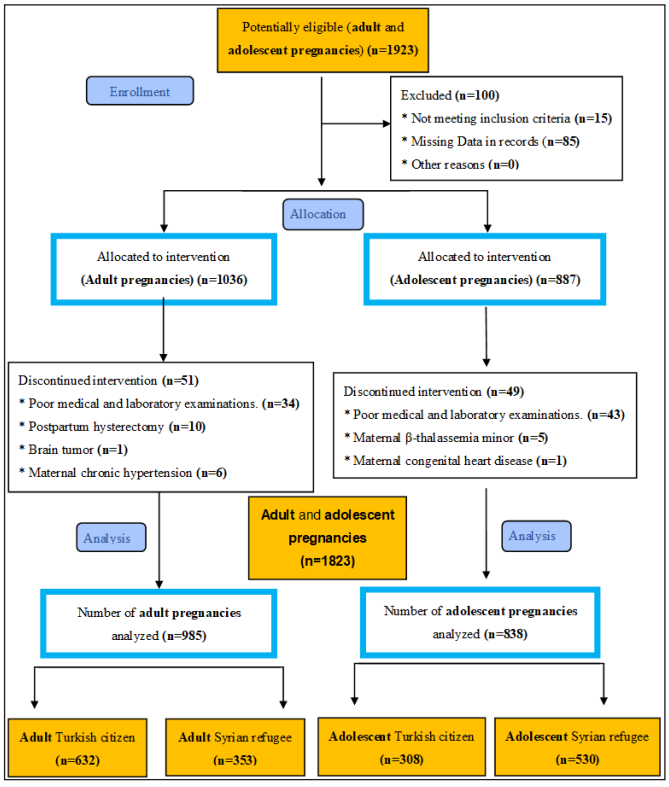

Figure 1. Flow diagram of participants

Pregnancies less than 24 weeks, pregnant women with known chronic systemic diseases (oncologic, cardiovascular, autoimmune, endocrinologic), and patients with missing laboratory and clinical information on the epicrisis form were excluded from the study. The gestational weeks of the pregnant women who took part in the study were based on their last menstrual dates and the crown-rump length values measured during the

first trimester ultrasounds (10). Our study looked at the APGAR score in the 5th minute because the APGAR score in the 1st minute could not accurately predict the newborn's prognosis. Numerous studies have connected a low APGAR score at the fifth minute to an increased risk of neonatal mortality and a broad spectrum of mental disorders (11, 12). When we looked at CS rates, group size, and contribution to CS in RTGCS (4), we tried to figure out the rise in CS rates among Syrian refugees and Turkish citizens.

Statistical Method

The data were analyzed using IBM SPSS V23. For this purpose, Pearson chi-square and Yates' correction for continuity tests were used to analyzing categorical data by nationality in adolescent and adult pregnancies. The Kolmogorov-Smirnov test was used to determine the normal distribution of quantitative data, and the Mann-Whitney U test was used to assess group comparisons. The risk variables for adolescent pregnancy were identified using logistic regression analysis. For quantitative data, the mean±standard deviation and median (minimum-maximum) were used, whereas the frequency (percent) was used for categorical data. The level of significance was set at $p<0.05$.

Power analysis: A minimum sample size of 300 in each group is necessary to detect a significant difference using this test (600 in total), considering type 1 error (alfa) of 0.05, power (1-beta) of 0.95, the effect size of 0.295, and two-sided alternative hypothesis (H1) (3). The study was completed on 838 individuals (distribution by nationality in adolescent pregnancies), and the power of the test was obtained at 98.4%.

RESULTS

The age distribution of mothers differs according to nationality in both adolescent and adult pregnant women ($p>0.05$). When analyzed according to education level, the rate of Syrian pregnant women with primary and secondary education is lower than Turkish pregnant women ($p<0.001$). Pregnancy rates in adolescent pregnant women differ according to nationality ($p<0.001$). The single pregnancy rate is higher among Turkish citizens. For Syrians, 2nd, 3rd, fourth, and eighth pregnancy rates are high. The number of pregnancies in the adult pregnant group is also related to nationality ($p<0.001$). While the first and second pregnancy rates in Turks are high, the third and fourth to eighth pregnancy rates are lower (**Table 2**)

RTGCS varies significantly between nationalities of pregnant adolescents ($p<0.001$). Syrian adolescents in Robson groups 2, 5, and 6 have a lower prevalence among Syrian adolescents, whereas Robson groups 3 and 10 have a higher prevalence. In the adult group of pregnant women, there was also a significant relationship between RTGCS and nationality ($p<0.001$). Robson's first and second groups were lower in Syrian adult pregnant women, while the third and fourth groups were higher (**Table 3**).

Birth types differ according to the adolescent pregnant group ($p<0.001$). While SVD was higher in Syrians, cesarean section rates were higher in Turks. There is no difference between the initial SVD rates. Similarly, birth types differ according to the adult pregnant group ($p<0.001$). While the rate of vaginal delivery is higher in Syrians, the cesarean section rate is higher in Turks. There

Table 2. Comparison of the number of pregnancies by nationality and educational level among adolescent and adult pregnant women

	Adolescent Pregnancies (13–19 years) (n, %)			Adult Pregnancies (20–47 years) (n, %)		
	Turkish citizen	Syrian refugee	p	Turkish citizen	Syrian refugee	p
Maternal age						
≤15	3 (1)	14 (2.6)		---	---	0.842[a]
16–19	305 (99)	516 (97.4)	0.163[b]	---	---	
20–34	---	---		533 (84.3)	296 (83.9)	
≥35	---	---		99 (15.7)	57 (16.1)	
Level of education						<0.001[a]
Illiterate	12 (3.9)[a]	104 (19.6)[b]		9 (1.4)[a]	85 (24.1)[b]	
Primary education	244 (79.2)[a]	368 (69.4)[b]	<0.001[a]	320 (50.6)[a]	241 (68.3)[b]	
Secondary education	52 (16.9)[a]	58 (10.9)[b]		251 (39.7)[a]	27 (7.6)[b]	
High education	---	---		52 (8.2)[a]	0 (0)[b]	
Number of pregnancies						
1	250 (81.2)[a]	370 (69.8)[b]		157 (24.8)[a]	51 (14.4)[b]	<0.001[a]
2	56 (18.2)[a]	131 (24.7)[b]	<0.001[a]	225 (35.6)[a]	113 (32)[a]	
3	2 (0.6)[a]	27 (5.1)[b]		107 (16.9)[a]	72 (20.4)[a]	
4–8	0 (0)[a]	2 (0.4)[a]		143 (22.6)[a]	117 (33.1)[b]	

[a]Pearson Chi-square test; bYates correction, cMann-Whitney U test; dIndependent-Samples T test; F: Fisher's Exact test; a-b: There is no difference between the ratios of columns with the same letter in each row; notation: mean±standard deviation, median (minimum—maximum) frequency (percent).

is no difference between the initial SVD rates. We found a statistically significant difference between the distribution of cesarean section indications according to nationality within the adult group (p=0.027). The abnormal fetal lie and presentation indication rate were higher in Syrian adult pregnant women. The median values of maternal hemoglobin and hematocrit differ according to nationality (p<0.001). In adolescent pregnancy, the median for Turks was higher than the median for Syrians. Likewise, among pregnant women, the median of Turkish women was higher than that of Syrians. The length of hospital stays for adolescent pregnancies varies according to nationality (p=0.009). The average length of hospital stays is higher in Turks. There is a difference in hospitalization durations according to nationality in pregnant adult women (p=0.001). Two days is the average hospital stay for pregnant women in two nationalities. However, the difference is due to the rank average. While the average rank for Turks is 513.28, the average rank for Syrians is 456.70 (**Table 4**).

Table 3. Comparison of RTGCS by nationality between adolescent and adult pregnant women

	Adolescent Pregnancies (13–19 years) (n, %)			Adult Pregnancies (20–47 years) (n, %)		
	Turkish citizen	Syrian refugee	p	Turkish citizen	Syrian refugee	p
Robson classification						
Group 1	111 (36)[a]	174 (32.8)[a]		67 (10.6)[a]	23 (6.5)[b]	
Group 2	79 (25.6)[a]	54 (10.2)[b]		55 (8.7)[a]	14 (4)[b]	
Group 3	21 (6.8)[a]	65 (12.3)[b]		135 (21.4)[a]	124 (35.1)[b]	
Group 4	10 (3.2)[a]	31 (5.8)[a]		36 (5.7)[a]	48 (13.6)[b]	
Group 5	11 (3.6)[a]	6 (1.1)[b]		190 (30.1)[a]	59 (16.7)[b]	
Group 6	4 (1.3)[a]	1 (0.2)[b]	<0.001[a]	6 (0.9)[a]	2 (0.6)[a]	<0.001[a]
Group 7	0 (0)[a]	5 (0.9)[a]		14 (2.2)[a]	14 (4)[a]	
Group 8	2 (0.6)[a]	9 (1.7)[a]		7 (1.1)[a]	2 (0.6)[a]	
Group 9	–––	–––		0 (0)[a]	2 (0.6)[a]	
Group 10	70 (22.7)[a]	185 (34.9)[b]		122 (19.3)[a]	65 (18.4)[a]	

[a]Pearson Chi-square test; bYates correction, cMann-Whitney U test; dIndependent-Samples T test; F: Fisher's Exact test; a-b: There is no difference between the ratios of columns with the same letter in each row; notation: mean±standard deviation, median (minimum – maximum) frequency (percent)

Table 4. Comparison of birth types, cesarean section indications, maternal anemia, and length of stays in hospital by nationality between adolescent and adult pregnant women

	Adolescent Pregnancies (13–19 years) (n, %)			Adult Pregnancies (20–47 years) (n, %)		
	Turkish citizen	Syrian refugee	p	Turkish citizen	Syrian refugee	p
Type of Delivery						
Cesarean Delivery	107 (34.7)[a]	115 (21.7)[b]	<0.001[a]	368 (58.2)[a]	138 (39.1)[b]	<0.001[a]
First vaginal birth	171 (55.5)[a]	300 (56.6)[a]		88 (13.9)[a]	36 (10.2)[a]	
Vaginal Birth	30 (9.7)[a]	115 (21.7)[b]		176 (27.8)[a]	179 (50.7)[b]	
Caesarean section indications						
Abnormal Fetal Lie and Presentation	12 (11.2)	3 (2.6)	0.170[a]	15 (4.1)[a]	17 (12.3)[b]	0.027[a]
Acute Fetal Distress (AFD)	35 (32.7)	40 (34.8)		47 (12.8)[a]	13 (9.4)[a]	
Cephalopelvic disproportion (CPD)	17 (15.9)	24 (20.9)		30 (8.2)[a]	7 (5.1)[a]	
Fetal Macrosomia	8 (7.5)	7 (6.1)		12 (3.3)[a]	4 (2.9)[a]	
Intrauterine Growth Restriction	2 (1.9)	2 (1.7)		5 (1.4)[a]	0 (0)[a]	
Multiple pregnancies (twin pregnancy)	2 (1.9)	9 (7.8)		6 (1.6)[a]	2 (1.4)[a]	
Placenta previa	---	---		8 (2.2)[a]	0 (0)[a]	
Placental Abruption	3 (2.8)	4 (3.5)		2 (0.5)[a]	1 (0.7)[a]	
Preeclampsia	8 (7.5)	6 (5.2)		16 (4.3)[a]	4 (2.9)[a]	
Previous Caesarean Section	20 (18.7)	20 (17.4)		227 (61.7)[a]	90 (65.2)[a]	
Length of stay in hospital / day	1.7±0.8	1.6±0.8	0.009[a]	1.9±0.7	1.8±0.8	0.001[a]
	2.0 (1.0-5.0)	1.0 (1.0-5.0)		2.0 (1.0-6.0)	2.0 (1.0-6.0)	
Maternal hemoglobin (g/dL)	11.2±1.2	10.4±1.0	<0.001[c]	11.7±1.3	10.9±1.1	<0.001[c]
	11.4 (6.8-13.3)	10.3 (6.5-12.8)		11.7 (6.6-15.5)	10.9 (6.8-14.1)	
Maternal hematocrit (%)	33.1±3.1	31.1±2.6	<0.001[c]	34.1±3.4	32.5±2.9	<0.001[d]
	33.6 (22.1-39.8)	31.1 (20.4-40.2)		34.2 (20.6-42.9)	32.9 (22.4-40.1)	

[a]Pearson Chi-square test; bYates correction, cMann-Whitney U test; dIndependent-Samples T test; F: Fisher's Exact test; a-b: There is no difference between the ratios of columns with the same letter in each row; notation: mean±standard deviation, median (minimum – maximum) frequency (percent)

In addition, there is a significant difference in newborn weight distribution according to nationality in adolescent pregnancies (p=0.001). Similarly, there is a significant difference between the median newborn heights of pregnant adolescents according to nationalities (p<0.001) (**Table 5**).

Nationality influences adolescent pregnancies (p<0.001). The probability of adolescent pregnancy is 3.081 times greater among Syrian refugees than among Turkish natives. When educational attainment is considered, the risk of adolescent pregnancy is 2.757 times greater among primary school graduates than among secondary school graduates (p<0.001). The incidence of adolescent pregnancy is 3.119 times greater among illiterate students than among secondary school graduates (p<0.001) (**Table 6**).

The probability of Syrian nationality in late preterm pregnancies is 1.51 times higher in adolescent women compared to term pregnancies. There is no statistically significant effect of the week of delivery in adult pregnancies or total (p>0.050) (**Table 7**).

Among pregnant adolescents, those with LBW are 2,041 times more likely to be of Syrian nationality than normal ones. There was no significant effect on newborn birth weight in pregnant adult women (p>0.050). Regardless of age group, LBW people are 2.33 times more likely to be Syrian citizens than normal ones (**Table 8**).

Table 5. Comparison of neonatal outcomes by nationality between adolescent and adult pregnant women

	Adolescent Pregnancies (13–19 years) (n, %)			Adult Pregnancies (20–47 years) (n, %)		
	Turkish citizen	Syrian refugee	p	Turkish citizen	Syrian refugee	p
Gestational week						
>41 weeks	2 (0.6)	2 (0.4)		3 (0.5)	0 (0)	
37–41 weeks	252 (81.8)	404 (76.2)		523 (82.8)	300 (85)	
32–36 weeks	46 (14.9)	111 (20.9)	0.217a	94 (14.9)	46 (13)	0.177a
28–31 weeks	7 (2.3)	9 (1.7)		9 (1.4)	2 (0.6)	
<28 weeks	1 (0.3)	4 (0.8)		3 (0.5)	5 (1.4)	
APGAR Score (5. minute)						
4–6	43 (14)	83 (15.7)		25 (4)	15 (4.2)	0.956b
≥7	265 (86)	447 (84.3)	0.507a	607 (96)	338 (95.8)	
Newborn weight (gram)						
≥2500 (g)	246 (79.9)a	355 (67)b		588 (93)	330 (93.5)	
LBW 1500 – 2499 (g)	55 (17.9)a	162 (30.6)b	0.001a	38 (6)	16 (4.5)	0.331a
VLBW 1000 – 1499 (g)	6 (1.9)a	9 (1.7)a		3 (0.5)	2 (0.6)	
ELBW <1000 (g)	1 (0.3)a	4 (0.8)a		3 (0.5)	5 (1.4)	
Newborn height (cm)	49.0±3.0	47.9±3.0	<0.001c	50.0±2.2	50.0±2.6	0.667c
	50.0 (36.0 – 54.0)	48.0 (33.0 – 54.0)		50.0 (35.0 – 53.0)	50.0 (34.0 – 55.0)	

[a]Pearson Chi-square test; bYates correction, cMann-Whitney U test; dIndependent-Samples T test; F: Fisher's Exact test; a-b: There is no difference between the ratios of columns with the same letter in each row; notation: mean±standard deviation, median (minimum – maximum) frequency (percent)

Table 6. Examination of risk factors influencing pregnancy in adolescents

	Adolescent Pregnancies (13-19 years) (n, %)	Adult Pregnancies (20-47 years) (n, %)	Total	OR (%95 CI)	p
Nationality					
Turkish citizen	308 (36.8)	632 (64.2)	940 (51.6)	Reference	
Syrian refugee	530 (63.2)	353 (35.8)	883 (48.4)	3.081 (2.544 – 3.731)	<0.001
Level of education					
Illiterate	116 (13.8)	94 (9.5)	210 (11.5)	3.119 (2.197 – 4.427)	<0.001
Primary education	612 (73)	561 (57)	1173 (64.3)	2.757 (2.15 – 3.536)	<0.001
Secondary education	110 (13.1)	278 (28.2)	388 (21.3)	Reference	
High education	0 (0)	52 (5.3)	52 (2.9)	---	

*Adolescent pregnancies are not included in the analysis because they are not graduates of higher education. OR (95%CI): Odds Ratio (95% Confidence Interval)

Table 7. The impact of gestational week on nationality

Pregnancies	Birth (weeks)	Nationality		Total	OR (%95 CI)	p
		Syrian refugee	Turkish citizen			
Adolescent	Term	406 (76.6)	254 (82.5)	660 (78.8)	Reference	
	Late preterm	111 (20.9)	46 (14.9)	157 (18.7)	1.51 (1.035 – 2.203)	0.033
	Very Preterm	9 (1.7)	7 (2.3)	16 (1.9)	0.804 (0.296 – 2.187)	0.67
	Extremely Preterm	4 (0.8)	1 (0.3)	5 (0.6)	2.502 (0.278 – 22.515)	0.413
Adult	Term	300 (85)	526 (83.2)	826 (83.9)	Reference	
	Late preterm	46 (13)	94 (14.9)	140 (14.2)	0.858 (0.587 – 1.255)	0.43
	Very Preterm	2 (0.6)	9 (1.4)	11 (1.1)	0.39 (0.084 – 1.815)	0.23
	Extremely Preterm	5 (1.4)	3 (0.5)	8 (0.8)	2.922 (0.693 – 12.314)	0.144
Total	Term	706 (80)	780 (83)	1486 (81.5)	Reference	
	Late preterm	157 (17.8)	140 (14.9)	297 (16.3)	1.239 (0.965 – 1.59)	0.092
	Very Preterm	11 (1.2)	16 (1.7)	27 (1.5)	0.76 (0.35 – 1.648)	0.486
	Extremely Preterm	9 (1)	4 (0.4)	13 (0.7)	2.486 (0.762 – 8.108)	0.131

*Adolescent pregnancies are not included in the analysis because they are not graduates of higher education. OR (95%CI): Odds Ratio (95% Confidence Interval)

Table 8. The impact of newborn weight on nationality.

Pregnancies	Newborn weight (gram)	Nationality		Total	OR (%95 CI)	p
		Syrian refugee	Turkish citizen			
Adolescent	≥2500 (g)	355 (67)	246 (79.9)	601 (71.7)	Reference	
	LBW 1500 – 2499 (g)	162 (30.6)	55 (17.9)	217 (25.9)	2.041 (1.444 – 2.886)	<0.001
	VLBW 1000 – 1499 (g)	9 (1.7)	6 (1.9)	15 (1.8)	1.039 (0.365 – 2.958)	0.942
	ELBW <1000 (g)	4 (0.8)	1 (0.3)	5 (0.6)	2.772 (0.308 – 24.949)	0.363
Adult	≥2500 (g)	330 (93.5)	588 (93)	918 (93.2)	Reference	
	LBW 1500 – 2499 (g)	16 (4.5)	38 (6)	54 (5.5)	0.75 (0.412 – 1.366)	0.347
	VLBW 1000 – 1499 (g)	2 (0.6)	3 (0.5)	5 (0.5)	1.188 (0.197 – 7.145)	0.851
	ELBW <1000 (g)	5 (1.4)	3 (0.5)	8 (0.8)	2.97 (0.705 – 12.505)	0.138
Total	≥2500 (g)	685 (77.6)	834 (88.7)	1519 (83.3)	Reference	
	LBW 1500 – 2499 (g)	178 (20.2)	93 (9.9)	271 (14.9)	2.33 (1.778 – 3.054)	<0.001
	VLBW 1000 – 1499 (g)	11 (1.2)	9 (1)	20 (1.1)	1.488 (0.613 – 3.612)	0.38
	ELBW <1000 (g)	9 (1)	4 (0.4)	13 (0.7)	2.739 (0.84 – 8.934)	0.095

*Adolescent pregnancies are not included in the analysis because they are not graduates of higher education. OR (95%CI): Odds Ratio (95% Confidence Interval).

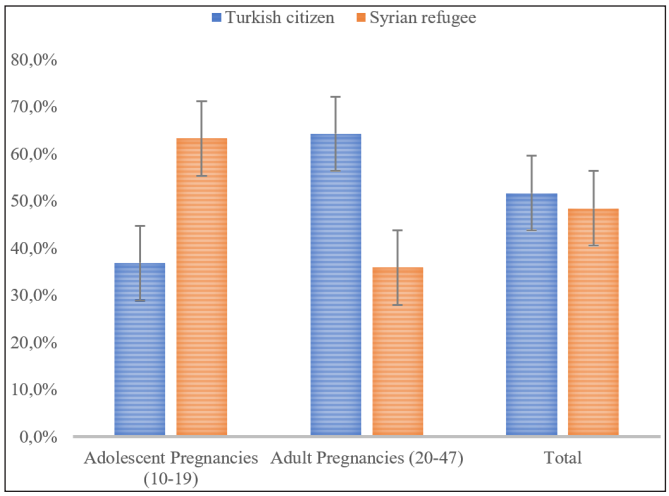

Figure 2. Percentage distribution of nationalities.

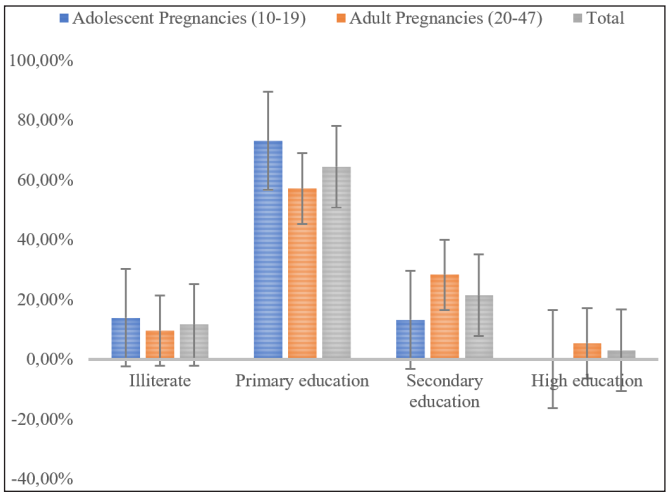

Figure 3. Percentage distribution of education level.

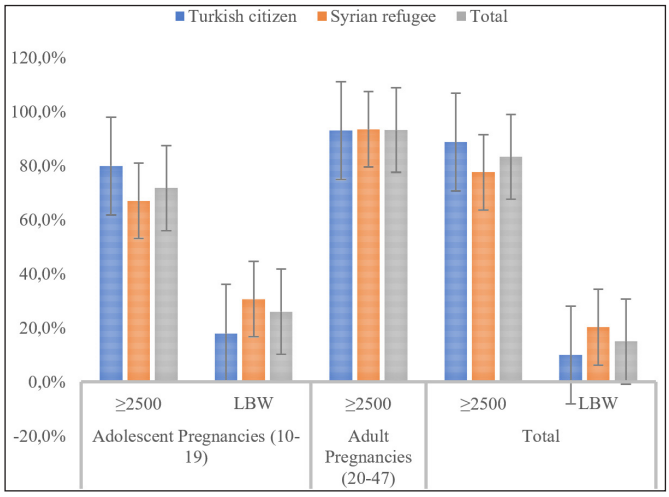

Figure 4. Percentage distribution of birth weight by nationality.

DISCUSSION

This study aimed to compare the clinical features and obstetric and neonatal outcomes of Turkish and Syrian adult and adolescent pregnant women. Also, we used the RTGCS to compare and analyze the birth characteristics of Turkish and Syrian pregnant adolescents and adults. According to the results of our study, adolescent pregnancy is exceptionally high among low-education young females and Syrian adolescent girls. Pregnant adolescent women are more likely than pregnant adult women of reproductive age to experience obstetric and neonatal complications. Robson Group 10 is more common in Syrian adolescent pregnant women than in Turkish pregnant women. This means that Syrian adolescent pregnant women have a higher rate of preterm births than Turkish adolescent pregnant women.

Our study shows that the risk of adolescent pregnancy is three times higher in Syrian refugees than in Turkish citizens ($p<0.001$). While the average fertility rate is 2.3 in Turkey, the regional fertility rate reaches the highest value at 3.2 in Eastern Anatolia. The total fertility rate among Syrian refugees has been 5.3, and 93% of births occur in health facilities (13). This shows that countries hosting numerous Syrian refugees, especially Turkey, need to make severe social, economic, political, and security plans and take comprehensive measures (13). Vural et al. (14) showed in their study that pregnant Syrian refugee women are younger and have a shorter gestation period. They also underlined those Syrian refugees have a higher risk of adolescent pregnancy. Similar studies in the literature support our findings (15,16). According to Turkay et al. (3), the pregnancy rate among young Syrian refugees was 10.8%, and the pregnancy rate of the Turkish adolescent population was 2.4%. In our study, while this rate is 63.2% for Syrians, it is 36.8% for Turks. This difference is due to the different regional distribution of Syrian refugees.

When our study is analyzed by education level, primary school graduates have a 2.7 times higher risk of adolescent pregnancy than secondary school graduates ($p<0.001$). Fertility increases as the education level falls, and illiterate women have two more children than women who have achieved a high school diploma or above (17). According to certain studies, education level may even affect preterm birth rates (18,19). It is even stated that the high CS rates in Turkey may be affected by education and literacy levels (20).

When we compared teenage and adult pregnancies by nationality in our study, we determined Syrian refugees had a greater rate of pregnancies and thus a higher rate of live births than Turkish residents. While the single pregnancy rate in the adolescent pregnant group is high in Turks, the rate of two or more pregnancies is higher in Syrians. In the adult population, Turkish women have a greater rate of first and second pregnancies, while Syrian women have a higher rate of three or more pregnancies. While we found no difference between the number of pregnancies among Syrian adolescents and Turkish adolescent pregnancies in Turkay et al. (3), we found a significant difference between the 1st, second, and third pregnancies. This difference is due to the different regional distribution and socio-cultural levels of Syrian refugees. In a similar study, Sayili et al. (21) found that Syrian refugees had a considerably greater parity than Turkish citizens ($p=0.010$) and a significantly shorter first gestational age ($p=0.034$).

In our present study, according to RTGCS (5), the high rate of Robson Group 10 among Syrian adolescent pregnant women shows that Syrian adolescent pregnant women give birth more prematurely than Turkish adolescent pregnant women. In the study by Vural et al. (14), the causes of premature births, which are more common in Syrian pregnant women, were associated with inadequate antenatal care, unemployment, malnutrition, inadequate iron supplementation, and low socioeconomic and cultural levels. In Robson Group 1, the proportion of Turkish adult pregnant women is higher than that of Syrian adult pregnant women. This could be because Turkish pregnant women become pregnant at a older age (15,16). This is probably the fear of childbirth, low sociocultural level, the pressure of malpractice laws on physicians, and especially Turkish women's negative expectations about SVD (22).

In our study, when we compared adolescent and adult pregnant women according to their nationalities, Robson Group 5 rates in RTGCS were higher in favor of Turkish pregnant women. Due to the increasing contribution of Robson Group 5 to overall cesarean rates, more women will need to have repeat cesarean sections in the future (23). Tontus et al. (24) observed that each CS delivery

in Robson groups 1-4 results in the addition of new cesarean candidates to Robson group 5, which already has the highest cesarean rate in subsequent pregnancies. As a result of Group 5's increased contribution to overall CS rates (23), more women will require a repeat CS as CS rates rise. Lefevre (25) asserts that various factors influence an obstetrician's attitude toward cesarean delivery. These factors include CSs being more profitable than SVDs due to financial incentives, the fear of malpractice, and physicians' desire to spend more time socializing.

When teenage and adult pregnancies were compared according to their national origins, we determined that Syrian pregnant women had a greater rate of SVD. According to Birge et al. (26), the rate of CS in adolescent pregnancies in Turkey is 36.7 percent, and 20.1 percent in Syrian adolescent pregnancies. In their study (26), they reported that CS rates were considerably higher ($p<0.001$) in Turkish adolescent pregnancies. In their study, Karacam et al. (27) discussed the rise in CS rates in Turks. More women are having CS surgery, an increase in CS surgeries, fear of vaginal birth by expectant mothers, IVF pregnancies, and more pregnant women over the age of 35. Turkay et al. (3), reported no difference in cesarean delivery rates between Syrian adolescent pregnant women and Turkish adolescent pregnant women. On the contrary, our study found a substantial difference in the proportion of CS of teenage Syrian refugees (21.7%) compared to Turkish adolescents (34.7%). Another result of our study is that while there is no difference between the first SVD rates of Syrian and Turkish adolescent pregnant women, subsequent vaginal delivery rates are higher than Syrian adolescent women (21.7% vs. 9.7%). It is seen that adolescent women are adversely affected not only by their first pregnancy and first birth but also by subsequent pregnancies and births. Therefore, adolescent pregnancies are also a risk factor for subsequent pregnancies and births of young mothers (28).

In this current study, we saw that the indications for cesarean section among adolescent pregnant women are not different between Turks and Syrians. When the adult pregnancy group was examined, we found that only the abnormal fetal lie and presentation rates were higher in favor of Syrian pregnant women.

According to our study, pregnant adults had a greater rate of previous CS than adolescent pregnant women, regardless of their nationality. Consistent with the literature, although there is no statistically significant difference between Turkish and Syrian pregnant women, the most common reason for CS indications is previous CS (14,29). Considering this data, if we want to decrease the CS rates in line with the WHO recommendations (9), we must first decrease the primary CS rates.

In our study, when adolescent and adult pregnancies were analyzed by nationality, the length of hospital stays was remarkably higher in favor of Turkish pregnant women in both groups. Vural et al.'s (14) study showed that Turkish pregnant women have a more extended hospitalization period than Syrian pregnant women. In our country, by WHO standards (30), we require women and newborns to stay in the hospital for at least 24 hours following SVD and at least 48 hours following cesarean delivery in the absence of postpartum problems. Because of the high rate of CS among pregnant Turkish women, we believe that this difference is reflected in the length of hospital stays.

Anemia adjusted for altitude and smoking was defined by the WHO (31) as values less than 11 mg/dL in pregnant women. In our study, when adolescent and adult pregnancies were compared according to their nationalities, the hemoglobin ratios of Syrian refugee pregnant women were lower. In their meta-analysis study, Karacam et al. (27) reported lower hemoglobin values in adolescent pregnancies ($p<0.001$). Contrary to our study, in Genc et al. (32), Hb and Htc values in adolescent pregnant women and adult pregnant women were within the WHO-recommended ranges, and they observed no significant difference. We can attribute the high rate of anemia in adolescent pregnancies to their low education level and low socioeconomic status. Adolescent pregnant women may not be aware of the necessity of regular prenatal follow-ups, oral iron supplementation, and laboratory tests to prevent anemia (14).

In our study, we found a proportionate difference in gestational weeks between teenage and adult pregnant women regardless of their nationality. While the delivery of adult pregnancies ended at the 37th and 41st gestational weeks, the delivery of adolescent pregnancies took place at the 32nd and 36th gestational weeks. We found that the probability of Syrian nationality in late preterm delivery in adolescent pregnancies is 1.51 times higher than at the term gestational week. In the study by Genc et al. In the study by Genc et al. (32), pregnant women aged 16 years and younger had a shorter gestational week. They noted in the same study that the pervasiveness of premature delivery was substantially higher in teenage pregnant women than in adult pregnant women ($p=0.0001$). In the study of Vural et al. (14), the prevalence of preterm birth was higher among Syrian refugee women, and they associated it with low sociocultural level, inadequate pre-pregnancy care, malnutrition, and post-traumatic stress disorder. In the study by Turkay et al. (3), as with our study, no difference in preterm birth rates was observed between Syrian and Turkish teenagers. In Karacam et al.'s meta-analysis study (27), they stated that preterm birth rates are 2.12 times more common in adolescent

pregnancies, and gestational weeks are shorter than adult pregnancies (p<0.001). According to Korencan et al. (33), preterm birth rates are higher in adolescent pregnant women than in adult pregnant women. The possible cause of premature birth in adolescent pregnant women is related to increased prostaglandin secretion due to insufficient uterine cervical blood flow. Other factors influencing the frequency of preterm birth in adolescent pregnancies include a small uterine volume and a short uterine cervical length of less than 25 mm (33).

When we compared the 5th minute APGAR scores of newborns born to teenage and adult pregnant women based on their nationality, we discovered that Turkish newborns had APGAR scores of 7 and above. We determined that neonates of pregnant adults, regardless of their nationality, had higher 5th minute APGAR ratings than newborns of teenage pregnant women. In the study published by Vural et al. (14), the APGAR score in infants of Syrian refugees was found to be below 7, like our study. As in Turkay et al.'s (3) study, there was no difference in our study's APGAR score rates between Syrian and Turkish adolescents. As a result, we should not forget that babies born to Syrian refugees and pregnant teenagers may need the right equipment and medical attention after birth for possible resuscitation.

In our study, newborns who were born with an LBW were twice as likely to be Syrian nationals as those born with a normal birth weight (p<0.001). However, we identified that Syrian teenage pregnant women's newborns have a lower birth weight than Turkish teenage pregnant women's newborns. Similarly, when we compared the neonates of Turkish and Syrian pregnant women, we determined that Syrian newborns had a lower birth weight than Turkish newborns, regardless of their age. When the weights of neonates born to teenage and adult pregnant women were compared, we revealed that all infants born to teenage pregnant women were underweight, except for newborns weighing less than 1000 g. We identified no difference in preterm birth rates between a Syrian refugee and Turkish pregnant women regardless of age, but a significant difference in favor of Syrian pregnant women between 1500 g and <2500 g in LBW newborns. In our study, newborns born with a LBW were twice as likely to be Syrian nationals as those born with an average birth weight (p<0.001). Regardless of age, newborns with LBW were 2.3 times more likely to be Syrian nationals (p<0.001). Genc et al. (32) showed that infants of adolescent pregnant women gave birth to lower fetal weights and had a higher risk of LBW than infants of adult pregnant women. According to Moraes et al. (34), he attributes this to the fact that adolescent girls at the age of growth and development must share the nutrients they need with their babies. Although the risk of late preterm delivery (34th–36th gestational weeks) is significant in Syrian pregnant women, the average birth weight of the babies is over 2500 g, according to Vural et al. (14). This is because LBW is caused by preterm birth, IUGR, or both (35). The term LBW means <2500 g absolute weight regardless of gestational week. LBW is an important indicator of maternal health, malnutrition, access to health care, and low socioeconomic status (36). Infants born with LBW are twenty times more likely to die than those born with more than 2500 g (37). Economic studies in low-income countries have shown that cutting down on the LBW burden will save a lot of money for the healthcare system (35).

Strengths and Limitations

Among the study's strengths was the high-quality index of publications against which we compared our results. Our study includes a significant number of patients evaluating Syrian and Turkish adolescent pregnant women. Numerous limitations apply to our investigation. In the information note it published (38), WHO drew attention to maternal mortality rates due to adolescent pregnancies. However, in our study, we realized that we needed to conduct a separate study with the anesthesia and reanimation clinic of our hospital to get sufficient data and evidence about maternal mortality due to adolescent pregnancies. Additionally, the limited sample size of our adolescent pregnant group of young girls aged 10–15 years may introduce bias into the results.

CONCLUSION

As a consequence of our study, the maternal age of pregnant Syrians is younger, and the risk of adolescent pregnancy is threefold that of Turkish nationals. According to education level, the risk of adolescent pregnancy in primary school graduates is approximately three times higher than that of secondary school graduates. According to the RTGCS, Syrian refugees and adolescent pregnancies have higher rates of preterm birth. Among adolescent pregnant women, the probability of late–preterm pregnant women being Syrian nationals is one and a half times higher than that of term pregnant women. Among adolescents who have given birth, infants with LBW are almost twice as likely to be Syrian nationals. Syrian pregnant women give birth to approximately two and a half times more LBW babies than Turkish pregnant women.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Health Sciences University Adana City Training and Researches Hospital Clinical Research Ethics Committee (Date: 26.08.2020, Decision No: 1046).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Clinical value of heart type fatty acid binding protein (H-FABP) in acute pulmonary thromboembolism

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ABSTACT

Introduction: To investigate factors which could possibly be prognostic, to decide on thrombolytic therapy as soon as possible, and to determine the prognostic value of H-FABP, which is a new marker, in pulmonary thromboembolism.

Material and Method: In our study, a patient group, consisting of 58 patients diagnosed with PTE and a control group of 30 healthy individuals were investigated. According to their risk of mortality, patients were analyzed in three groups: high, moderate and low. The moderate mortality risk groups were categorized into two groups according to PAP and patients with PAP≥45 mmHg were "Group 1" and PAP<45 mmHg were "Group 2". Differences in levels of H-FABP and other cardiac prognostic markers between these groups were analyzed.

Results: H-FABP level was measured as 507.6±99.3 pg/ml in the control group and 3203.1±2389.3 pg/ml in the patient group. H-FABP level was found to be the highest in the high mortality risk group, and the lowest in the low mortality risk group ($p<0.001$). Moreover, in the subgroups of moderate mortality risk group, H-FABP levels were significantly higher in group 1 compared to group 2. For the evaluation of other cardiac markers in PTE subgroups, pro-BNP level was the highest in the high mortality risk group, and the lowest in the low mortality risk group.

Conclusion: The findings in this study show that, H-FABP is a superior marker in determining the prognosis compared to pro-BNP and troponin. High PAP level is one of the important prognostic markers that should be considered along with the electrocardiography findings.

Keywords: Pulmonary thromboembolism, H-FABP, cardiac enzymes, prognostic factors, thrombolytic treatment

INTRODUCTION

Pulmoner tromboembolism (PTE) is one of the most common diseases that is difficult to be diagnosed and that has high mortality and morbidity (1,2).

The diagnosis of PTE and initiation of proper treatment requires urgency. The severity of the disease should be determined swiftly because it might change the treatment approach for the patients diagnosed with PTE (3). In assessment of the severity of the disease, the following approaches are used: clinical evaluation (e.g., hyper-tension, shock), electrocardiograph (ECO), thorax computerized tomography angiography, brain natriuretic peptides (BNP), and cardiac troponins (4,5).

In instances of cardiac injury, heart type fatty acid binding protein (H-FABP) is released to blood stream faster than troponins and it shows up in blood circulation approximately 30 minutes after the symptom emerges. It reaches peak levels in six hours. Recent studies on PTE diagnosed patients suggest that H-FABP levels during first introductioncan predict early negative clinical incidents with higher accuracy and specificity than cardiac troponin can do (6-8). Moreover, H-FABP levels in Chronic thromboembolic pulmonary hypertension patients are found to be associated with risk of death in the long run (6,9). The main purpose in this study is to research the changes in H-FABP levels, as a new marker, based on the type of pulmonary thromboembolism and compare it with other prognostic markers.

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MATERIAL AND METHOD

The study was carried out with the permission of Yıldırım Beyazıt University Non-Interventional Clinical Researches Ethics Committee (Date: 18/06/2014; Decision No: 19). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. This study was conducted from January 2015 through June 2016 as part of a prospective study in our hospital.

Patients Inclusion and Study Design

The sample consists of 58 patients and a randomly selected healthy 30 individuals to serve as the control group. Patients with hematologic problems, those below the age of 18 and pregnant patients are not included in the study. Further, patients diagnosed with acute myocardial infarction and patients diagnosed with heavy cardiac insufficiency are not also included in the study because the H-FABP levels of such patients could increase as a result of their condition. Patients with systemic illness history are not included in the control group. All patients diagnosed with pulmonary thromboembolic disease using multi-sectorial (with 16 and 64 detectors) thorax computed tomography pulmonary angiography are included in the study.

Patients with venous thrombus are identified employing lower- and upper-extremity venous doppler ultrasonography. ECO results and all symptoms indicated by the patients are recorded along with the demographic characteristics such as age, sex, profession, symptoms on hospital admission, history of risk factors, vital signs, D-dimer, troponin T, CK-MB, Pro-BNP and CRP levels and other background information on the patients are also recorded.

The patients are classified into three mortality risk categories: (1) High risk, (2) Middle risk, (3) Low risk, based on the Acute Pulmonary Embolite Diagnosis and Treatment Manual, published by European Society of Cardiology in 2019 (4). Additionally, the patients are also subjected to a second layer of classification based on their pulmoner artery pressure (PAP) value: those who have a PAP value of 45mm Hg and above are classified in Group 1 (high mortality risk), those with a PAP value less than 45 mm Hg are considered in Group 2 (low mortality risk). Distribution of the patients in these groups are shown in **Table 1**. We have examined the differences of major cardiac prognostic markers such as H-FABP levels, troponin T, creatine kinase-muscle/brain /(CK-MB), pro-brain natriuretic peptide (pro-BNP).

Biochemical Analysis

We take 3 cc blood from PTE patients at the time of hospitalization using tubes containing lithium heparin and then analyse them in our biochemistry laboratory in less than two hours. Therefore, we have also analysed in these blood samples from the PTE patients to measure the the H-FABP levels with the sandwich method using Hycult biotech HK 401 and Human H-FABP Elisa kits.

Statistical Analysis

Statistical analyses were performed with SPSS for Windows version 17.0 (SPSS Inc., Chicago, III., USA). The Kolmogorov-Smirnov test was applied to determine the probability distribution. In comparing the mean differences between two groups, we used Student's t test, more than two groups we used One-Way-ANOVA (Analysis of Variance) when there were. Relationship between two quantitative variables is tested using Spearman's Correlation test. We have accepted $p<0.05$ as the threshold value for statistical significance.

RESULTS

The mean ages of the patient and control groups are 63.8±16.1 and 62.1±18.2, respectively. Control groups have not yielded any significant differences ($p>0.05$). The patient group was composed of 32 females (55.2%) and 26 males (44.8%). The control group was comprised of 13 females (43.3%) and 17 males (56.7%). Chi square test results have not shown any significant relationship between sexes and groups ($p>0.05$).

When we compared the pulmoner artery pressure (PAP) among different mortality risk groups of PTE patients, we found that patients in the high-risk group to have higher PAP values (mean: 55±8.7; range: 50-80) than those in the low-risk group and those in Group 2 of the moderate risk group ($p<0.001$) (**Table 2**).

The most common symptom at the time of first arrival to the hospital were shortness of breath (93.1%) and chest pain (55.2%). The frequency of syncope and chest pain among high mortality risk group was significantly higher than other PTE groups ($p<0.05$).

Table 1. Classification of patients based on mortality risk

	(n)	Shock or hipertension[a]	Right ventricle dysfunctions (ECO findings)
High risk (Massive)	15	+	+
Moderate risk (Submasssive)	Group 1	13	(+)b
	Group 2	12	(+)c
Low risk (Low emboli)	18	-	-

a: 40mm Hg decrease in daily average arterial tension or having an arterial tension of ≤90/60 mm Hg. b: If PAP≥45mm Hg c: If PAP<45mm Hg

Table 2. Distribution of pulmonary emboli groups based on PAP levels

		High risk (n=15)	Moderate risk		Low risk (n=18)	Significance
			Group 1 (n=13)	Group 2 (n=12)		
Sex	Female/Male	6/9	5/8	6/6	9/9	0.878
PAP	45mm Hg<n	0	0	12 (%100)	18 (%100)	<0.001
	45 mm Hg≥n	15 (%100)	13 (%100)	0	0	
PAP value* (mm Hg)		55±8.7 (50-80)	55 ±9.4 (45-70)	40± 4.6 (35-40)	Normal	<0.001

*: PAP values include mean±standard deviation and the minimum & maximum in parenthesis

The vital symptoms of the patients were found the following measures: an average pulsation count of 117±15/min, mean systolic tension of 112±16 mm Hg, mean diastolic tension of 71±12 mm Hg, an average respiratory rate of 24±6.8/min, and 88.6%±6.7 oxygen saturation rate. Significant differences were detected in vital measures across PTE sub-groups. For instance, the average number of puls and respiratory rates of patients in high risk group were significantly higher than all other PTE groups (p<0.001). The oxygen-free saturation among patients in the low mortality risk group were significantly higher than other PTE groups (p<0.001). Likewise, the systolic and diastolic blood pressure of patients in the low-risk group was significantly higher than other patients (p<0.001).

With radiological tests, we have detected segmentary thrombus in 26 patients (44.8%), sub-segmentary thrombus in 14 patients (24.1%), thrombus at the bilateral main pulmonary artheries in 13 patients (22.4%), thrombus at unilateral main pulmonary arthery in 5 patients (8.6%).

The amount of radiological congestion of thrombus across PTE groups showed significant differences. The existence of thrombus at the bilateral main pulmanory arthery among patients in the high mortality risk group was significantly higher than other groups (p<0.001). Moreover, the H-FABP levels of 6560.3 (1959.6-7912) pg/ml, segmentary value 1997.5 (446.3-6210) pg/ml, and sub-segmentary valu e of 535.5 (446.6-3071) pg/ml among patients with thrombus at their bilateral pulmonary arthery were significantly higher than those with thrombus at their pulmonary arthery(p<0.001).

The H-FABP level in the patient group was significantly higher than the H-FABP level in the control group (p<0.001). An analysis of the H-FABP levels across PTE sub-groups showed significant differences (p<0.001). Besides, H-FABP level was higher in the Group 1 than it was in the Group 2 of the moderate risk group (p<0.001). The **Figure 1** is scatter plot diagram that shows the H-FABP levels across patient and control groups.

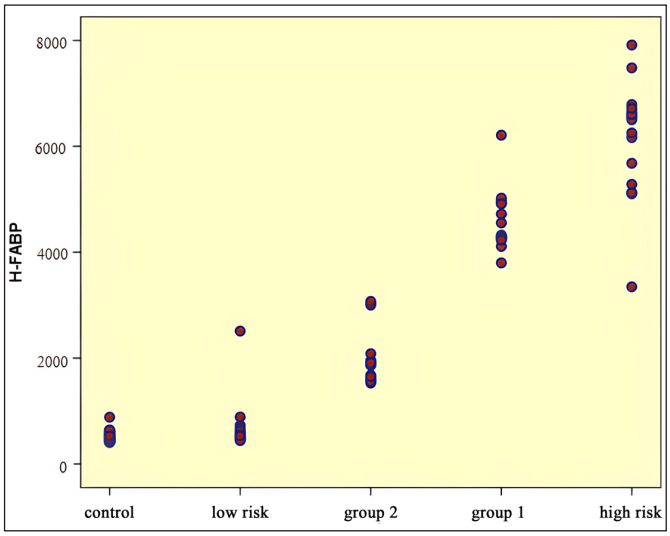

Figure 1. H-FABP levels in patient and control groups

Cardiac markers varied tremendously among PTE sub-groups (**Table 3**). For example, Pro-BNP level in high mortality risk group was the highes (3678 pg/ml) while it was the lowest in the low risk group (60 pg/ml). Troponin T level significantly higher in the high-risk group than both the moderate and low risk groups (p<0.001). Neither troponin T nor pro-BNP levels showed any significant differences between Group 1 and Group 2 of the moderate risk group. However, H-FABP levels were significantly higher than Group 2 (p<0.001).

Table 3. Cardiac markers, D-dimer & CRP levels across PTE groups

	Normal values	High risk (n=15)	Moderate risk		Low risk (n=18)
			Group 1 (n=13)	Group 2 (n=12)	
H-FABP (pg/ml)	*	6503.9 (3346.3-7912)	4553.0 (3798-6210)	1876.6 (1525.2-3071)	520.8 (446.3-886.8)
Pro-BNP (pg/ml)	0-198	3678 (102-9000)	320 (60-5578)	153.5 (60-9000)	60 (47-710)
Troponin T (pg/ml)	0-14	16 (3-55)	14 (7-63)	11 (7.5-19)	9.5 (3-66)
CK-MB (ng/ml)	0-4.9	2.3 (0.3-6.2)	1.6 (0.3-2.6)	1.5 (0.1-6.6)	1.2 (0.3-2.8)
D-dimer (ng/ml)	0-500	9300 (2300-10000)	3089 (1400-10000)	3421.5 (835-10000)	1590 (323-5632)
CRP (mg/dl)	0-0.8	9.4 (5.4-25)	8.3 (1.9-14)	6.7 (2.6-16.8)	2.0 (0.5-4.0)

When we divide the sample into two groups based on H-FABP levels, accepting 4000 pg/ml as the threshold value, we have found that having H-FABP values of 4000 pg/ml and above was related to a constellation of symptoms, accompanying venous thrombus, and atrial fibrillation in electrocardiography (ECG) (**Table 4**).

In this study, we have found significant positive correlations between H-FABP and PAP, pro-BNP, troponin T level, and oxygen saturation. The correlation of H-FABP with tropenin T was a slight correlation, with pro-BNP there was a mild correlation, and with PAP there was a strong correlation. With increases in PAP, pro-BNP, troponin T, and oxygen saturation levels, the H-FABP level was also increasing (**Figure 2**).

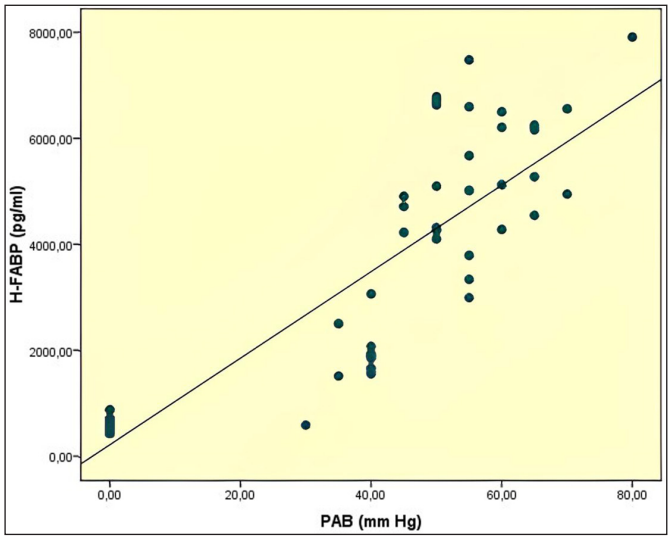

Figure 2. Correlation between H-FABP levels and PAP scores

DISCUSSION

In this study, we have found that the H-FABP level is a superior marker in determining prognosis compared to troponin T and pro-BNP because of H-FABP having a faster septicemia than other cardiac markers such as the aforementioned ones and having significant differences across all PTE sub-groups and having been influenced from PAP levels.

It is very important to determine the severity of the disease rapidly in order to decide treatment for patients diagnosed with PTE. In this way, we can make thrombolytic treatment decisions for required patients because it is scientifically proven that thrombolytic treatment reduces PTE related mortality and recurrence (10). In 2019, this document was revised and updated with the 2019 ESC Guidelines on the Diagnosis and Management of Acute Pulmonary Embolism (4,11). In this guide, mortality risk of acute pulmunorythromboly was groupped into three categories: high risk, moderate risk, and low risk. The moderate mortality group was further divided into low risk and high-risk groups. In this new classification, thrombolytic treatment is recommended for the high-risk sub-group of the moderate mortality risk group. Thus, while acute pulmunorythromboly was considered in two groups and only those in the high mortality risk group was offered thrombolytic treatment, now the number of patients for whom thrombolytic treatment decision could be made has been expanded with the new classification of three risk groups.

It is recommended to use PESI, sPESI, right ventricular dysfunction indicators and cardiac markers in classifying patients. From cardiac markers, BNP and NT-proBNP are released from the ventricule muscles as soon as high

Table 4. Comparison of major parameters that are influential in selecting the patient group that could have worse prognosis condition for thrombolic treatment decision of acute PTE patients across two H-FABP Levels

	H-FABP <4000 pg/ml (n=32)	H-FABP<4000 pg/ml (n=26)	Significance
Existence of malignite	3 (9.4%)	10 (38.5%)	0.008
Shortage of breath	28 (87.5%)	26 (100.0%)	0.120
Chest pain1	12 (37.5%)	20 (76.9%)	0.003
Syncope	0	5 (19.2%)	0.014
Hemoptysis	4 (12.5%)	2 (7.7%)	0.681
Sputum	13 (40.6%)	3 (11.5%)	0.014
Flank pain	18 (56.3%)	8 (30.8%)	0.052
Hipertansion2 or shock	1 (3.1%)	14 (53.8%)	<0.001
Heartbeat per minute	109.3±13.3	127.0±11.1	<0.001
Respiratory rate per minute	19.8±4.4	30.7±3.8	<0.001
Oxygen-free saturation3(%)	93.6±3.1	82.5±4.5	<0.001
Existence of deep venous thrombosis	17 (53.1%)	24 (92.3%)	<0.001
Existence of atrial fibrillation in ECO (electrocardiography)	5 (15.6%)	13 (50.0%)	0.005
PAP (mm Hg)4	0 (0-55)	55 (45-80)	<0.001

1: Existence of chest pain that cause tightness underneath sternum
2: 40mm Hg decrease in daily average arterial tension or having an arterial tension of ≤90/60 mm Hg
3: Figures include means and standard deviations
4: Figures include median and minimum-maximum in parentheses.

ventricule pressure is reached. Therefore, the level of BNP that is reased to the serum might increase when there is tension with the RV muscle fibresand when myocardial hypoxemia takes place (12). In a study conducted by Cavallazzi and his colleagues, it was found that high levels of BNP and NT-proBNP are associated with mortality and right ventricular dysfunction (13). In a study by Kostrubiec and his colleagues, while none of the patients with NT-proBNP <600 pg/ml levels had mortality or any complications, it was found that the mortality was increased by 6.7 times when the patients had NT-proBNP >7600 pg/ml levels (14). In our current study, pro-BNP increased significantly with pulmunory thrombolism and this increase was significantly the highest in the high mortality risk group and the lowest in the low mortality risk group. These findings are in line with other studies in the literature. Further, have found a positive milde correlation between H-FABP and pro-BNP. With an increase in pro-BNP the H-FABP levels was increasing.

Troponin I and T and a new marker, H-FABP are used as myocard injury markers. In Management Strategies and Prognosis of Pulmonary Embolism (MAPETT 2) study, a significant relationship between high troponin levels and mortality was found and because of this finding the authors proposed troponin as an indicator in predicting right ventricular microinfarctus. Increased serum troponin level is evidence for RV dysfunction (15). In a study conducted by Golpe and his colleagues, the authors report that high troponin I levels among hemodynamically stable PTE patients are associated with the seriousness of the disease (16). In another study by Sanchez and his colleagues, increased troponin levels were found to increase mortality 5.2 times in normotensive PTE patients (17). In our study, we found that troponin T level in low mortality risk group was significantly lower than moderate and high mortality risk groups. However, there was not a significant difference in troponin T levels between high and moderate mortality risk groups. Likewise, there was not any significant difference between Group 1 and Group 2 of the moderate mortality risk group. We have found a weak positive correlation between H-FABP and troponin T levels. As the troponin T levels increased, so did the H-FABP levels.

Recent studies in the literature show that H-FABP is a superior marker for acute PTE cases compared to troponin T and pro-BNP (18-20). Dellas and his colleagues' study on a sample of PTE patients found that, as one of the important markers of right ventricular dysfunction, H-FABP has 89% sensitivity, 82% specifity, 99% negative predictive value. In the same study, the authors also mention the value of utilizing H-FABP with tachycardiain prognosis (18). However, in another study by Jenab and his colleagues, patients and in the presence of NT-proBNP association was not with the short-term adverse events and long-term mortality in terms of H-FABP. The authors explained these contradictions with relatively low number of cases or the different inclusion criteria for their study (21).

We found significantly higher H-FABP values in Group 1 (PAP ≥45 mmHg) than Group 2 (PAP <45 mmHg). Otherwise, we could not find any significant differences between Group 1 and 2 in terms of troponin T and proBNP levels. There is enough research evidence on H-FABP to be a superior marker in PTE cases. Having higher values of H-FABP among patients with high pulmunory artery pressure led us to think that a high PAP value is one of the influential parameters on prognosis, but its value might be unnoticed when used together with other cardiac markers.

Moreover, in our research we have found a very strong positive correlation between PAP and H-FABP. H-FABP was increasing in parallel with the increase in PAP values. Particularly, having significantly high H-FABP values when PAP was equal to or higher than 45mm Hg values can be interpreted as an indicator of microinfarction and right ventricular infarction with the patients.

When the markers for right ventricular dysfunction are defined in 2019 ESC Guidelines on the Diagnosis and Management of Acute Pulmonary Embolism, we do not see any prognostic evaluation regarding PAP. In our study, we have proved that high PAP should be considered as a bad prognostic parameter in addition to the right ventricular dysfunctions in ECO. In a study conducted by Qian and his colleagues, significantly higher PAP values were found in high mortality risk group than other groups (9).

In another study on PTE cases, 6000 pg/ml was found as the cut-off threshold value for H-FABP level (18). In our study, we have considered this threshold value to be around 4000 pg/ml. With patients whose H-FABP value was 4000 pg/ml and above, we found senkop, hypertension, atrial fibrillation frequency values as significantly high. Additionally, we found significantly higher pulmunory artery pressure among patients whose H-FABP value was 4000 pg/ml or above. Findings of a study by Langer and his colleagues supports the hypothesis that H-FABP can be a promising prognostic marker of short-term mortality among low-risk PTE patients (19).

In addition to the findings above, we have also found strong negative correlation between H-FABP and oxygen saturation, which is one of the parameters in pulmunory embolism seriousness index. As the oxygen saturation decreased, the H-FABP levels increased. This finding shows that hypoxia should be considered as a bad prognostic marker.

The relationship between malignite and venous thromboembolism (VTE) is well known. Lee and his colleagues found VTE in 4-28% of the cancer patients (22). Similar to the Lee et al study, we found active malignite in 22.4% of the patients in our study. The earlier study showed 6-month VTE risk for cancer patients is currently 12-fold higher than in the general population (23). Parallel with other studies in literature on distribution of risk factors across PTE groups, we have also found significantly higher amounts of malignite and atrial fibrillation in high mortality risk group than the low-risk group. Therefore, we have come to conclude that atrial fibrillation is an important factor in developing pulmonary thromboembolism both in terms of its etiology and in the prognosis of these patients.

In this study, we have found that H-FABP level is a superior marker over other cardiac markers. There was not a significant difference in troponin T levels between high mortality risk group and moderate mortality risk groupH-FABP is a superior marker in determinin progranosis over pro-BNP and troponin because of its fast diffusion into blood stream and its significant variation across all PTE sub-groups, and its sensitivity for high PAP levels.

The H-FABP level was significantly higher with patients who had bilateral main pulmunory artery thrombus than it was with patients who had segmentary and sub-segmentary pulmunory artery thrombus. In one of our recent studies, cases with central thrombus had significantly higher troponin levels and frequent signs of Right ventricular dilatationon echocardiography, compared to cases with distal thrombus (24).

It is worth noting that our study has s ome limitations due to some factors such as collecting data in one hospital, having relatively low numbers of patients, and a lack of long-term follow up of patients.

In conclusion, diagnostic and treatment of PTE cases is an urgent matter. Therefore, in order to make proper treatment decisions, swift analyses must be made on PTE patients by evaluating clinical findings, markers of right ventricular dysfunction, cardiac markers and radiological findings. There is a much more sensitive approach regarding the classification of PTE patients in 2019 ESC Guidelines on the Diagnosis and Management of Acute Pulmonary Embolism (4). Nevertheless, this classification does not include any prognostic evaluations of PAP. We believe that having high PAP values should be considered as a prognostic parameter. Medium mortality risk group is a heterogenous group. The cardiac markers that are used for prognostic purposes must provide fast diagnosis capability, they must be sensitive and specific. In this regard, we have considered H-FABP as a superior marker over alter alternatives.

In risk classification of the disease, cardiac markers, right ventuculary dysfunctional findings, and pulmunory embolism seriousness index have begun to be used. In this connection, the clinical, physical and laboratory findings are evaluated altogether.

Limitations

Our study has some limitations such as being a single-centre study and having a limited number of patients.

CONCLUSION

According to the findings of our study, when cardiac markers are evaluated across PTE sub-groups:

- H-FABP is an important prognostic marker across all acute PTE groups.
- H-FABP is a superior marker over troponin T ve pro-BNP in determining prognosis.
- H-FABP levels in low mortality risk group was very close to H-FABP levels in healthy control group. This finding was interpreted as PTE group with low mortality risk to have good prognosis.

Strong positive correlation between H-FABP and PAP, and strong negative correlation between H-FABP and oxygen saturation led us to conclude that all three indicators are aggravating factors.

Making a decision for thrombolic treatment only after clinical, laboratory, and radiological evaluation will be in the best interest of normotensive patients as well. We need the more research to find out the risk coefficients factors of the aggravating factors and additional factors mentioned above.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Yıldırım Beyazıt University Non-interventional Clinical Researches Ethics Committee (Date: 18/06/2014; Decision No: 19).

Informed Consent: Written informed consent was obtained from all participants who participated in this study

Referee Evaluation Process: Externally peer-reviewed.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Histopathological examination of the placenta after delivery in pregnant women with COVID-19

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ABSTRACT

Introduction: COVID-19 is a viral disease generated by a new coronavirus named SARS-CoV-2. The consequences of this virus on the human placenta and the newborn are still unclear. IL-6 can disturb the placenta's immunological homeostasis and be employed as an inflammatory marker for the poor prognosis of COVID-19 infection. Bax has some features like being a key protein regulating apoptotic mechanisms and plays an important role in both maintaining dynamic balance and integrity in the placenta as in many tissues. This study aims to indicate the impact of COVID-19 on inflammation and apoptotic pathways in the placenta by using IL-6 and Bax antibodies.

Material and Method: COVID-19 positive (n:10) and COVID-19 negative (n:10) normotensive placentas were included. Haematoxylin-eosin staining and immunohistochemical staining (IL-6 and Bax antibodies) were applied. Statistical data of immunohistochemical (IL-6 and Bax expression) staining results were assessed by analyzing the H-score. Biochemical parameters were recorded. Group means were analyzed with a nonparametric Kruskal Wallis Test.

Results: In the COVID-19 group, increased syncytial knots, fibrin deposition, inflammation, fibrinoid necrosis, neutrophil accumulation were observed. The COVID-19 group had considerably higher levels of IL-6 and Bax expression than the control group. Furthermore, COVID-19 patients had statistically lower WBC and higher CRP values than normotensive patients.

Conclusion: COVID-19 has been linked to placental inflammation and trophoblast cell damage, both of which can result in major maternal and fetal problems during pregnancy. We found intense IL-6 expression in the placentas of pregnant women with COVID-19 infection. A rise in IL-6 levels triggers CRP production, and this increase is linked to the severity of COVID-19 as a risk factor. Also, we suggested that COVID-19 infection triggers the apoptotic process in placental tissue by increasing the expression of the proapoptotic Bax protein. It is clinically very significant to follow up COVID-19 positive pregnancies for maternal and fetal health. During this follow-up, IL-6 and Bax expression levels in the placenta, together with histopathological findings and serum CRP levels, can guide the evaluation of the prognosis, severity and response to treatment of the disease.

Keywords: COVID-19, placenta, IL-6, inflammation, Bax, apoptosis

INTRODUCTION

In December 2019, a new coronavirus-related epidemic broke out in Wuhan and World Health Organization labeled it a pandemic (1). The pathogenic factor was determined as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on phylogenetic research, and the disease caused by the virus was dubbed Coronavirus Disease 2019 (COVID-19) (2). The disease is diagnosed by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) (3). The clinic of COVID-19 viremia is similar to previous coronaviruses, which is a cause of severe acute respiratory syndrome (SARS). Clinical manifestations are fever, cough, fatigue, sputum production, loss of taste sense, myalgia, difficulties in breathing, etc. (2,3). Pregnant patients with COVID-19 disease and nonpregnant adult patients have similar clinical characteristics (4). Ground-glass opacity (56.4 percent) and bilateral patchy shadowing were found to be the most prevalent radiological abnormalities on computed tomography (51.8 percent). Lymphocytopenia, thrombocytopenia, leukopenia, and elevated C-reactive protein (CRP) levels are more common laboratory findings in patients with COVID-19 positive. COVID-19 is treated with remdesivir, anticytokinic

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biological agents (anti-interleukin-1 (IL-1) (Anakinra) and anti-interleukin-6 (IL-6) (Tocilizumab, TCZ), IVIG (intravenous gammaglobulin), and recently vaccines (e.g., Biontech, sinovac, moderna) are recommended (5,6).

Maternal morbidity is a health problem due to pregnancy. Infections and eclampsia are among the most common causes (7). Pregnancy diseases can adversely affect the fetus as much as the mother. Respiratory viral infections observed during pregnancy cause serious obstetric and neonatal consequences (8). Pregnancy-related complications associated with maternal and fetal morbidity have a close relationship with the placenta (9). However, the impacts of COVID-19 on human placenta and newborn are not fully understood (10). Since the COVID-19 reports are based on a limited number of cases (10,11), comprehensive studies are needed to define placental histopathological findings specific to COVID-19 infection.

In normal pregnancies, there is a mild systemic inflammatory response (12). Leukocyte count increases, neutrophils and monocytes are activated (13), and concentrations of circulating inflammatory cytokines (IL-6, Tumor necrosis factor-alpha) increase (14). The secretion levels of these cytokines with increasing concentrations are affected by each other (15). Many tissues release IL-6 as a pleiotropic immunomodulatory cytokine in response to infection and tissue damage. Acute phase responses, hematopoiesis, and immune cell development and activation are all regulated by it (16). IL-6, which are products of activated macrophages, has negative effects on pregnancy (17). It interferes with many events affecting fetal growth, such as nutrient transfer, anoxia, and vascular permeability, by acting in the feto-maternal gap (18). IL-6 can also disturb the placenta's immunological homeostasis (18) and be employed as an inflammatory marker for the poor prognosis of COVID-19 infection (19). IL-6 levels over a certain threshold are linked to the necessity for mechanical ventilation. Patients with high IL-6 levels (≥80pg/ml) are 22 times more likely to have respiratory failure than those with low IL-6 levels (20). Therefore, antisitokinic drugs such as TCZ, a monoclonal antibody against IL-6, are used as a treatment option for COVID-19 patients (21).

Apoptosis is programmed cell death and plays a significant role in tissue homeostasis, embryonic growth, and immunity. Bcl-2 family control apoptosis with proapoptotic proteins and antiapoptotic proteins (22). The balance between proapoptotic and antiapoptotic protein expression determines the outcome of apoptosis (23). Bax, one of these proteins, is a molecule that stimulates apoptosis and that causes cytochrome c to be released from the mitochondria when produced. When it is released, it activates caspase-9 and caspase-3, respectively (23). Bax has some features like being a key protein regulating apoptotic mechanisms and plays an important role in both maintaining dynamic balance and integrity in the placenta as in many tissues (24).

This study's purpose is to demonstrate the consequences of COVID-19 on inflammation and apoptotic pathways in the placenta by using IL-6 and Bax antibodies.

MATERIAL AND METHOD

The study was carried out with the permission of Siirt University Non-Interventional Clinical Researches Ethics Committee (Date: 23.09.2020, Decision No: E.11597). All procedures were realized in accordance with the ethical rules and the principles of the Declaration of Helsinki. Patients who were approved to participate in this study were informed about it and completed an informed consent form.

Placental tissue was obtained from patients submitted to the Obstetrics and Gynecology Department of the Siirt Training and Research Hospital. The study included placentas from pregnant women who tested positive for COVID-19 PCR during pregnancy (n:10) and placentas from pregnant women who tested negative for COVID-19 (n:10). This study did not include patients in both groups with secondary or chronic diseases (such as any respiratory infection, pregnancy complications, or other systemic diseases).

Obtaining and Following-up Tissues

Placental tissue was processed for routine histological examination, fixed in %10 formaldehyde, passed through an ascending alcohol series, and then incubated in xylene and in paraffin. 5 μm thick sections were stained with hematoxylin-eosin (HE) staining and immunohistochemical staining. Biochemical blood parameters were recorded for all patients.

Immunohistochemical Staining

Sections were brought to distilled water and soaked in EDTA buffer solution (pH:8.0, lot number: ab93680, Abcam) for epitope retrieval in a microwave oven at 700 Watts for 10 minutes. For endogenous peroxidase blocking, hydrogen peroxide solution (lot number: TA-015-HP, Thermo Fischer) was dropped into this section and incubated for 20 minutes. After blocking solution, sections were overnight with IL-6 (catalog no:sc-32296, Santa Cruz Biotechnology) and Bax (catalog no: sc-20067, Santa Cruz Biotechnology) antibodies at 1/100 dilution. Diaminobenzidine (DAB) (lot number: TA-001-HCX, Thermo Fischer) was used as chromogen. After counterstaining with Harris haematoxylin, sections were mounted with entellan (lot number:107961, Sigma-Aldrich) and analyzed with Zeiss Imager A2 (Germany) by Zen 3.2 lite software.

The H-score (HS) was used to assess the outcomes of immunohistochemical staining. HS= Σ (1 + i) x pi. Where i indicates the staining intensity (0=no expression, 1=light, 2=medium, 3=dense, and 4=very dense) and pi indicates the percentage of staining intensity (25). Results were given as±standard error. Statistical analysis of the results obtained was made.

Statistical Analysis

IBM SPSS software (ver. 25) was used for statistical analysis. The nonparametric Kruskal Wallis Tests were used to examine the mean values of groups. All data were given as a mean with a standard deviation (S.D.). Any p value<0.05 was admitted statistically significant.

RESULTS

Laboratory Statistics

In terms of RBC (red blood cell), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), HGB (hemoglobin), HTC (hematocrit), PLT (platelet), neutrophil, lymphocyte, monocyte, eosinophil, and basophil values, there was no significant difference (p>0.05) between experimental (COVID-19) and control groups. They are in normal range in both groups. The WBC value in the experimental group (COVID-19) was considerably lower than the control group (p<0.05), whereas CRP value in the experimental group (COVID-19) was considerably higher than the control group (p<0.05) (**Table 1**).

Table 1. Laboratory findings (mean) and statistical comparison between groups

Parameter	Control (mean±SD)	COVID-19 (mean±SD)	Paired-comparison
WBC (10^3/mm³)	10.72±1.83	8.66±1.84	p=0.034
RBC (10^{12}/L)	4.54±0.43	4.24±0.45	p=0.096
HGB (g/dl)	12.62±1.68	11.68±1.21	p=0.257
HCT (%)	39.01±4.51	36.86±2.97	p=0.472
PLT (10^3/mm³)	254.2±70.61	262.30¬±77.51	p=0.940
MCV (fL)	88.50±5.08	87.53±7.19	p=0.910
MCH (pg)	29.05±1.52	28.42±2.13	p=0.406
Neutrophil (%)	75.49±3.95	69.28±22.84	p=0.940
Lymphocyte (%)	18.34±4.32	17.22±4.16	p=0.449
Monocyte (%)	5.71±1.26	4.78±1.01	p=0.130
Eosinophil (%)	0.78±0.27	0.95±0.54	p=0.422
Basophil (%)	0.22±0.10	0.29±0.14	p=0.229
CRP (mg/dl)	10.22±7.60	22.51±12.83	p=0.026

SD: Standard deviation

Immunohistochemical Statistics

Statistical data of immunohistochemical (IL-6 and Bax expression) staining results indicated in **Table 2** were evaluated by investigating the H-score. The levels of IL-6 and Bax expression in COVID-19 group were found to be considerably higher than in the control group (p<0.05).

Histopathological Findings

Haematoxylin-eosin staining of placental tissues belonging to COVID-19 group are shown in **Figure 1** and **Figure 2,** respectively. IL-6 and Bax immunostaining of placental tissues belonging to the control and COVID-19 groups are shown in **Figure 3** and **Figure 4**, in sequence. In the control group, normal histology was observed with cytotrophoblasts, syncytiotrophoblasts, fetal capillaries, and villous stroma. No pathology was observed in the stromal connective tissue and vascular structures (not shown in figures). In the placenta of COVID-19 group, increased syncytial knots, fibrin deposition, fibrinoid necrosis in the vascular wall, intervillous hemorrhage, inflammation, neutrophil accumulation, fetal villi with chorangiosus and oedema were observed (**Figure 1** and **2**). IL-6 and Bax expression levels were increased considerably in COVID-19 group competing with the control group (p<0.05). IL-6 expression was mostly negative in the control group (**Figure 3a**), yet it was intense in syncytial knots and syncytiotrophoblast cytoplasm (**Figure 3b**) in COVID-19 group. Bax expression was mostly negative in the syncytial knots and tertiary villi in the control group (**Figure 4a**). In COVID-19 group, Bax reaction was positive predominantly in syncytiotrophoblast cytoplasm (**Figure 4b**).

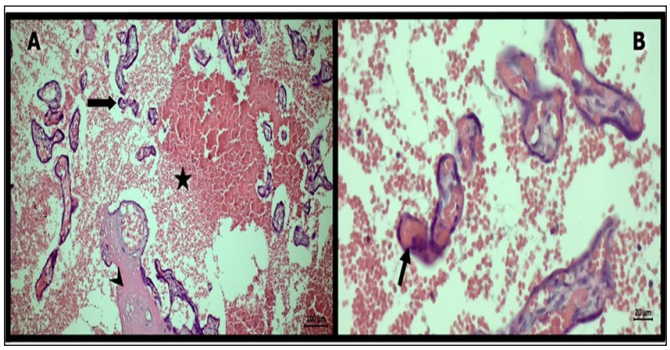

Figure 1. HE staining of placental tissues belonging to the COVID-19 group (A), İncreased syncytial knots (arrow), fibrin deposition (arrowhead), and intervillous hemorrhage (star) (X10) (B) Fetal villi with oedema and chorangiosus (arrow) (X40)

Table 2. Statistical data of immunohistochemical (IL-6 and Bax expression) parameters

	Control Placentas						COVID-19 Placentas						Comparison between groups
	Number	Mean	Std deviation	Median	Min	Max	Number	Mean	Std deviation	Median	Min	Max	
IL-6	10	0.5	0.52	0.5	0	1	10	3.8	0.42	4	3	4	p<0.01
Bax	10	0.4	0.52	0	0	1	10	2.4	0.52	2	2	3	p<0.01

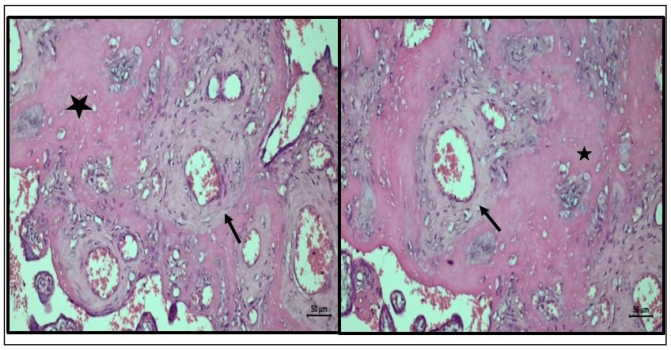

Figure 2. HE staining of placental tissue: fibrin deposition (star) and fibrinoid necrosis in vascular wall (arrow) in the placenta belonging to the COVID-19 group (X20)

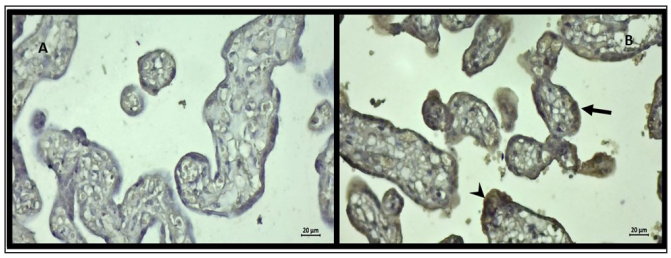

Figure 3. IL-6 immunohistochemical staining of placental tissue (A) Generally negative expression was observed in the control group (B) IL-6 expression was observed mostly in syncytiotrophoblast cytoplasm (arrow) and syncytial knots (arrowhead) in COVID-19 placentas (X40)

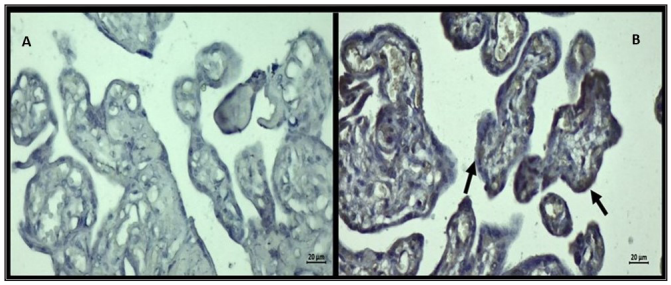

Figure 4. Bax immunohistochemical staining of placental tissue (A) Generally negative expression was determined in the control group (B) Bax expression was determined mostly in syncytiotrophoblast cytoplasm in COVID-19 placentas (arrows) (X40)

DISCUSSION

The placenta develops dynamically during pregnancy and may undergo various changes depending on pregnancy pathology. Changes in the placenta provide clues about maternal and fetal morbidity (26). Inflammatory and infectious diseases are among the indications that require histopathological evaluation of the placenta (27). However, the relationship of some diseases to pregnancy is not fully understood because some placental lesions do not have pregnancy-specific changes and some placental lesions are not associated with perinatal mortality and morbidity (28). COVID-19 infection during pregnancy is also one of the diseases whose maternal and fetal effects are not yet very clear (29). Therefore, the diagnosis and treatment of the illness during pregnancy is essential to control maternal and infant complications correlate with the disease.

Former coronavirus pandemics, such as SARS and the Middle East respiratory disease (MERS), have been linked to maternal and perinatal complications, including infections, mortality, and spontaneous abortion during pregnancy (30). Similarly, related to COVID-19 disease, conditions such as miscarriage, preterm birth and stillbirth have been reported (31). Therefore, the results of the histopathological analysis of the placenta may have significant contributions to maintaining of maternal and fetal health.

COVID-19 is a disease caused by SARS-CoV-2 that induces inflammation (32). In some studies, it is stated that COVID-19 infection is not present in the placenta, and vertical contamination is quite infrequent (33). Accordingly, the placental alterations produced by COVID-19 may be due to inflammation and maternal infection rather than fetal illness (32). In our study, the laboratory values of pregnant women who have COVID 19 agreed with literature. Additionally, we found that CRP values were high, and patients had leukopenia (**Table 1**).

During pregnancy, viral infections can cause specific placental alterations (32). In the histopathological examination of placentas from SARS patients, decreased placental perfusion, subchorionic and intervillous fibrin aggregation, achorionic villus areas, and fetal thromboangiopathy were observed (30,34). On the other hand, how COVID-19 affects placental pathology during pregnancy is not completely known yet (35). Shanes et al. (32) study on placentas from infected pregnant women with COVID-19 demonstrated that maternal fibrinoid necrosis, vascular malperfusion, decidual arteriopathy and atherosis. Authors also suggested that; perivillous fibrin accumulation, intervillous thrombi, villous oedema, and chorangiosis in some placental tissues. According to these findings, chorangiosis is linked to a decrease in maternal oxygen saturation (32). In another research of placentas from COVID-19 patients, perivillous fibrin accumulation, locally increased syncytial knots, and pathology of fetal vascular malperfusion (thrombosis, intramural fibrin deposition, stromal vascular karyorrhexis, avascular villi) were reported. In addition, in some cases, chronic villitis associated with obliterative vasculopathy has also been observed (10).

In our study, placentas with COVID-19, increased syncytial knots, fibrin deposition, intervillous hemorrhage, inflammation, neutrophil accumulation, fetal villi with chorangiosus, and oedema findings were observed (**Figures 1** and **2**). Both our study and other studies (32) reveal that the observation of various histopathological changes in the placenta suggests that it may be linked to COVID-19.

In the study, placentas were analyzed by immunohistochemical methods in symptomatic COVID-19 in second trimester pregnancy. It has been reported that in the feto-maternal gap of the placenta, the SARS-CoV-2 virus is mostly found in syncytiotrophoblast cells by SARS-CoV-2 spike protein immunostaining. In addition, histological examination showed macrophages and T-lymphocyte infiltration suggesting a histiocytic intervillocyte by CD68 and CD3 immunostaining (35). In our study, we showed inflammation and apoptosis in the placenta by using IL-6 and Bax immunostaining (**Table 2**).

Cytokines, which act as immunomodulators during pregnancy, are of great importance for a healthy pregnancy (36). Implantation, placental development, extravillous trophoblast invasion, cytotrophoblast proliferation, angiogenesis, spiral artery remodeling, cell growth, and apoptosis are all regulated by the maternal-fetal space to the existence of cytokines. IL-6 is an important proinflammatory cytokine that plays a role in the acute phase response to injury and infection (37). Furthermore, increased levels of this cytokine in the feto-maternal gap are associated with fetal loss (38). Therefore, elevated IL-6 values may be important in the follow-up of the threat of miscarriage in pregnant women.

Patients with COVID-19 infection usually have high serum inflammatory markers, including high CRP and cytokine (IL-6) levels (39). We found intense IL-6 expression in the placentas of pregnant women with COVID-19 infection, especially in syncytial knots and syncytiotrophoblast cytoplasm (**Figure 3**). Similarly, CRP values were considerably higher in these patients. As a result, IL-6 and CRP readings can be utilized together as indicators to assess the severity of the illness of COVID-19 patients throughout pregnancy.

Monoclonal antibodies are currently used to diagnose, research, prevent, and treat diseases (40). In the treatment of some COVID-19 patients, the effectiveness of monoclonal antibodies developed against cytokines such as IL-6 are evaluated (41,42). In cytokine release syndrome and acute inflammation, there is a significant role of IL-6 (43). Additionally, a rise in IL-6 levels triggers CRP production, and this increase is linked to the severity of COVID-19 as a risk factor (44). Thus, some drugs may be effective in COVID-19 patients by blocking the signal transduction pathway of IL-6 (43). Studies have reported that these drugs cause a significant decrease in CRP levels, an increase in lymphocyte count, and a decrease in COVID-19 clinical symptoms and prognosis (41,45). Tocilizumab is an IL-6 inhibitor and is used in the context of clinical trials in the handling of COVID-19 (46). This drug may be safe due to the small amount of transplacental interaction during pregnancy (47). On the other hand, as the teratogenic effects of such drugs in pregnancy are not fully known, more comprehensive studies are needed to be used in these cases.

Increases in the number of COVID-19 patients lead to the emergence of new variants (Alpha, Beta, Gamma, Delta, and Omicron). Of these, emerging variants such as Omicron adversely affect the success of monoclonal antibody therapy due to mutations in the spike protein receptor binding sites (48). The fact that newly emerged variants (Delta and Omicron) can lead to unsuccessful results in monoclonal antibody treatment (48) reveals that new variants should be taken into account when making arrangements for the treatment of pregnant women with COVID-19.

Apoptotic cell death in endothelial cells of vascular structures due to SARS-CoV-2 infection has been demonstrated in various organs (49). Furthermore, apoptosis has important roles in placental homeostasis, growth, and reform. Abnormalities in the expression of Bax protein, which is involved in apoptosis, are associated with some pathological placental changes (24). In a study, Bax protein was discovered to be a good indicator of apoptotic alterations in placentas with gestational diabetes and preeclampsia (50). In another study, it was reported that the Bax gene could be responsible for pregnancy loss, and variation of this gene would be useful in the evaluation of recurrent pregnancy loss (51).

In our study, Bax expression was observed in the syncytiotrophoblast cytoplasm in the COVID-19 group (**Figure 4**). It is founded that the cytokine storm and oxidative stress seen in some COVID-19 cases lead to reactive oxygen species-dependent apoptosis of endothelial cells (39). In addition, cytokines such as IL-6 produced in the cytokine storm seen in COVID-19 patients are known to cause apoptosis in lymphoid organs (52). Apoptosis in lymphoid organs due to COVID-19 may also apply to syncytiotrophoblast cells in the placenta. As a matter of fact, the fact that IL-6 and Bax expressions were found to be high together in COVID-19 positive placentas in our study supports this idea. All these data suggest that COVID-19 infection triggers the apoptotic process in placental tissue by increasing the expression of the proapoptotic Bax protein. At this point, it may be beneficial to use Bax and IL-6 expression as a marker to determine apoptotic changes in the placenta in COVID-19.

Limitations

Our study has some limitations; first, the limited number of patients limits the accuracy of the results, necessitating comprehensive studies of large numbers of patients. Second, IL-6 and Bax antibodies were used for immunohistochemistry in this study. Therefore, the scope of research could be expanded in the future by using other markers of inflammation and apoptosis.

CONCLUSION

We believe that COVID-19 causes placental inflammation and damage to trophoblast cells, which can lead to severe maternal and infant disease during pregnancy. Therefore, clinical follow-up of pregnant women infected with COVID-19 is of great significance. During this follow-up, IL-6 and Bax expression levels in the placenta, together with histopathological findings and serum CRP levels, can guide the evaluation of the prognosis, severity and response to treatment of the disease. More comprehensive research is needed to support our recommendations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Siirt University Non-Interventional Clinical Researches Ethics Committee (Date: 23.09.2020, Decision No: E.11597).

Informed Consent: Patients who approved to participate in this study were informed about it and completed an informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: All authors state that there is no conflict of interest.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Role of red cell distribution width in colorectal cancer diagnosis and prognosis

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ABSTRACT

Objective: We aimed to assess whether red cell distribution width (RDW) was associated with pre-operative clinical features or post-operative clinicopathological outcomes in patients with colorectal cancer (CRC), and to determine the utility of RDW as a diagnostic or prognostic marker of CRC.

Material and Method: This retrospective cohort study was conducted between January 2018-May 2021 at a university hospital in Turkey. A total of 188 patients histologically diagnosed with CRC who had undergone surgery were included in the study.

Results: Our study included 118 (62.77%) male patients, and the mean age of the patients was 66.28±11.71 years. We found that RDW values were significantly higher in females compared to males (p=0.033), in patients with T3 or T4 tumors compared to those with T1 or T2 tumors (p<0.001), in patients with stage 2 and stage 3 tumors compared to stage 1 patients, those with early mortality (p=0.012), in patients with right or transverse colon tumors compared to those with descending colon or sigmoid colon or rectum tumors (p<0.001), and those that died during follow-up compared to survivors (p=0.001). Additionally, age (r=0.233, p<0.001), tumor size (r=0.229, p=0.002) and length of stay in hospital (r=0.167, p=0.022) were positively correlated with RDW values. RDW had 75.7% sensitivity and 67.5% specificity to predict mortality for the cut-off point of 15.7 (AUC: 0.704, 95.0%CI: 0.615-0.793, p<0.001).

Conclusion: These results show that RDW has a potential function as a biomarker for the diagnosis and prognosis of CRC.

Keywords: Colorectal cancer, RDW, prognosis, progression, overall survival

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide, with more than 1 million new cases and 600.000 deaths annually (1). It is the second leading cause of cancer-related death among both men and women, and leads to increased healthcare expenditure (2).

Although the mechanisms underlying CRC are not fully understood, appropriate screening and accurate prognostic assessment can reduce deaths from CRC. Stool occult blood testing, stool DNA testing, colonoscopy, and computed tomography (CT) have long been used as screening tools in CRC, but these screening tools have many limitations, such as low sensitivity and specificity, invasiveness, and high cost. Thus, simple, inexpensive and readily available biomarkers for the diagnosis and prognosis of CRC are urgently needed (3). Red blood cell distribution width (RDW) is an important classical element of routine blood examination, mainly reflecting the uniformity of the volume and size of red blood cells. Relatively recent studies have focused on the identification of blood-derived biomarkers that could facilitate the early diagnosis of CRC, but there are currently no widely available markers that can be used to diagnose CRC (4). The RDW value has been shown to be associated with prognosis in various tumors such as renal cell carcinoma, gastric cancer, lung cancer, ovarian cancers, esophageal cancer, endometrial cancer, and breast cancer (3,5). Notably, Ay et al. (6) found that RDW was significantly higher in patients with colon cancer than those with colon

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polyps, and that RDW could be used as an early indicator for solid colon tumors. Additionally, two recent studies showed that RDW was associated with cancer stage and survival in patients with CRC (7,8). Taken together, these results suggest that RDW may be useful in the diagnosis and prognosis of CRC (3).

The aim of the present study was to assess whether RDW level was associated with pre-operative clinical features and post-operative clinicopathological outcomes of patients with CRC, and to determine the possible utility of RDW as a diagnostic and/or prognostic parameter in CRC.

MATERIAL AND METHOD

This was a retrospective single center study conducted between January 2018-May 2021 at Department of General Surgery, Eskişehir Osmangazi University. The protocol of this study was approved by the Non-Interventional Clinical Researches Ethics Committee of Eskişehir Osmangazi University (Date:15.06.2021, Decision No: 09), and carried out in accordance with the ethical standards stated in the Declaration of Helsinki and its amendments. As the study has a retrospective nature, the Medical Ethics Committee of the Eskişehir Osmangazi University did not require written informed consent from patients. All samples and information were recorded anonymously.

Study Population and Follow-up

A total of 188 patients histologically diagnosed with CRC who had undergone surgery were included in the study. Other inclusion criteria were: being aged older than 18 and younger than 90 years, having a complete blood count result obtained and studied two weeks before the surgery, and being followed for at least 12 months after surgery. Patients who received neoadjuvant chemotherapy and/or radiotherapy, had an active infection when the blood sample was taken, those in which necessary data were incomplete, patients who were lost to follow-up, subjects with a history of other serious diseases that affect survival outcomes (such as cardiovascular and cerebrovascular diseases, pulmonary diseases, blood diseases, infectious diseases, other malignant tumors, cerebral infarction, pulmonary infarction, uncontrolled hypertension, HIV infection etc.) were excluded from the study.

The following information of each patient was acquired from hospital records: demographic characteristics including age and gender; tumor characteristics including location, size, pathological diagnosis, number of lymph nodes, number of metastatic lymph nodes, differentiation, surgical margin positivity, perineural invasion, lymphovascular invasion, TNM stage and clinical stage (reported according to the pathological classification criteria of the 7th Edition of the American Joint Committee on Cancer guidelines and the Union International Contre Le Cancer criteria for CRC), liver metastasis. Additionally, surgical characteristics including type and extent of surgery, whether ostomy was opened, laboratory measurements (including complete blood count; CBC) length of stay in hospital, follow-up time; complications including leakage, infection and recurrence, and finally, mortality state (early mortality was defined as death occurring within 30 days of surgery).

Patients were called for check-up at regular intervals for an average of 21 months (0-40) postoperatively, and necessary examinations were performed. All outcomes such as leakage, infection, recurrence and death were recorded.

Laboratory Analysis

Blood samples were acquired from the antecubital vein for the measurement of the CBC before the operation. CBC, including hemoglobin and hematocrit values, white blood cell, neutrophil, lymphocyte, platelet counts, and MPV and RDW values were measured via use of routine devices (Sysmex XE-5000, Japan and Roche, Cobas E601, Switzerland) within 2 weeks prior to the date of surgery.

Pathological Analysis

All of the specimens obtained from fully resected tumors were sent to the pathology unit of the Eskişehir Osmangazi University for pathological examinations. Lymph node metastasis, depth of infiltration and tumor size, pathological type and degree of differentiation, radial surgical margin positivity, distal surgical margin positivity, perineural invasion, and lymphovascular invasion were reported by qualified pathologists.

Statistical Analysis

All study data were entered into an SPSS v25 (SPSS Inc., Chicago, IL, USA) database and analyses were performed. Q-Q plots and histograms were used to assess quantitative variable distributions. Quantitative variables were depicted with mean±standard deviation or median (1st quartile - 3rd quartile) values with regard to normality of distribution (normal and non-normal, respectively), and as frequency (percentage) for categorical data. Between-group comparisons were done with the Mann-Whitney U test or the Kruskal-Wallis test depending on the number of groups being compared, and subsequent post-hoc analyses after Kruskal-Wallis tests were performed with the Bonferroni correction method. Spearman correlation coefficients were calculated to evaluate relationships between quantitative variables. Mortality prediction performance of the RDW was assessed by using Receiver Operating Characteristic (ROC) curve analysis. Optimal cut-off point was determined by using Youden index. Two-tailed p values were calculated and values of $p<0.05$ were considered to show statistical significance.

RESULTS

Seventy female and 118 male patients were included in our study, and the mean age of the patients was 66.28±11.71 (range 36 - 87) years. Patients and tumor characteristics, laboratory measurements and data obtained throughout clinical follow-up studies are depicted in **Table 1**.

The median RDW value of patients with T3 or T4 tumors was found to be significantly higher than that of patients with T1 or T2 tumors ($p < 0.001$). We also found a significant relationship between RDW and gender (female > male, p=0.033), tumor stage (stage 1 values lower than stage 2 and 3 values, p=0.010) and mortality. A total of 7 patients had early mortality (postoperative days 0, 2, 3, 5, 14, 22 and 29). The preoperative RDW values of patients who died during this period were significantly higher compared to the other patients (p=0.012). There were no relationships between RDW and any other parameters analyzed (**Table 2**)

When tumor localizations were evaluated, a significant difference was found between the RDW values of patients with right colon tumor (n=67, 35.64%) and patients with rectal tumor (n=74, 39.36%) (p=0.004). Since the number of patients with tumors in other localizations such as the transverse colon (n=15, 7.98%), descending colon (n=16, 8.51%), sigmoid colon and rectosigmoid region (n=16, 8.51%) was insufficient, reliable statistical evaluations could not be performed with respect to specific sites (**Figure 1a**, **Table 1**). However, when tumor localizations were grouped, the median RDW values of patients with tumors in the right or transverse colon were significantly higher than that of patients with tumors in the descending or sigmoid colon or the rectum (p<0.001) (**Figure 1b**).

In addition, the median RDW value of patients who died was significantly higher compared to those who survived (p=0.001) (**Figure 2**).

There were significant weak positive correlations between RDW and several continuous variables, including age (r=0.233, p<0.001), tumor size (r=0.229, p=0.002) and length of stay in the hospital (r=0.167, p=0.022). There were no significant correlations between RDW values and the number of lymph nodes or the number of metastatic lymph nodes (**Table 3**).

Mortality prediction success of the RDW was found to be statistically significant (AUC: 0.704, 95.0% CI: 0.615 - 0.793, p<0.001) (**Figure 3**). RDW had 75.7% sensitivity, 67.5% specificity, 69.1% accuracy, 36.4% positive predictive value and 91.9% negative predictive value to predict mortality for the cut-off point of 15.7 (equal or higher values predict mortality) (**Table 4**).

Table 1. Summary of patients and tumor characteristics and laboratory measurements

Age	66.28±11.71
Gender	
Female	70 (37.23%)
Male	118 (62.77%)
Location	
Right colon	67 (35.64%)
Transverse colon	15 (7.98%)
Descending colon	16 (8.51%)
Sigmoid colon & rectosigmoid region	16 (8.51%)
Rectum	74 (39.36%)
Pathological diagnosis	
Non-mucinous adenocarcinoma	138 (73.40%)
Mucinous adenocarcinoma	50 (26.60%)
Tumor size	40 (27.5 - 60)
Number of lymph nodes	23 (16 - 34.5)
Number of metastatic lymph nodes	0 (0 - 1)
Differentiation	
Poor	20 (10.64%)
Moderate	144 (76.60%)
Well	24 (12.77%)
Radial surgical margin positivity	4 (2.13%)
Distal surgical margin positivity	3 (1.60%)
Perineural invasion	42 (22.34%)
Lymphovascular invasion	66 (35.11%)
T stage	
T1	6 (3.19%)
T2	33 (17.55%)
T3	118 (62.77%)
T4	31 (16.49%)
N stage	
N0	117 (62.23%)
N1	48 (25.53%)
N2	23 (12.23%)
Stage	
Stage 1	31 (16.49%)
Stage 2	85 (45.21%)
Stage 3	72 (38.30%)
Liver metastasis	1 (0.53%)
Type of surgery	
Laparoscopy	33 (17.55%)
Open surgery	155 (82.45%)
Operation	
Right hemicolectomy	53 (28.19%)
Transverse hemicolectomy	10 (5.32%)
Left hemicolectomy	21 (11.17%)
Anterior resection	24 (12.77%)
Low anterior resection	62 (32.98%)
Abdominoperineal resection	17 (9.04%)
Other	1 (0.53%)
Ostomy	76 (40.43%)
Hemoglobin	12.32±2.12
Hematocrit	37.89±5.56
White blood cell (x1000)	7.34 (5.90 - 9.60)
Neutrophil (x1000)	4.83 (3.91 - 6.71)
Lymphocyte (x1000)	1.48 (1.05 - 2.01)
Platelet (x1000)	279 (223 - 372)
MPV	9.56±1.15
RDW	14.65 (13.30 - 17.55)
Length of stay in hospital, days	6 (5 - 9)
Follow-up time, months	21 (12.5 - 28)
Leakage	7 (3.72%)
Infection	30 (15.96%)
Recurrence	12 (6.38%)
Mortality	37 (19.68%)
Early mortality (≤30 days)	7 (3.72%)

Data are given as mean±standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables

Table 2. Summary of RDW with regard to patients and tumor characteristics

	Median (1st quartile-3rd quartile)	p
Gender		0.033
Female	15.5 (13.7 - 17.9)	
Male	14.55 (13.2 - 17.2)	
Location		<0.001
Right colon & Transverse colon	16.25 (14.0 - 19.1)	
Descending colon & Sigmoid colon & Rectum	14.2 (13.3 - 16.6)	
Pathological diagnosis		0.149
Non-mucinous adenocarcinoma	14.9 (13.5 - 17.8)	
Mucinous adenocarcinoma	14.35 (13.2 - 17.2)	
Differentiation		0.477
Poor	13.95 (13.1 - 17.4)	
Moderate	14.65 (13.4 - 17.35)	
Well	16.1 (13.5 - 17.65)	
Perineural invasion		0.504
No	14.65 (13.4 - 17.8)	
Yes	14.65 (13.3 - 17.2)	
Lymphovascular invasion		0.211
No	14.9 (13.3 - 17.8)	
Yes	14.4 (13.3 - 17.1)	
T stage		0.001
T1 & T2	13.8 (13.2 - 15.2)	
T3 & T4	15.3 (13.5 - 18.1)	
N stage		0.586
N0	14.8 (13.5 - 17.7)	
N1	14.85 (13.3 - 16.9)	
N2	14.3 (13.1 - 18.4)	
Stage		0.010
Stage 1	13.9 (13.2 - 15.3)	
Stage 2 & 3	15.1 (13.5 - 17.9)	
Leakage		0.271
No	14.6 (13.3 - 17.2)	
Yes	17.9 (13.2 - 19.4)	
Infection		0.263
No	15.1 (13.3 - 17.8)	
Yes	14.35 (13.5 - 15.7)	
Recurrence		0.086
No	14.6 (13.3 - 17.2)	
Yes	17.2 (15 - 18.75)	
Status		<0.001
Alive	14.4 (13.3 - 17.2)	
Exitus	17.1 (15.7 - 19.1)	
Early mortality (≤30 days)		0.012
No	14.6 (13.3 - 17.2)	
Yes	18.6 (17.1 - 20.2)	

Same letters denote the lack of statistically significant differences between groups

Table 3. Relationships between RDW and continuous variables

	r	p
Age	0.233	0.001
Tumor size	0.229	0.002
Number of lymph nodes	0.048	0.516
Number of metastatic lymph nodes	-0.059	0.420
Length of stay in hospital	0.167	0.022

r: Spearman correlation coefficient

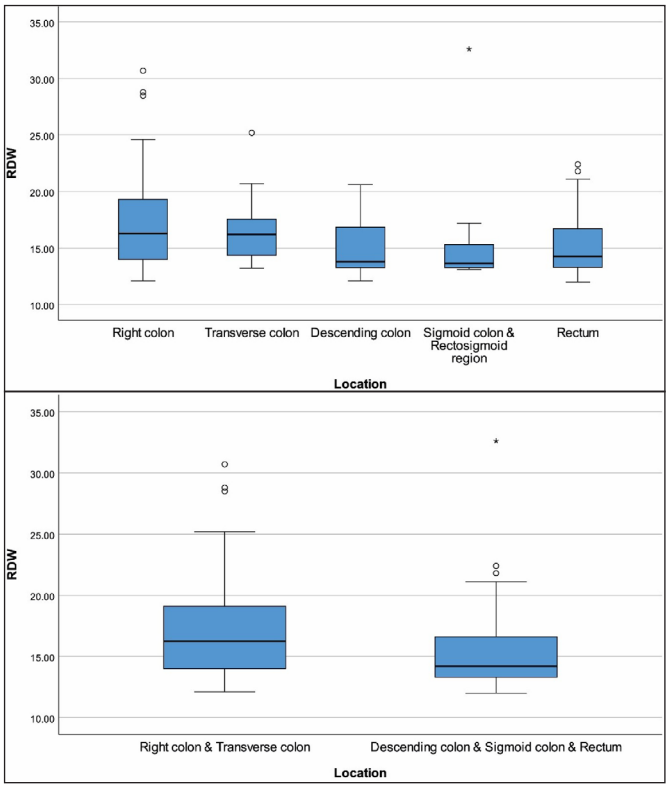

Figure 1a, 1b. Relationships between RDW and tumor site

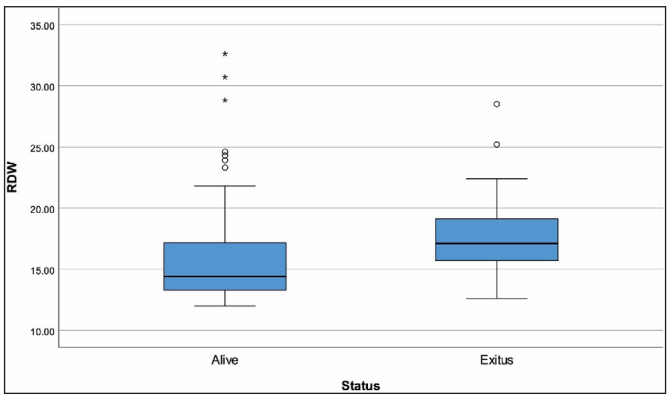

Figure 2. Relationship between RDW and mortality

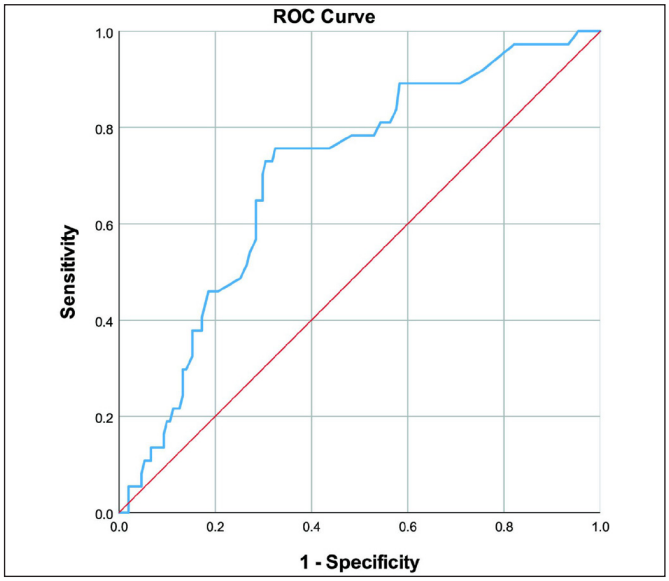

Figure 3. ROC curve of the RDW to predict mortality

Table 4. Performance of the RDW to predict mortality	
Cut-off	≥15.7
Sensitivity	75.7%
Specificity	67.5%
Accuracy	69.1%
PPV	36.4%
NPV	91.9%
AUC (95.0% CI)	0.704 (0.615 - 0.793)
p	<0.001

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under ROC curve, CI: Confidence intervals

DISCUSSION

According to the results we obtained, it was found that the RDW values showed significant differences with respect to gender, age, tumor localization, T stage, clinical stage, duration of post-operative hospitalization, tumor size, early death, and death during follow-up.

CRC is the third most common cancer in both men and women and the second leading cause of cancer-related death (2,9,10). For these reasons, early diagnosis, proper treatment and prognostic assessment of CRC are critical. Blood tests are of great importance for diagnosis, treatment and prognosis, and help clinicians in assessing patients. In addition, the fact that blood tests are easier and less costly supports the focus of interest in the search for new markers associated with the diagnosis, treatment and prognosis of CRC (11-14). RDW is an indicator of the heterogeneity of red blood cell volume and is used in the diagnosis and prognosis of several diseases like anemia, some cardiovascular and infectious diseases and some types of cancer including lung, stomach, esophageal, hepatocellular, breast cancers, and, in recent years, CRC (15-23). In some studies, it has been shown that the RDW level of patients with CRC is higher than that of controls (3,4,7,8,24). The underlying cause of the relationship between RDW and cancer is unknown (1,25), but several possible mechanisms have been considered. The first is the hypothesis that inflammation and oxidative stress around the tumor may increase RDW. The second is that the tumor may indirectly cause changes in erythropoiesis by causing malnutrition, which may increase RDW. Finally, it is also possible that iron deficiency anemia due to bleeding seen in patients with CRC may increase RDW (26-28). All these results show that high RDW may be an independent risk factor for CRC (13).

In some studies, it has been shown that high RDW values might be a negative predictors of survival in several types of malignancies including lung, gastric, esophageal, hepatocellular cancers and breast cancer (1,15,17-20,29). A similar relationship has been suggested to exist between elevated RDW and CRC (8,30). Prior studies have also reported that RDW can independently assess the prognosis of patients with colorectal cancer (13). Pedrazzani et al. (1) have shown that CRC patients with high RDW have a lower 10-year overall survival compared to those with lower RDW. Zhang et al. (30) found high RDW to be associated with poorer overall and disease-free survival in their study of 625 patients with rectal cancer who underwent curative surgery without neoadjuvant therapy. Li et al. (13), in a retrospective analysis of 168 colorectal cancer patients, found a positive relationship between RDW values and both 3- and 5-year overall and disease free survival. Similarly, high RDW was found to be associated with worse overall survival by Kust et al. (7) in a retrospective study of 90 patients with CRC; however, this relationship was only present in subjects with stage II cancer. Several other studies have also shown similar results (1,4,31). In this study, we also showed that the preoperative mean RDW values of patients who died during postoperative follow-up were significantly higher than those who survived. Also, interestingly, we found that patients who died within the first 30 days after surgery had higher RDW values. Additionally, it was observed that patients with high RDW had longer hospital stay after surgery. This significant relationship between high RDW and CRC-related deaths may be due to chronic inflammation due to cancer, iron deficiency anemia due to chronic blood loss in CRC, folate deficiency, changes in erythropoiesis, dyslipidemia and other metabolic abnormalities (32-36).

Today, TNM stage is accepted as the most significant prognostic factor for CRC (3). Many researchers have shown that RDW was significantly associated with clinical stage, T stage, N stage, M stage and tumor size in subjects with CRC. For instance, Song et al. (32) found that RDW values were associated with TNM stage, pT stage, and pM stage, similar to the results put forth by Yang and colleagues (8). Importantly, they also found that the level of RDW was associated with tumor size. However, there was a difference between the two studies with regard to the relationship between pN stage and RDW level. While the study by Yang et al. (8) found a significant positive correlation between RDW and pN stage, the study by Song et al. (32) did not. Moreover, Yang et al. (8) showed that RDW values in stage 3 and 4 CRC were higher compared to stage 1 and 2, similar to our results. In another research, RDW values were found to be associated with clinical stage, and T status, but not N or M status (4). Consistent with the results by Yang et al. (8), RDW values of CRC patients at the T3 and T4 stages in our study were found to be significantly higher than those with disease stages T1 and T2. We found similar results with regard to comparisons based on clinical staging. That is, the RDW levels of stage 1 patients were lower compared to patients with stage 2 or 3 disease. Likewise, we found that, as the tumor size increases, the RDW value also increases. Taking into

account these studies and our findings, it appears that increased tumor burden in CRC patients is associated with RDW elevation. Despite the fact that there are various studies showing the aforementioned relationship, the underlying causes for said relationship is not known exactly, but the increase in inflammatory activity around the tumor is likely a primary factor.

The relationship between the site of the CRC tumor and RDW has also been an interesting subject. In a study, RDW level was found to be significantly higher in right sided CRC tumors than left sided CRC tumors, similar to the present study (37). We also found higher RDW in patients with CRC tumors localized in the right or transverse colon compared to those with tumors in the descending colon, sigmoid colon or rectum. This interesting association may be a consequence of iron deficiency anemia. Right colon tumors are known to have a greater frequency of demonstrating bleeding and associated symptoms. As a result, anemia is a relatively more common symptom in tumors of the right colon compared to other regions of the colon (38).

Additionally, significant positive correlations between age and RDW levels have been reported previously (1,34,39), and we found supportive results in the current study. The relationship between gender and RDW is still unclear. Some studies have shown that the RDW is slightly higher in females (40), while others have found no significant association between RDW and gender. In our study, we also found that the RDW values of females were significantly higher than that of males; however, current data is not sufficient to draw conclusions regarding this matter.

Although our study showed significant relationships between RDW and various CRC characteristics that were largely consistent with previous studies, it has several limitations that must be noted. First, this is a single center and retrospective cohort study and has relatively few patients. Additionally, a control group was not included in this study, and therefore, comparisons with data from healthy patients were not possible. These may have led to various types of bias. Second, the number of patients in several sub-group analyses could have limited statistical reliability, and therefore, comparisons based on parameters such as tumor localization, pathological diagnosis, differentiation, T stage and N stage should be cautiously evaluated. Considering the presence of various studies showing some degree of relationship between elevated RDW and CRC prognosis, it appears that there is a need for further prospective, long-term multicenter studies with a larger number of patients to accurately assess the diagnostic and/or prognostic value of RDW in patients with CRC.

CONCLUSION

Both our study and similar studies have shown that CRC patients with high RDW levels have shorter postoperative survival or greater likelihood of death. Furthermore, elevated RDW seems to be associated with greater tumor size and more advanced clinical and TNM stages. All these relationships indicate a positive correlation between RDW values and the severity of CRC, and suggest that RDW may have a potential function as a diagnostic or prognostic marker in patients with CRC. For instance, it will not be surprising to suspect higher tumor burden in subjects with relatively elevated RDW –which could potentially be used to make decisions on surgical approach. However, in order for RDW to be accepted as a molecular marker associated with prognosis and survival in CRC, more comprehensive studies which can perform longer-term follow-up are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The protocol of this study was approved by the Non-Interventional Clinical Researches Ethics Committee of Eskişehir Osmangazi University (Date:15.06.2021, Decision No: 09).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Comparison of ocular emergencies in the COVID-19 pandemic and pre-pandemic period in the tertiary hospital of Turkey

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ABSTRACT

Aim: To evaluate clinical characteristics and alterations in the of patients admitted to the ocular emergency department (ED) of a tertiary hospital during coronavirus disease period in 2019 (COVID-19) and pre-pandemic period. Also, we intended to share our strategy and experience to prevent disease transmission to health-care staff during patient submission.

Material and Method: In this study, 45901 patients who applied to ED between January and May in 2020 were reviewed retrospectively for ocular manifestations. The five months divided into two groups as the pre-pandemic period and the pandemic period. Clinical and demographic data were collected. The proportion of urgent and non-urgent cases in the pre-pandemic and pandemic period was compared.

Results: A total of 30,576 patients (66.6%) admitted to ED before COVID-19 and 15,325 patients admitted (33.4%) during COVID-19 era. Five hundred thirty-eight (1.8%) of cases admitted in the pre-pandemic period, and 395 (2.6%) of the cases admitted in the pandemic period were in the real urgent category. Conjunctivitis, blepharitis and hordeolum, dry eye diseases, corneal diseases were the most common conditions in both pre-pandemic and pandemic periods.

Conclusion: This study showed that admissions to ED for ocular conditions during the pandemic period decreased significantly, and the rate of real urgent cases increased. Yet even during the pandemic period, non-urgent patients continue to come to the ED.

Keywords: Coronavirus disease, COVID-19, emergency department, patient triage, ophthalmology

INTRODUCTION

Emergency departments (ED) are one of the most crowded units of hospitals. Overcrowding in EDs causes poor quality of care, increased waiting times, dissatisfaction, and increased anxiety of patients, increased cost (1) The most crucial cause of congestion in EDs seems to be an increased proportion of patients with non-urgent complaints. It is probably tempting for patients to be examined in ED in a shorter time and at any time. ED in our hospital serves only eye patients and shares similar problems with General EDs. Overcrowding of EDS has always been and will probably continue to be a problem, but this situation has been even more critical during the COVID-19 pandemic due to the transmission of the virus.

The coronavirus disease 2019 (COVID-19) emerged first in Wuhan, Hubei province, China, in December 2019 by leading an outbreak and turned into a global pandemic (2). The virus caused the disease was called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (3). As the disease spread all over the world in a short time. It was declared as a pandemic by the World Health Organization (4). The countries had to take various precautions such as mask use, social distancing, and stay-at-home rules. The first confirmed case of COVID-19 was declared in Turkey on March 11, 2020, and the early death due to COVID-19 occurred on March 18, which is the date when restrictions began throughout the country. As it is known, due to the high risk of nosocomial spread of the virus, measures were also taken in the hospitals, including delivering only urgent and emergent care, rescheduling visits, postponing elective procedures, and enhancing

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personal protective measurements. Although all health-care workers have a risk of transmission of the virus, the ophthalmologist may be at increased risk of infection in examining patients with short working distance, especially during slit-lamp examination.

In this study, we aimed to evaluate the alterations in the number and proportion of ocular emergencies admitted to our hospital's ED during the COVID-19 period and pre-pandemic period in Ankara. We also wanted to share our strategy and experience to prevent the spread of the virus transmission to health-care staff.

MATERIAL AND METHOD

The study was carried out with the permission of Ankara Training and Research Hosptal, Non-Invasive Clinical Researches Ethics Committee (Date: 20.08.2020, Decision No:2020/374). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Participants and Study Design

A total of 45,901 patients admitted to our ocular ED between January and May in 2020 were included in this retrospective study. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written consent was taken from all adult cases and the parents of the child cases. Our hospital was not assigned by the government for COVID-19 treatments. Therefore, it was one of the only hospitals where the eye patients admitted to in Ankara and neighbouring cities in the COVID-19 period and maintained care intensity like in the pre-COVID-19 period.

Data of all the cases admitted to the ED were collected from patients' records, including the patient's age, gender, and diagnosis. The cases were divided into groups according to their ages as the cases younger than 17-year-old, between 17 and 40-year old, and the instances older than 40-year old. The five months were divided into two groups as before COVID-19 and COVID-19 era, based on March 15. The reason for the baseline of March 15 is the first COVID-19 case was confirmed in our country on March 11 and then, as of March 15, official COVID-19 measures were started to be taken in hospitals. We compared the number of patients admitted to the ED 2.5 months before and after March 15.

In Turkey, the patients attending ED are divided into zones according to their priority. Green zone refers to care patients with the least emergent situation, while the red zone is for more urgent cases. After examination, patients are marked with green or red on the system according to immediate status. In this study, we determined the proportions of urgent and non-urgent cases using the zones.

Strategies to Prevent COVID-19 Spread in the Emergency Departments

With the onset of the covid 19 pandemic, the working strategies of the emergency room has changed markedly, as in all departments of our hospital. In order to exclude Covid 19, symptoms such as fever, a history of contact with someone with COVID-19 in the last 14 days, and cough were questioned in all patients who applied to the emergency department. If there were any of them, they were referred to an assigned pandemic hospital. Patients without symptoms or contact history were admitted to the emergency department. All patients who applied were encouraged of the obligation to wear masks and social distance rules. Waiting rooms were readjusted according to 1,5-meter social-distant. Health care providers applied universal personal protective measures like wearing surgical/N95 mask, gloves in the necessity, protective clothing, face shield, and googles. They were informed about the importance of hand washing and disinfection. Slit lamps were meticulously cleaned after each patient examination, and a transparent barrier was placed in slit lamps between ophthalmologists and patients. When examining, both examiner and patient had to be masked, and the room was well ventilated. In hospitals assigned pandemic, patients who are not in the urgent or emergent category were informed about the necessity of examination under elective conditions, not in ED, as the intensity of the ED was tried to be reduced. But this situation could not be applied in hospitals designated as non-pandemic.

Statistical Analysis

Statistical analyses were performed with SPSS 21 (IBM, New York, USA) program. Frequencies and percentages were presented for categorical variables. An independent t-test was used to compare differences between groups because the data were normally distributed.

RESULTS

The mean age of 28,334 (61.7%) male and 17,567 (38.3%) female cases were 37.84±19.73 (range; 0-100). The age distribution of patients were 7,315 (16%) in younger than 17-year-old, 17,908 (39%) in between 17 and 40-year-old, 20,678 (45%) in older than 40-year-old.

A total of 30,576 patients (66.6%) admitted to ED before the COVID-19 era (before March 15) and 15,325 patients admitted (33.4%) during COVID-19 era (**Table 1**). Five hundred thirty-eight (1.8%) of cases admitted pre-pandemic era, and 395 (2.6%) of the cases admitted pandemic era were in real urgent category. It was determined that a significant decrease in the number of patients who applied to ED in the COVID-19 era. ($p<0,05$) The percentage of rel urgent cases during the COVID-19 era was higher than before the COVID-19 era (**Figure 1** and **Table 1**).

Table 1. Admissions rates to the emergency department in pandemic and pre-pandemic period

	Total Count	Non-urgent Count	Urgent Rate	Count	Rate
Pre-pandemic period	30,576	30,038	98.2%	538	1.8%
Pandemic period	15,325	14,930	97.4%	395	2.6%

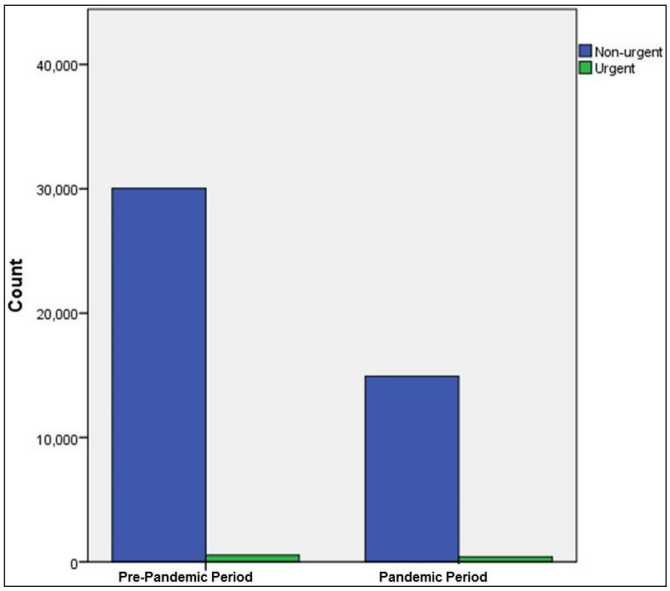

Figure 1. Urgent and non-urgent cases in pandemic and pre-pandemic period

Conjunctivitis (including allergic, chronic), blepharitis and hordeolum, dry eye diseases (feeling of ocular surface discomfort and meibomitis were also evaluated in this category), corneal diseases (including corneal foreign body and keratitis) were the most common conditions both pre-pandemic and pandemic period. Some situations were summarized in **Table 2**. The distribution of diagnoses of the cases by the COVID-19 period and months were summarized in **Table 2**, **Figure 2**, and **Figure 3**.

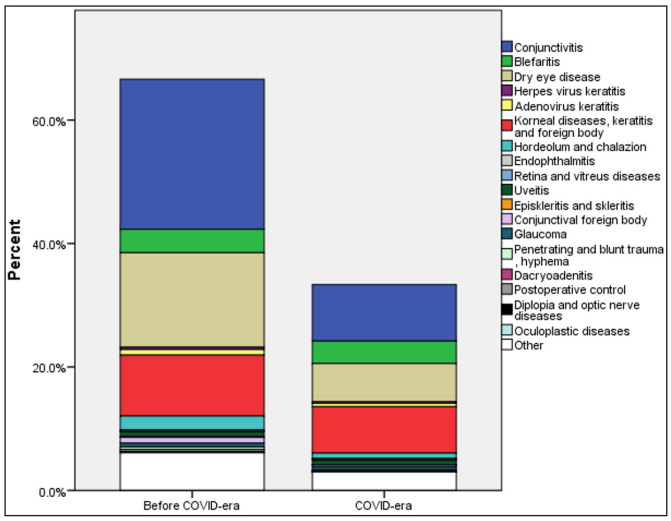

Figure 2. The distribution of diagnoses of the cases by the pandemic and pre-pandemic period

Table 2. Distributions of the patients with pandemic and pre-pandemic period in terms of common diagnosis

Diagnosis	Pre-Pandemic Period		Pandemic Period	
	Count	Column N %	Count	Column N %
Conjunctivitis	11158	36.5%	4203	27.4%
Blefaritis	1747	5.7%	1680	11.0%
Dry eye disease	7020	23.0%	2842	18.5%
Herpes virus keratitis	170	0.6%	111	0.7%
Adenovirus keratitis	424	1.4%	249	1.6%
Korneal diseases, keratitis and foreign body	4527	14.8%	3459	22.6%
Hordeolum and chalazion	1056	3.5%	418	2.7%
Endophthalmitis	6	0.0%	2	0.0%
Retina and vitreus diseases	109	0.4%	119	0.8%
Uveitis	351	1.1%	308	2.0%
Episkleritis and skleritis	72	0.2%	34	0.2%
Conjunctival foreign body	423	1.4%	124	0.8%
Glaucoma	279	0.9%	231	1.5%
Penetrating and blunt trauma , hyphema	177	0.6%	86	0.6%
Dacryoadenitis	17	0.1%	16	0.1%
Postoperative control	188	0.6%	34	0.2%
Diplopia and optic nerve diseases	7	0.0%	10	0.1%
Oculoplastic diseases	55	0.2%	17	0.1%
Other	2789	9.1%	1382	9.0%

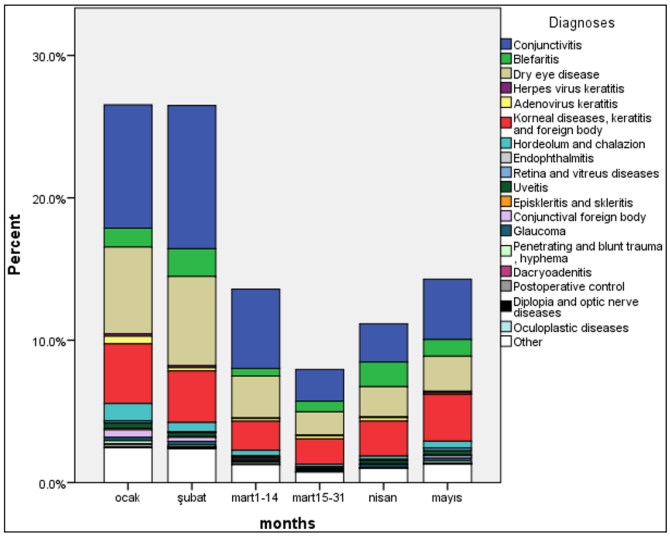

Figure 3. The distribution of diagnoses of the cases by months

DISCUSSION

The ED of our hospital in Ankara, Turkey, serves about the number of 12 500 patients a month (data in 2019) as one of the most crowded EDs. The total amount of ED examinations between January and May in 2020 was 49.501 patients. When we compared the amounts of ED examinations before and after March 15, we found that a significant decrease in the number of patients admitted to ED during the COVID-19 period. At the same time, we observed an increased proportion of real urgent cases during COVID-19. The restriction of outdoor explains

the reduction during this period. Besides, the fear of being infected by going to the hospital and statement of government and health-care professionals like keeping away from the hospital except for urgent situations could be efficient.

As in other EDs in Turkey, in our ocular ED, the most common problem is overcrowding, which puts both personnel and patients in a problematic situation. The most important reason for the congestion of EDs is the application of patients with non-urgent complaints consistent with the literature (2,5). The people in Turkey may be applied to EDs with the financial concerns, the expectation of rapid service, or avoiding the necessity of appointment (2). The other significant reason is the feasible hours of operation. Open 24 hours a day, seven days a week, and EDs are a great convenience to the community (6). Also, some patients applying to ED may think that their ocular condition is emergent. In our study, only 1.8% (538 of 30,038 in total) of the cases accessed to ED in the 2.5 months before COVID-19 were in the real urgent category (marked red zone). During the COVID-19 era, the rate increased to 2.6% (395 of 14,930 in total). Consistent with our results, it has been reported in the literature that less than 3% of patients applying to EDs need vital interventions (7). One of the essential findings detected in this study is that non-urgent patients continue to come to the ED even during the pandemic period.

Carvalho and José (8) reported that 55% of the cases admitted to the ocular emergency had inflammatory conditions such as conjunctivitis, blepharitis, chalazion, or hordeolum, all of which need to be treated in primary or secondary care units. In our report, similar to this study, 33.5% had conjunctivitis, 21.5% had dry eye or meibomitis, 17.4% had corneal diseases (keratitis and corneal foreign body), and 7.5% had blepharitis, respectively, in both periods.

The demographic characteristics of our sample were similar to the previous reports (9,10), and the number of males admitted to ED for ocular conditions was higher than females. (61.7% to 38.3%). The average age of our sample was 37.84 years, and most of the patients (45%) had within the range 41 to 100 years. Similar to our study, in a study in which epidemiology of ocular emergencies was evaluated, the majority of admissions were reported as middle-aged men (11).

Transmission of SARS-CoV2 is known to occur by respiratory droplet, aerosol, and contact. Ophthalmologists can be considered as one of the most risky professions in the covid-19 pandemic, since the examination performed by them with slit lamp and ophthalmoscope is shorter than the safe social distance of 1.5-2 meters (12). During the severe acute respiratory syndrome (SARS) outbreak in 2003, many health care providers infections alerted medical institutions to take more severe precautions in case of a future epidemic (13). Unfortunately, thousands of health care workers have been infected during COVID-19. As mentioned above, a strategy was developed for protection during clinical practice according to guidelines. Triage was one of the most important of them. A detailed COVID-19 and ocular history were taken from the patients before entering the waiting room. All personal protective measures were taken, even if contact history and fever were regular, as the ability to spread the infection was known during the asymptomatic period. Patients who were not urgent in ocular history were turned back to protect both health care workers and patients from the risk of infection caused by crowding in the ED of the pandemic hospital. During this period, none of the health care providers working in our hospital was infected with SARS-CoV2. Although a significant decline in the total number of patient admissions in the COVID-19 period, a tiny proportion of patients had a genuinely urgent medical condition. This determination is crucial to demonstrate the inappropriate utilization of the ED, even during the COVID-19 period.

CONCLUSION

As our knowledge, this is the first study to evaluate diagnoses and alterations in the number of patients admitted to the ocular ED during the COVID-19 period. The most common conditions for emergency admissions were inflammatory ocular surface conditions that do not need to be urgently treated in ED. This study revealed that the majority of patients entries to the ocular emergency department were not real urgent neither COVID-19 period nor before.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Ankara Training and Research Hosptal, Non-invasive Clinical Researches Ethics Committee (Date: 20.08.2020, Decision No: 2020/374)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Fear of COVID-19: reflections on disease severity, sleep and anxiety in fibromyalgia patients

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ABSTRACT

Aim: This study aimed to assess the effects of COVID- 19 fear and anxiety on disease severity, pain, sleep quality and health-related quality of life in fibromyalgia patients.

Material and Method: Sixty-four patients with diagnosis fibromyalgia and sixty-five healthy volunteers over 18 years of age were enrolled in the study. Sociodemographic features were evaluated. Fibromyalgia Impact Questionnaire (FIQ), Short Form 36 (SF-36), Coronavirus Anxiety Scale (CAS), Obsession with COVID-19 Scale(OCS), and Pittsburgh Sleep Quality Index (PSQI) were administered to all participants.

Results: The mean values of CAS, FIQ, PSQI, and BDI were found to be higher in the fibromyalgia group than the control group ($p<0.05$). The median/mean value of CAS, OCS, and BDI were higher in patients with losing a first-degree relative patient due to COVID-19 in fibromyalgia group, these results were statistically significant ($p<0.05$).

Conclusion: According to the results of this study, fibromyalgia may be affected more by psychological stress which affects disease severity during COVID 19 pandemic.

Keywords: Fibromyalgia, COVID-19, anxiety

INTRODUCTION

COVID-19 disease was reported from Wuhan, China in December 2019 as viral pneumonia of unknown origin (1). On January 7, 2020 the new type of coronavirus SARS- CoV-2 was detected as the viral pneumonia agent. The disease has spread all over the world rapidly and the World Health Organization (WHO) defined disease as the novel coronavirus disease (COVID-19) and declared a pandemic (2). The prognosis of COVID 19 disease varies according to age, comorbidities and gender (3). The researches on the etiology, prognostic factors, prevention and treatment of COVID-19 disease continues (4).

Fibromyalgia is a chronic pain disease that includes the musculoskeletal system. The symptoms of fibromyalgia are generalized pain (head-to-toes), fatigue, morning stiffness not exceeding 60 minutes, regional pain syndromes such as migraine, dysmenorrhea, irritable bowel syndrome, autonomic disturbances as orthostatic hypotension, feeling of instability, xerostomia. Anxiety and stress are important factors for fibromyalgia severity and psychological mood problems are seen more frequently in fibromyalgia patients than healthy individuals. The prevalence of anxiety for fibromyalgia patients is 60 % and depression is 14-36% (5).

There are many studies in the literature evaluating fibromyalgia severity during the COVID-19 pandemic. We aimed to evaluate the effects of COVID-19 fear and anxiety on disease severity, pain, sleep quality and health related quality of life in fibromyalgia patients.

MATERIAL AND METHOD

Study Design and Setting

The study protocol was approved by the Kırklareli University Health Sciences Institute Ethics Committee (Date: 12.07.2021, Decision No: E-69456409-199-17531). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.The participants were informed about the aim of study before applying the study, all participants were asked whether they accepted to complete the study and thus all participants have declared voluntary for the study. The informed consent form was applied to all the participates.

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Participants

Sixty-four patients with diagnosis fibromyalgia and sixty-five healthy volunteers were participated to the study. The following inclusion criteria were applied: (a) having a diagnosis as primary fibromyalgia for more than 1 year according to the American College of Rheumatology criteria (b) being more than 18 years old age (c) being volunteer to participate study. The following exclusion criteria were applied (a) a diagnosis with skeletal muscle diseases, chronic renal diseases, chronic liver failure, malignancy and neurologic diseases; (b): having cognitive impairment that affects answering the questionnaires; (c): being pregnant.

Sociodemographic features including age, gender, body mass index, fibromyalgia duration, education level, having history of COVİD-19 disease in participants and loosing first degree relatives were analyzed. The Fibromyalgia İmpact Questionnaire (FIQ), Short Form 36 (SF36), Coronavirus Anxiety Scale (CAS), Obsession with COVID-19 Scale (OCS), and Pittsburgh Sleep Quality Index (PSQI) were administered to all participants.

Outcome Measures

The beck depression inventory (BDI): It is a scale developed by Beck A. using clinical observations in depressed psychiatric patients and rarely in non-depressed psychiatric patients (6). BDI assesses depression symptoms with 21 multiple choice items. Each item has a 4-point scale variable between 0 (absent) and 3 (severe). A minimum of 0 and a maximum of 63 points can be obtained from the scale The Turkish validity and reliability study of the questionnaire was carried out by Hisli (7).

Pittsburgh sleep quality index (PSQI): PSQI was developed and widely used by Buysse et al. In order to evaluate sleep quality in the last month, the questionnaire consisting of 24 questions includes 19 questions about sleep duration, duration of latency, and the frequency and severity of certain sleep-related problems. The last 5 questions are used for clinical purposes and are answered by the patient's bed or room partner. PSQI consists of 7 components and the score obtained from the components represents the total score. The total score is in the range of 0-21, and an increase in the score indicates poor sleep quality (8). The Turkish validity and reliability study of the scale was performed by Agargün et al. in 2004 (9).

The short form 36 (SF-36): Short Form 36 has eight parts that include general health (5 items), vitality (4 items), physical functioning (10 items), bodily pain (2 items), physical role functioning (4 items); emotional role functioning (3 items); mental health (5 items); and social functioning (2 items). The total score was obtained the combination of the eight sections. Higher scores are related to well quality of life. The SF-36 was adapted into Turkısh in1999 by Koçyigit H et al., and the study results suggest it is useful for clinical studies (10).

The fibromyalgia impact questionnaire (FIQ): FIQ has three parts including function, symptoms and overall impact. High scores indicate worse functionality and increased symptom severity and affects the person more The FIQ was adapted into Turkısh in 2000 by Sarmer S et al, and the study results suggests it is useful for clinical studies (11).

Coronavirus anxiety scale all questions (CAS): COVID-19 related anxiety (CAS) was developed by Lee. The questionnaire is a five-item mental health scale that assesses physiological effects as anxiety related to COVID-19. A cut-off point of ≥ 9 demonstrates the anxiety related to COVID-19 (12). The Turkısh validity and reliability studies was performed by Evren et al.(13).

Obsession with COVID-19 scale (OCS): Obsession with COVID-19 Scale (OCS) is a 4-item scale that helps to assess persistent and disturbing thinking about COVID-19 (14). OCS was found an effective and valid tool for clinical practice. OCS indicates dysfunctional thinking associated with a total score ≥7. The Turkısh validity and reliability studies was performed by Evren et al.(13).

Statistical Analysis

Mean±standard deviation and median (minimum–maximum) were used for continuous variables, whereas categorical data was reported with numbers and percentages. The Kolmogorov-Smirnov goodness-of-fit test was used to perform normality analyses in the cross-group analysis of continuous variables. Evaluation of the groups with the normal distribution of continuous variables was done by using the independent samples t-test. Cross-group comparisons of variables not eligible for normal distribution were analyzed with the Mann-Whitney U test. The chi-square test (Fisher's exact test when necessary) was used for the comparison of categorical data. The analyses were performed with the Statistical Package for the Social Sciences (SPSS) software program version 26.0 (IBM Corporation, Armonk, NY, USA). The statistical significance level was set at $p<0.05$.

RESULTS

A total of 129 participants were enrolled in the study. The participants were grouped into Group 1 (fibromyalgia, n=64) and Group 2 (control, n=65). The mean age, body mass index (BMI), the ratio of female gender, the ratio of educational status as primary school, the ratio of marital status, and usage of drugs (painkiller, antidepressant, and myorelaxant) were found to be statistically significantly higher in fibromyalgia group than control group ($p<0.05$).

However, there were no statistically significant differences between the two groups in terms of comorbidities, usage of anticonvulsant, history of COVID-19, hospitalization due to COVID-19 and losing a first-degree relative due to COVID-19 (p>0.05). Comparisons of the demographic and some clinical features of groups are presented in **Table 1**.

Table 1. Comparison of demographic and clinical features of participants between two groups

	Fibromyalgia (n=64)	Control (n=65)	p
Age (mean±SD)	41.46±9.42	27.33±5.17	<0.001*
BMI (kg/m²) (mean±SD)	26.38±4.41	23.23±3.88	<0.001*
Gender (n, %)			<0.001**a
Female	62 (96.9%)	48 (73.8%)	
Male	2 (3.1%)	17 (26.2%)	
Educational status (n, %)			<0.001**
Primary school dropout	8 (12.5%)	0 (0.0%)	
Primary school	29 (45.3%)	0 (0.0%)	
Secondary school	8 (12.5%)	0 (0.0%)	
High school	11 (17.2%)	3 (4.6%)	
University	8 (12.5%)	62 (95.4%)	
Marital status (n, %)			<0.001**
Single	7 (10.7%)	45 (69.2%)	
Married	55 (85.9%)	19 (29.2%)	
Divorced	0 (0.0%)	1 (1.5%)	
Widowed	2 (3.1%)	0 (0.0%)	
Comorbidities (n, %)			0.368**
No	64 (98.4%)	64 (98.5%)	
Yes (Hypothyroidism)	1 (1.6%)	0 (0.0%)	
Yes (others)	0 (0.0%)	1 (1.5%)	
Usage of painkiller (n, %)			<0.001**a
Yes	32 (50.0%)	0 (0.0%)	
No	32 (50.0%)	65 (100.0%)	
Usage of antidepressant drugs (n, %)			<0.001**a
Yes	14 (21.9%)	0 (0.0%)	
No	50 (78.1%)	65 (100.0%)	
Usage of myelorelaxant drugs (n, %)			<0.001**a
Yes	14 (23.4%)	0 (0.0%)	
No	49 (76.6%)	65 (100.0%)	
Usage of anticonvulsant drugs (n, %)			0.244**a
Yes	2 (3.1%)	0 (0.0%)	
No	62 (96.9%)	65 (100.0%)	
History of COVID-19 (n, %)			0.666**
Yes	25 (39.1%)	23 (35.4%)	
No	39 (60.9%)	42 (64.6%)	
Hospitalization due to COVID-19 (n, %)			0.244**a
Yes	2 (3.1%)	0 (0.0%)	
No	62 (96.9%)	65 (100.0%)	
History of COVID-19 in a first-degree relative (n, %)			0.650**
Yes	37 (57.8%)	35 (53.8%)	
No	27 (42.2%)	30 (46.2%)	
Losing a first-degree relative due to COVID-19 (n, %)			0.203**a
Yes	6 (9.4%)	12 (18.5%)	
No	58 (90.6%)	53 (81.5%)	
Total	64 (100.0%)	65 (100.0%)	

* Independent Samples T Test, ** Mann Whitney U Test, *** Chi-square Test (aFisher's exact test)

The mean values of CAS, FIQ, PSQI and BDI were found to be higher in the fibromyalgia group than the control group (p=0.044, p<0.001, p=0.009 and p<0.001 respectively). Also, the mean values of subscales of SF-36 as physical function, pain, physical role functioning, general health, social functioning, and vitality were found to be lower in the fibromyalgia group than the control group (p<0.001, p<0.001 p=0.004, p<0.001, p<0.001, p=0.015 respectively). However, the mean values of OCS, emotional role functioning and mental health were found lower in the fibromyalgia group but the differences were not statistically significant (p>0.05). Comparisons of the outcomes scores among the groups is shown in **Table 2**.

Table 2. Comparison of results of Fibromyalgia Impact Questionnaire, Pittsburgh Sleep Quality Index, sub scales of Short Form 36 , Obsession with COVID-19 Scale, Coronavirus Anxiety Scale and Beck Depression Inventory between groups

	Fibromyalgia (n=64)	Control (n=65)	p
CAS	1.05±2.32	0.36±1.35	0.044*
OCS	2.57±3.24	2.35±2.58	0.664*
FIQ	50.0 (3.6-94.4)	27.4 (0-80.9)	<0.001**
PSQI	10.0 (0-17.0)	6 (1-16)	0.009**
BDI	16 (0-45)	12 (0-36)	<0.001**
Physical Function	65 (0-100.0)	95 (40-100.0)	<0.001**
Pain	32.0 (0-90.0)	62.0 (22-100.0)	<0.001**
Physical Role Functioning	25.0 (0-100.0)	75.0 (0-100.0)	0.004**
General Health	43.5 (5-97.0)	62.0 (15-100.0)	<0.001**
Social Functioning	62.5 (0-100.0)	75.0 (12.5-100.0)	<0.001**
Vitality	45.0 (0-90.0)	50.0 (5-100.0)	0.015**
Emotional Role Functioning	50.0 (0-100.0)	66.6 (0-100.0)	0.173**
Mental Health	64.0 (8-92.0)	68.0 (20-100.0)	0.162**

* Independent Samples T Test, ** Mann Whitney U Test, CAS, Coronavirus Anxiety Scale; OCS, Obsession with COVID-19 Scale; FIQ,Fibromyalgia Impact Questionnaire; PSQI, Pittsburgh Sleep Quality Index; BDI, Beck Depression Inventory

The mean value of CAS was found to be statistically significantly higher in patients with COVID-19 history (2.00±3.27) than patients without COVID-19 history (0.43±1.07) in fibromyalgia group (p=0.007). However, there were no statistically significant differences of the mean values of OCS, BDI, FIQ, PSQI and the subscales of SF-36 according to having COVID-19 history in fibromyalgia group (p>0.05) (**Table 3**).

When the history of losing a first-degree relative due to COVID-19 was compared in fibromyalgia group, the median/mean value of CAS, OCS, and BDI were higher in patients with losing first-degree relative and the differences were statistically significant (p=0.029, p<0.001 and p=0.005, respectively). Also, the mean values of physical role functioning, social functioning

and mental health were lower in patients with losing a first degree relative and the differences were statistically significant (p=0.011, p=0.007, p=0.033 and p=0.002, respectively). But there were no statistically significant differences according to FIQ and PSQI scores (p=0.336 and p=0.175 respectively) (**Table 4**).

Table 3. Comparison of results of Fibromyalgia Impact Questionnaire, Pittsburgh Sleep Quality Index, sub scales of Short Form 36 , Obsession with COVID-19 Scale, Coronavirus Anxiety Scale and Beck Depression Inventory in fibromyalgia group in terms of COVID-19 history

	History of COVID-19 (n=25)	No History of COVID-19 (n=39)	p
CAS	2.00±3.27	0.43±1.07	0.007*
OCS	3.00±3.31	2.30±3.20	0.409*
FIQ	54.7 (9.7-81.7)	47.1 (3.6-81.7)	0.175**
PSQI	10.0 (1-17.0)	8 (0-17)	0.317**
BDI	19 (0-45)	15 (0-35)	0.158**
Physical Function	65 (25.0-90.0)	65 (0-100.0)	0.392**
Pain	31.0 (0-90.0)	32.0 (0-90.0)	0.502**
Physical Role Functioning	25.0 (0-100.0)	50.0 (0-100.0)	0.655**
General Health	35.0 (5-82.0)	45.0 (5-97.0)	0.544**
Social Functioning	62.5 (0-100.0)	62.5 (0-100.0)	0.801**
Vitality	35.0 (0-90.0)	50.0 (5-100.0)	0.310**
Emotional Role Functioning	66.6 (0-100.0)	66.6 (0-100.0)	0.625**
Mental Health	64.0 (8-84.0)	68.0 (8-92.0)	0.384**

* Independent Samples T Test, ** Mann Whitney U Test, CAS, Coronavirus Anxiety Scale; OCS, Obsession with COVID-19 Scale; FIQ,Fibromyalgia Impact Questionnaire; PSQI, Pittsburgh Sleep Quality Index; BDI, Beck Depression Inventory

Table 4. Comparison of results of Fibromyalgia Impact Questionnaire, Pittsburgh Sleep Quality Index, sub scales of Short Form 36 , Obsession with COVID-19 Scale, Coronavirus Anxiety Scale and Beck Depression Inventory in fibromyalgia group in terms of History of COVID-19 in a first-degree relative

	Loosing first degree relative due to COVID-19 (n=6)	No Loosing first degree relative due to COVID-19 (n=58)	p
CAS	3.00±3.46	0.84±2.10	0.029*
OCS	6.83±3.81	2.13±2.86	<0.001*
FIQ	54.9 (45.1-57.1)	49.5 (3.6-94.4)	0.336**
PSQI	11.0 (10.0-15.0)	8 (0-17)	0.175**
BDI	27 (17-36)	15.5 (0-45)	0.005**
Physical Function	60 (0-75.0)	65 (20.0-100.0)	0.252**
Pain	25.5 (0-41.0)	32.0 (0-90.0)	0.198**
Physical Role Functioning	0.0 (0-25.0)	50.0 (0-100.0)	0.011**
General Health	38.5 (5-67.0)	43.5 (5-97.0)	0.506**
Social Functioning	25.0 (0-62.5)	62.5 (0-100.0)	0.007**
Vitality	30.0 (0-45.0)	45.0 (0-90.0)	0.033**
Emotional Role Functioning	16.6 (0-66.6)	66.6 (0-100.0)	0.242**
Mental Health	34.0 (8-52.0)	68.0 (8-92.0)	0.002**

* Independent Samples T Test, ** Mann Whitney U Test, CAS, Coronavirus Anxiety Scale; OCS, Obsession with COVID-19 Scale; FIQ,Fibromyalgia Impact Questionnaire; PSQI, Pittsburgh Sleep Quality Index; BDI, Beck Depression Inventory

DISCUSSION

The presence of COVID-19 fear and the relationship with disease severity in fibromyalgia patients were evaluated in this study. The psychological effects as anxiety and COVID-19 fear were higher in fibromyalgia patients than healthy participants. The study revealed that the fibromyalgia patients with history of COVID-19 disease had more COVID-19 fear and losing first degree relative due to COVID-19 may improve COVID-19 fear, depression symptoms, fibromyalgia disease severity, sleep problems and effect the health related quality of life in fibromyalgia patients.

Fibromyalgia is defined as a syndrome characterized by chronic and widespread musculoskeletal pain. Insomnia, fatigue, mood disorders, anxiety, depression are the common symptoms (15). The pathophysiological factors of fibromyalgia are not known exactly. Most fibromyalgia patients are hypersensitive to have pain and psychologic factors may be associated occurrence of the pain (16). Epstein et al. (17) have been showed the prevalence of psychological comorbidities as anxiety disorders or depression are 60% in fibromyalgia patients. Also depressive patterns are associated with worse prognosis so that greater pain severity was observed in fibromyalgia patients with comorbidity of depression symptoms than healthy groups (18) .

COVID-19 disease as well as the various restrictions taken by the states to prevent the spread of the disease, have also affected the recovery process of patients, especially their mental and physical functions (19). Studies have reported individualis'loneliness level had increased during the pandemic due to lack of social interactions. Increased of individualis'loneliness has been associated with anxiety and depression. (20) During COVID-19 pandemic many assessment tools have been used to evaluate psychological effects of COVID-19. In this current study CAS and OCS were used and the fibromyalgia patients with history of COVID-19 had higher scores demonstrates anxiety associated with coronavirus and thinking about COVID-19 too much. As we know the severity of fibromyalgia symptoms such as higher perception of tenderness after pressure is applied to tender points have been increased with anxiety (21). Batres-Marroquín et al. (22) reported that the fibromyalgia symptoms as pain, anxiety and depression have been worsening COVID-19 pandemic lockdown. Also increased sympathetic nervous system activity play a role on sleeping problems and COVID-19 lockdown- associated life style changes could worsen sympathetic nervous system. Previous studies showed that fibromyalgia patients have norepinephrine-evoked pain (23) .

The relationship between sleep disorders and fibromyalgia is known. It has been shown in recent studies to be associated with sleep disturbance and widespread musculoskeletal pain. The effect of insomnia on pain formation and pain persistence is reported (24,25). The relationship between COVID-19 disease and sleep disturbances is known. Sleep duration and timing in COVID-19 positive individuals were affected by the illness behavior of sleep pattern at 30 days post-illness (26). It was not surprising that PSQI scores were higher in the fibromyalgia group in this study. However, sleep scores were found to be high in fibromyalgia patients with COVID-19 history. This suggests that COVID-19 may be an effect on sleep disorders in fibromyalgia patients.

Dell'Osso et al. (27) showed that loss events and the severity of illness and health related quality of life in fibromyalgia patients. Similarly, in this current study the patients with losing first degree relatives due to COVID-19 had higher CAS, and OCS scores besides disease severity and low health related quality of life scores. Death and mourning process are universal and refers to losses that can occur at any stage of life. The grieving process reflects affective, cognitive, behavioral, physiological responses which affect individual and family system (28). Losing first degree relative and mourning process have difficulties to accept and adapt for most people. During pandemic period the restrictions, lack of social relations and legal limits on the number of people who can attend funeral could be increase these difficulties of mourning process. The main limitation of this study is its relatively small sample size and a single center study.

CONCLUSION

Psychological disorders are related with fibromyalgia severity and symptoms of disease. Fibromyalgia patients may be more effected in accordance of cognitive, mental and psychological features. These patients should be evaluated closely and treatment of fibromyalgia patients assessed. With the completion of researches on the long-term effects of COVID-19, the effects of COVID-19 disease will be better understood and the management of fibromyalgia will be reviewed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kırklareli University Health Sciences Institute Ethics Committee (Date: 12.07.2021, Decision No: E-69456409-199-17531).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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Is there an association between thyroid function tests and 18F FDG PET/CT parameters in untreated cancer patients?

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ABSTRACT

Objectives: We aimed to investigate the association between the extent of disease, 18F FDG PET/CT parameters (SUVmax and the highest SUVmax) and thyroid function tests (TFT) (TSH, FT4, FT3, FT3/FT4 ratio, AntiTG, and AntiTPO) in untreated cancer patients.

Material and Method: One hundred and seventy-nine patients who underwent FDG PET/CT for metabolic characterization and staging in our clinic between May 2020 and November 2020 were included in the study. Patients were divided into two groups as malignant and benign according to histopathology findings. Thyroid function tests were ordered from all patients at the time of PET/CT imaging. The association between the presence of local lymph node metastasis, distant metastases and thyroid function tests as well as the association between PET/CT parameters and thyroid function tests in benign and malignant groups were statistically analyzed.

Results: Thyroid function tests did not exhibit a significant difference between patients with malignant and benign disease ($p > 0.05$). Univariate logistic regression analysis revealed that age, FT4 value, and the FT3/F4 ratio were significant parameters in predicting distant metastases. These parameters were also significant in predicting mortality. Multivariate logistic regression analysis showed that age was an independent prognostic factor predicting mortality.

Conclusion: Thyroid function tests are not decisive in differentiating malignant and benign lesions. While no statistically significant correlation was observed between thyroid function tests and PET/CT parameters, univariate analyses revealed that especially FT4 and FT3/FT4 ratio were significant in predicting disease extent and mortality in malignant disease. Age was found to be an independent prognostic factor in predicting mortality.

Keywords: PET/CT, SUVmax, thyroid hormones, malignancy, distant metastasis

INTRODUCTION

Thyroid hormones are regulated by the hypothalamic-pituitary-thyroid and peripheral tissue axes and affect cell development, differentiation, and growth (1). Thyroid hormones specifically bind to the membrane and nuclear receptors that activate various oncogenic pathways, resulting in the promotion of cell growth, inhibition of apoptosis, and stimulation of angiogenesis. As a result of these multiple actions, thyroid hormones can play a major role in carcinogenesis. Numerous studies have pointed to an association between the increased risk of both solid organ and hematological malignancies and elevated thyroid hormone levels and/or suppressed thyrotropin (TSH) levels (2-4). Also, many in vitro studies revealed the tumor-promoting effects of thyroid hormones (5-8). Hyperthyroidism is associated with poorer prognosis and increased mortality in cancer patients, while hypothyroidism is associated with improved prognosis and prolonged mortality (9,10).

The most common hormone pattern in euthyroid sick syndrome is normal T4 and thyroid stimulating hormone levels, and low total T3 and free T3 levels. Changes in hormone parameters are thought to be a response to systemic disease in different ways in response to oxidative stress. In addition, it can be observed in acute and chronic diseases, after operations and malignancies. It is not a true syndrome and approximately 75% of hospitalized

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patients have significant changes in the hypothalamic-pituitary-thyroid axis (11,12).

18F-fluorodeoxyglucose positron emission computerized tomography (18F FDG PET/CT) is one of the most commonly used functional imaging methods in clinical practice. This imaging technique is based on the demonstration of in vivo glucose metabolism (13,14). FDG uptake is routinely measured using the maximum standardized uptake value (SUVmax), which is an accurate and reliable imaging biomarker. 18F FDG PET/CT and SUVmax are widely used in the diagnosis, staging, and evaluating treatment response of various malignant diseases (15-19). A few recent systematic reviews and meta-analyses have shown that SUVmax can be used as a prognostic factor in numerous malignancies (20-23). SUVmax is closely associated with tumor size, number of metastatic lymph nodes, occurrence and progression of distant metastases, and mortality predictions (24).

In this study, we aimed to investigate whether thyroid function tests (TSH, FT4, FT3, FT3/FT4 ratio, AntiTG, and AntiTPO) exhibit any difference between patients with untreated malignant or benign disease, and analyze the association between the extent of disease, FDG PET/CT parameters and thyroid function tests among these patients.

MATERIAL AND METHOD

The study was conducted with the permission of the Health Sciences University Gazi Yaşargil Training and Research Hospital Clinical Researches Ethics Committee (Date: 26.03.2020, Decision No: 736). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 179 patients who underwent FDG PET/CT for metabolic characterization and staging in our Nuclear Medicine Clinic between May 2020 and November 2020 were reviewed retrospectively. Patients were divided into two groups as malignant and benign according to histopathology findings. Thyroid function tests were ordered from all patients at the time of PET/CT imaging. SUVmax values were measured from all primary lesions as well as local lymph node and distant metastases in patients with malignant disease. The highest SUVmax was recorded for all patients. The association between the presence of local lymph node metastasis, distant metastases and thyroid function tests as well as the association between PET/CT parameters and thyroid function tests in benign and malignant groups were statistically analyzed.

Inclusion Criteria

Patients who had their thyroid function tests measured at the time of PET/CT imaging, did not yet undergo surgery or medical treatment, and had a histopathological diagnosis were included in the study.

Exclusion Criteria

Patients who are under thyroid hormone replacement for any reason and patients who had a previous history of Iodine-131 radionuclide treatment or thyroid surgery were excluded. Patients who underwent surgical treatment or chemotherapy/radiation therapy for the primary lesion or metastases were also excluded.

18F FDG PET/CT Protocol

All patients were asked to fast and stop intravenous (IV) glucose intake for at least 6 hours before undergoing scans. We confirmed blood glucose values to be ≤140 mg/dl by finger-stick method before FDG injection. One hour after the 18F FDG injection of 3.5 MBq/kg–5.5 MBq/kg, we obtained the CT images (120 kV, 80 mAs/slice, 700 mm transaxial FOV, no gap, 64x0.625 mm collimation, pitch 1.4, 0.5 s rotation time, 3.3 mm slice thickness, 512x512 matrix) from the vertex to the middle of the thigh in the supine position using the Discovery IQ 4 ring 20-cm axial FOV PET/CT device (GE Healthcare, Milwaukee, WI, USA). We then obtained the bedside PET (3D FOV 20 cm, ordered subset expectation-maximization algorithm [OSEM] 5 iterations/12 subset, full width at half maximum [FWHM] 3 mm) images at 2.5 minutes thereafter.

Image Analysis

Images were evaluated by two nuclear medicine attendings with at least 10-years of PET/CT experience. Volumes of interest (VOIs) were drawn from the primary lesion, local lymph nodes, and distant metastases. SUVmax of all lesions and the highest SUVmax of all patients were measured and recorded.

Statistical Analysis

IBM SPSS Statistics for Mac, version 25.0 (IBM Corporation, Armonk, New York) was used for statistical analyses. Normality of data was tested with Kolmogorov-Smirnov test. Comparison of two independent groups with nonparametric distribution was performed using Mann-Whitney U test, while categorical variables were compared with Pearson's chi-squared test using the results of Fisher's exact test. The prognostic role of thyroid parameters in predicting local lymph node metastasis, distant metastasis, and mortality was evaluated with univariate and multivariate logistic regression analysis. Quantitative data were expressed as mean±SD (standard deviation) or median (minimum-maximum), while categorical variables were expressed as percentage (%). Data were evaluated within 95% confidence interval. $p< 0.05$ was considered statistically significant.

RESULTS

Among the total of 179 patients, 97 (54.2%) were male. The mean age of all patients was 60 years (3-94). Fifty-three patients (29.6%) had benign and 126 (70.4%) had malignant histopathology findings. Histopathological diagnoses of the patients are listed in **Table 1**. Eighty-six (68.2%) out of 126 patients with malignant disease had local lymph node metastases and 44 (34.9%) had distant metastases.

Table 1. Patient numbers and tumor types in malignant and benign groups

Malignant Group (126)	**N**
Lung cancer	28
Breast cancer	21
Gastric cancer	15
Colorectal cancer	13
Prostate cancer	9
Pancreatic cancer	6
Lymphoma	5
Hepatic cancer	3
Cervical cancer	2
Endometrial cancer	2
Ovarian cancer	2
Esophageal cancer	2
Duodenal cancer	2
Multiple myeloma	2
Bladder cancer	2
Renal cancer	2
Gallbladder cancer	2
Tongue cancer	2
Glioblastoma Multiforme	1
Neuroblastoma	1
Paraganglioma	1
Liposarcoma	1
Ampullary tumor	1
Peritoneal carcinomatosis	1
Benign Group (53)	**N**
Solitary pulmonary nodule	21
Pleural thickening	8
Cervical-Mediastinal-Abdominal lymphadenopathy	5
Bone lesion	5
Tumor of unknown origin	3
Pancreatic mass	3
Hepatic mass	2
Cerebral mass	1
Esophageal mass	1
Breast lump	1
Cervical mass	1
Testicular mass	1
Peritoneal lesionv	1

Our patients had a median TSH value of 1.25 mU/L (0.01-131.00), a median free thyroxin (FT4) value of 1.36 ng/dL (0.10-2.58), and a median free triiodothyronine (FT3) of 3.03 pg/mL (0.61-6.20). The median anti-thyroglobulin antibody (AntiTG) level was 13.73 IU/mL (1.71-3125.00) and the median anti-thyroid peroxidase antibody (AntiTPO) level was 13.80 IU/mL (5.08-538.20). The median FT3/FT4 ratio vas 2.29 (0.79-6.10). A primary lesion was observed in 157 of our patients with a median SUVmax of 8.84 (0.59-91.17) and the median highest SUVmax of 156 patients (primary or metastatic lesion) was 10.34 (0.59-91.17) (**Table 2**).

The Association of Malignant/Benign Lesions with Thyroid and PET/CT Parameters

The median values of age, TSH, FT4, FT3, FT3/FT4 ratio, AntiTG, and AntiTPO levels did not exhibit a statistically significant difference between patient with malignant or benign disease. The median primary lesion SUVmax and the median highest SUVmax were significantly higher in malignant lesions than benign lesions (p<0.001 for both) (**Table 3**).

Table 2. Descriptive Parameters

	Total			
	N	**Mean**	**Std. Deviation**	**Median (Min-Max)**
Age	179	58.41	17.111	60 (3-94)
TSH	178	2.2185	9.76901	1.25 (0.01-131)
FT4	179	1.3676	0.30567	1.34 (0.1-2.5)
FT3	173	3.0601	0.73522	3.03 (0.61-6.2)
FT3/FT4	173	2.3385	0.70569	2.29 (0.79-6.1)
AntiTG	165	55.544	266.148	13.73 (1.71-3125)
AntiTPO	168	27.5317	67.2994	13.80 (5.08-538.2)
Primary Lesion SUVmax	157	11.4343	11.7915	8.84 (0.59-91.1)
Highest SUVmax	156	12.2935	11.7178	10.34 (0.59-91.1)

TSH: Thyrotropin, FT4: Free thyroxin, FT3: Free triiodothyronine, AntiTG: Anti-thyroglobulin, AntiTPO: Anti-thyroid peroxidase, SUVmax: Maximum standardized uptake value

Table 3. The Association of Malignant/Benign Lesions with TFT and PET/CT Parameters

	Benign			**Malign**			
	N	**Median (Min-Max)**	**Std. Deviation**	**N**	**Median (Min-Max)**	**Std. Deviation**	**p**
Age	53	55 (7-80)	15.01	126	63 (3-94)	17.82	0.079
TSH	53	1.35 (0.01-6.84)	1.36	125	1.23 (0.01-131)	11.63	0.104
FT4	53	1.32 (0.78-1.92)	0.26	126	1.34 (0.1-2.58)	0.32	0.769
FT3	50	3.11 (1.94-6.2)	0.75	123	2.97 (0.61-4.96)	0.72	0.126
FT3/FT4	50	2.44 (1.27-5.27)	0.71	123	2.23 (0.79-6.1)	0.69	0.142
AntiTG	49	13.94 (10-185)	38.11	116	13.46 (1.71-3125)	316.01	0.268
AntiTPO	50	14.56 (6.76-538.2)	85.1	118	13.55 (5.08-515.1)	58.1	0.154
Primary Lesion SUVmax	45	2.17 (0.59-24.1)	4.66	112	12.11 (1.2-91.17)	12.46	0
Highest SUVmax	44	2.35 (0.59-24.1)	5.12	112	13.39 (1.2-91.17)	12.19	0

TSH:Thyrotropin, FT4:Free thyroxin, FT3:Free triiodothyronine, AntiTG:Anti-thyroglobulin, AntiTPO:Anti-thyroid peroxidase, SUVmax: Maximum standardized uptake value

The Correlation between Thyroid and PET parameters

There was no significant correlation between the primary tumor SUVmax, the highest SUVmax, and thyroid function tests in 126 patients with malignant disease. AntiTG level was significantly correlated with sex (r: -1.99, p: 0.033). Age also had a significant correlation with TSH, FT3, AntiTG, and FT3/FT4 ratio (r: .242, p: 0.020; r: .780, p: <0.001; r: .307, p: <0.001; and r: .033, p: 0.006, respectively). Local lymph node metastasis was significantly correlated with FT3/FT4 ratio as well (r: .462, p: 0.045). Distant metastasis, on the other hand, was significantly correlated with age, FT4 level, and FT3/FT4 ratio (r: -.193, p: 0.030; r: -.221, p: 0.013; and r: .241, p: 0.007, respectively). Mortality was also significantly correlated with age, FT4 level, and FT3/FT4 ratio (r: -.280, p: 0.002; r: -.242, p: 0.006; and r: .245, p: 0.005, respectively) (**Table 4**).

Logistic Regression Analysis on Predicting Local Lymph Node Metastasis

Univariate logistic regression analyses for age, TSH, FT4, FT3, FT3/FT4 ratio, AntiTG, and AntiTPO levels failed to demonstrate a significant parameter in predicting local lymph node metastasis (**Table 5**).

Logistic Regression Analysis on Predicting Distant Metastasis

Univariate logistic regression analyses revealed age, FT4 levels, and FT3/FT4 ratio to be significant for predicting distant metastasis (OR: .977, p: 0.046; OR: .172, p: 0.008; and OR: .957, p: 0.005, respectively). However, multivariate logistic regression analyses failed to demonstrate an independent prognostic variable (**Table 6**).

Table 4. Correlation of TFT and PET/CT Parameters among Patients with Malignant Disease

		Sex	Age	Primary Lesion SUVmax	Highest SUVmax	Local Lymph Node Metastasis	Distant Metastasis	Mortality
Sex	r		.146	.008	-.010	.090	.022	.029
	p		.104	.930	.918	.319	.811	.750
Age	r	.146		.107	.155	-.056	-.193	-.280
	p	.104		.259	.104	.531	.030	.002
TSH	r	-.106	-.208	-.098	-.076	.017	-.004	-.041
	p	.242	.020	.308	.428	.853	.963	.649
FT4	r	-.075	.067	-.058	-.062	-.104	-.221	-.242
	p	.405	.454	.547	.517	.246	.013	.006
FT3	r	.026	-.344	-.080	-.135	.159	.115	.096
	p	.780	.000	.408	.162	.079	.206	.293
FT3/FT4	r	.093	-.319	.000	-.071	.181	.241	.254
	p	.307	.000	.999	.462	.045	.007	.005
AntiTG	r	-.199	-.253	-.075	-.128	-.077	.147	.117
	p	.033	.006	.450	.195	.410	.115	.212
AntiTPO	r	-.092	.004	-.050	-.171	.097	-.006	-.116
	p	.325	.966	.609	.080	.294	.949	.212

TSH:Thyrotropin, FT4:Free Thyroxin, FT3:Free Triiodothyronine, AntiTG:Anti-Thyroglobulin, AntiTPO:Anti-Thyroid Peroxidase, SUVmax:Maximum Standardized Uptake Value

Table 5. Logistic Regression Analysis on Predicting Local Lymph Node Metastasis

	Univariate analysis					Multivariate analysis				
	B	OR	95% C.I.for EXP(B)		p	B	OR	95% C.I.for EXP(B)		P
			Lower	Upper				Lower	Upper	
Sex	.393	1.481	-	-	.316	-	-	-	-	-
Age	-.008	.992	3	94	.456	-	-	-	-	-
TSH	-.037	.964	.01	131.00	.731	-	-	-	-	-
FT4	-.575	.563	.10	2.58	.352	-	-	-	-	-
FT3	.456	1.578	.61	4.96	.100	-	-	-	-	-
FT3/FT4	.447	1.564	.79	6.10	.116	-	-	-	-	-
AntiTG	.001	1.001	1.71	3125.00	.314	-	-	-	-	-
AntiTPO	.001	1.001	5.08	515.10	.818	-	-	-	-	-
Primary Lesion SUVmax	.014	1.014	1.20	91.17	.363	-	-	-	-	-
Highest SUVmax	.005	1.005	1.20	91.17	.749	-	-	-	-	-

TSH:Thyrotropin, FT4:Free thyroxin, FT3:Free triiodothyronine, AntiTG:Anti-thyroglobulin, AntiTPO: Anti-thyroid peroxidase, SUVmax: Maximum standardized uptake value

Logistic Regression Analysis on Predicting Mortality

Univariate logistic regression analyses revealed age, FT4 levels, and FT3/FT4 ratio to be significant for predicting distant metastasis (OR: .947, p: 0.004; OR: .223, p: 0.042; and OR: .3.970, p: 0.006, respectively). Age was determined to be significant prognostic marker for mortality in multivariate analyses (OR: .949, p: 0.018) (**Table 7**).

DISCUSSION

In this prospective study investigating the association of thyroid function tests with the extent of malignant disease in patients with a tumoral lesion, we did not observe a significant difference in thyroid function tests between malignant and benign groups. Also, PET/CT parameters and thyroid function tests did not exhibit a statistically significant correlation. FT3/FT4 ratio was significantly correlated with local lymph node metastasis and was shown to be a prognostic factor predicting distant metastasis and mortality in univariate analyses.

Thyroid hormones specifically bind to the membrane and nuclear receptors that activate various oncogenic pathways, resulting in the promotion of cell growth, inhibition of apoptosis, and stimulation of angiogenesis. Therefore, thyroid hormones can play a major role in carcinogenesis. Numerous studies indicate an association between increased risk of both solid organ and hematological malignancies and elevated thyroid hormone levels and/or suppressed TSH levels (2-4).

In a study performed with 158 patients with various types of cancer and 100 healthy controls, no statistically significant difference was observed between the groups in terms of the prevalence of thyroid dysfunction (16% and 14%, respectively; p: 0.51). However, the authors reported that thyroid dysfunctions are usually overlooked in cancer patients (25). In their prospective study with more than ten thousand participants, Khan et al. (26) found that elevated FT4 levels were associated with a higher risk of solid organ malignancies, but this association was weaker when corrected for patients under thyroid medications. Still, the highest FT4 level was associated with 13% increase in the risk of solid organ malignancies compared to the lowest FT4 level. TSH level was not associated with the overall risk of cancer. In our study, we did not observe any difference in thyroid function tests between the malignant and benign groups.

Table 6. Logistic Regression Analysis on Predicting Distant Metastasis

	Univariate analysis					Multivariate analysis				
	B	OR	95% C.I. for EXP(B)		p	B	OR	95% C.I. for EXP(B)		p
			Lower	Upper				Lower	Upper	
Sex	.091	1.095	-	-	.809	-	-	-	-	-
Age	-.023	.977	3	94	.046	-.019	.981	-	-	.127
TSH	.029	1.030	.01	131.00	.658	-	-	-	-	-
FT4	-1.762	.172	.10	2.58	.008	-1.239	.290	-	-	.127
FT3	.229	1.257	.61	4.96	.391	-	-	-	-	-
FT3/FT4	.957	2.605	.79	6.10	.005	.434	1.544	-	-	.318
AntiTG	.009	1.009	1.71	3125.00	.317	-	-	-	-	-
AntiTPO	.010	1.010	5.08	515.10	.413	-	-	-	-	-
Primary Lesion SUVmax	.009	1.009	1.20	91.17	.598	-	-	-	-	-
Highest SUVmax	-.012	.988	1.20	91.17	.458	-	-	-	-	-

TSH: Thyrotropin, FT4:Free Thyroxin, FT3:Free Triiodothyronine, AntiTG:Anti-Thyroglobulin, AntiTPO:Anti-Thyroid Peroxidase, SUVmax: Maximum Standardized Uptake Value

Table 7. Logistic Regression Analysis on Mortality

	Univariate analysis					Multivariate analysis				
	B	OR	95% C.I. for EXP(B)		p	B	OR	95% C.I. for EXP(B)		p
			Lower	Upper				Lower	Upper	
Sex	.160	1.174	-	-	.748		-	-	-	-
Age	-.054	.947	3	94	.004	-.052	.949	-	-	.018
TSH	.024	1.025	.01	131.00	.772	-	-	-	-	-
FT4	-1.501	.223	.10	2.58	.042	-1.189	.304	-	-	.262
FT3	.359	1.432	.61	4.96	.328	-	-	-	-	-
FT3/FT4	1.379	3.970	.79	6.10	.006	.658	1.931	-	-	.300
AntiTG	.010	1.010	1.71	3125.00	.567	-	-	-	-	-
AntiTPO	.003	1.003	5.08	515.10	.666	-	-	-	-	-
Primary Lesion SUVmax	-.018	.982	1.20	91.17	.295	-	-	-	-	-
Highest SUVmax	-.016	.985	1.20	91.17	.397	-	-	-	-	-

TSH: Thyrotropin, FT4: Free Thyroxin, FT3: Free Triiodothyronine, AntiTG: Anti-Thyroglobulin, AntiTPO: Anti-Thyroid Peroxidase, SUVmax: Maximum Standardized Uptake Value, OR: Odds Ratio, CI: Confidence Interval

Among the various values determined with 18F-FDG PET/CT, the most widely used parameter is SUVmax, which measures the metabolic rate of glucose uptake by tumor cells. Recent systematic reviews and meta-analyses have shown that SUVmax can be used as a prognostic factor in numerous malignancies (20-23). Increased SUVmax is associated with aggressive tumor behavior and patients with higher SUVmax have a higher risk of recurrence and progression (27). Therefore, aggressive treatments are more effective in patients with higher SUVmax and are associated with progression free survival (PFS) and/or overall survival (OS) advantage. Tumor size and the number of metastatic lymph nodes are well-defined prognostic factors in various types of cancer as they are closely related to distant metastasis and progression (28). However, there is no study in the literature about how SUVmax and the highest SUVmax, which are PET/CT parameters, and thyroid parameters are related. In the current study, SUVmax and the highest SUVmax had no significant correlation with thyroid function tests.

In their study evaluating the relationship of suppressed TSH levels and mortality, Ittermann et al. (29) failed to show a significant association between low serum TSH levels and cancer mortality. Similarly, Zhang et al. (30) observed no association between thyroid hormone levels within the reference range and mortality. In concordance with the above-mentioned studies, we found no statistically significant association between TSH levels and tumor extent and mortality.

The effect or ineffectiveness of thyroid hormones on tumor progression and the efficacy of systemic anticancer therapies has been previously described but has not yet been clearly demonstrated or thoroughly understood. The association between worse survival and decreased FT3 levels, also named as low T3 syndrome, is documented in a variety of clinical scenarios (31,32). The mortality rate of critical patients with distinct alterations in serum thyroid parameters is significantly increased. Also, preclinical experiences point to an association between low serum FT3 levels and mortality in the frail elderly (33,34). In their study on the association of thyroid hormone levels within the reference range with mortality, Zhang et al. (30) found that FT3 levels were negatively correlated with cancer mortality. In another study on patients with lung cancer, 33% of patients demonstrated abnormalities in their thyroid function tests, and most of them had euthyroid sick syndrome (ESS) (a low T3 variant), indicating poorer prognosis (35). In our study, FT3 levels did not significantly predict mortality and were not associated with the extent of primary tumor.

Pinter et al. (36) reported consistent data indicating elevated FT4 levels to be a poor prognostic factor in patients with advanced hepatocellular carcinoma. On the other hand, some studies have shown a direct association between high FT4 levels and better physical performance or survival in elderly patients (34,37). In their before-mentioned study, Zhang et al. (30) did not observe any association between FT4 and mortality. The univariate analyses of the current study showed FT4 level to be a factor predicting distant metastasis and mortality.

Serum levels of the active forms of thyroid levels depend on the iodothyronine deiodinases, an enzyme family that can convert biological precursor T4 to active T3 (deiodinases 1 and 2) and inactive rT3 and T2 (deiodinase 3) (38, 39). Aside from their roles, the three deiodinases differ in their tissue of expression: D1 is expressed in liver and kidneys, while D2 is expressed in skeletal muscle cells where most of the T3 is produced. D3 is accepted as an inactivating enzyme and is vital for placental and fetal tissues (39). Chronic diseases, cachexia, hepatic or renal failure, and chronic systemic inflammation lower the activity of D1 and D2, but augment the activity of D3, causing decreased FT3 levels (40,41). These clinical scenarios are prevalent in cancer patients, especially in the terminal stage, and are typically associated with worse prognosis. Most of our patients had malignancy, some of them were being followed up in the intensive care unit due to chronic disease and malignancy. Therefore, changes in thyroid hormones may be affected by euthyroid sick syndrome.

Using FT3/FT4 ratio rather that the mean of two hormone levels can act as a more functional marker for peripheral deiodination activity and can even help classifying patients with normal FT3 levels (32). To date, limited data are available regarding the prognostic impact of thyroid hormone levels in patients with advanced cancer and the potential role of the FT3/FT4 ratio remains undiscovered (36,42,43). Two recently published studies have shown that low FT3/FT4 ratio better predicts OS and PFS in patients with metastatic colorectal cancer, independent of other prognostic factors (44,45).

Another recent study on hospitalized elderly patients with acute illnesses showed that peripheral thyroid hormone conversion dysfunctions evaluated by FT3/FT4 ratio is closely associated with frailty and survival, even if FT3 is within reference range (38). Low FT3/FT4 ratio is reported to be a strong prognostic factor for both PFS and PS in patients with metastatic renal cell carcinoma (46). In the current study, univariate analyses revealed the FT3/FT4 ratio to be a prognostic factor for both the extent of disease and mortality.

Aging is known to trigger functional and structural changes in the hypothalamic-pituitary-thyroid axes, causing ESS (47). Tellini et at. (48) investigated the

incidence of ESS in 220 geriatric patients hospitalized for cancer and observed that the risk of ESS is higher in elderly cancer patients with recent major weight loss and worse clinical conditions. The incidence of thyroid dysfunction in the normal population may vary depending on iodine deficiency, geographical differences, and ethnic factors. NHANES III, a wide population screening study, showed that subclinical and clinical hyperthyroidism and hypothyroidism potentially contributes to morbidity in especially the elderly in the US (49). Patients with malignant disorders have a higher risk of thyroid dysfunction than the normal population. Increasing age and lower performance score is also significantly associated with thyroid dysfunction (50). In our study age was both correlated with thyroid function tests and was a factor predicting the extent of disease in univariate analyses and an independent prognostic factor predicting mortality.

The limitations of our study include the limited number of patients and therefor the inability to group then according to tumor types, the numerical inequality in malignant and benign groups and the inability to follow-up patients for long term.

CONCLUSION

Thyroid function tests did not exhibit a significant difference between malignant and benign lesions. No statistically significant correlation was observed between thyroid function tests and PET/CT parameters. Univariate analyses revealed that especially FT4 and FT3/FT4 ratio were significant in predicting disease extent and mortality in malignant disease, while age was found to be an independent prognostic factor in predicting mortality. Even though further studies are needed, we recommend cancer patients with advanced age to have thyroid function test as a part of their first clinical screening.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was conducted with the permission of the Health Sciences University Gazi Yaşargil Training and Research Hospital Clinical Researches Ethics Committee (Date: 26.03.2020, Decision No: 736).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Impact of patient satisfaction with insulin pens on glycemic control

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ABSTRACT

Aim: Adherence to the insulin regimen is poor. The use of an insulin pen contributes positively to glycemic control by increasing patient satisfaction and adherence. The aim of this study is to analyze the influence of patients' opinions of insulin pen use on glycemic control in type 2 diabetes mellitus (T2DM).

Material and Method: 126 patients with T2DM who use insulin and inject it with insulin pens were included in the study. Patients' evaluations about the pens (ergonomics, ease of reading the dosage scale, dose selection, needle change, and ease of use of the insulin pen in general) were assessed. Glycemic parameters, demographic characteristics, and treatment protocol were recorded.

Results: Patients who perceived the use of the insulin pen as ergonomically 'excellent' had a significantly lower HbA1c (8.0±1.4%) (p=0.04). HbA1c was significantly lower in patients who perceived needle tip replacement as 'very easy' (8.0±1.6%) (p=0.04). No statistically significant relationship was found between the ease of reading the dosage scale and the HbA1c value (p=0.53). The HbA1c value decreased significantly in patients who rated the dosage selection as 'very easy' (8.1±1.7%) (p=0.02). The HbA1c value increased significantly in patients who rated the pen as 'difficult' to use (12.2±1.6%) (p=0.01).

Conclusion: In our study, we found that patients' opinions of insulin pen use may influence glycemic control parameters. HbA1c was better in patients who found the insulin pen as easy to use and good in ergonomics. In T2DM, patient assessment of insulin pen injection is related to glycemic control. New studies are needed to say whether this situation is related to the appropriate dose of insulin injection or adherence to therapy.

Keywords: Diabetes mellitus, insulin, injection, HbA1c

INTRODUCTION

The incidence of diabetes mellitus (DM) is increasing in our country (13.7%) as in the whole world (1). The American Diabetes Association (ADA) recommends a glycemic target of glycated hemoglobin (HbA1c) < 7% (53 mmol/mol) for the treatment of type 2 DM (T2DM) (2). Despite new pharmacological agents, many patients do not reach glycemic target values (3). A major obstacle to effective treatment of DM is lack of adherence (4). People with type 1 DM take insulin only. Some people with T2DM fail to control their blood glucose with diet, exercise, and oral hypoglycemic agents and require insulin. However, adherence to the insulin regimen is poor (5,6). There are numerous factors that influence patients' adherence to therapy. These include age, duration of disease, adverse events such as hypoglycemia or weight gain, injection method, pain on injection, number of daily injections, and patient confidence in treatment (7). Improving the adherence to insulin treatment helps to reduce HbA1c levels and improve metabolic control (8). In addition, studies show that the injection method (such as the technology used, the prevention of lipohypertrophy by changing the injection site each time) can affect glycemic control by providing the appropriate dose and absorption of insulin, apart from patient compliance (9). Insulin therapy is administered with a syringe, insulin pen, or insulin pump. In patients with T2DM, insulin is usually injected with insulin pens. Instead of the syringe, the use of insulin pens contributes positively to glycemic control by increasing patient satisfaction and adherence (10). Over the years, different insulin pens have been developed to improve accuracy and ease of use. In order to increase the ease of use, many features of insulin pens

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have been tried to be developed. These include portability, ease of reading the dose scale, ease of dose adjustment, ergonomic design, sturdiness, safety, and the ability to distinguish between pens in patients using multiple pens (different colors or thickness etc (11). Nowadays, there are various insulin pens with different characteristics and innovations among the conventional (with replaceable cartridge) and disposable (with single cartridge) insulin pens. Studies have shown that using the insulin pen that the patient prefers and is satisfied with will increase the success of the treatment (12) The aim of this study is to analyze the influence of patients' opinions of insulin pen use on glycemic control in T2DM.

MATERIAL AND METHOD

This cross-sectional study included patients with T2DM who were treated between September 2013 and March 2014 in Endocrinology Department of Kırıkkale University Faculty of Medicine Hospital. The study was approved by the Clinical Researches Ethics Committee of the Kırıkkale University (Date: 17.07.2013, Decision No: 14/01). All procedures were performed in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients aged 18 to 70 years who had been using an insulin pen regularly for at least one month, used an insulin needle tip of the same brand and size, were motor and intellectually capable of self-administering an insulin pen, had no severe retinopathy affecting vision and had no severe neuropathies or motor deficits were enrolled in the study. Exclusion criteria were that patients were under 18 years of age and older than 70 years of age, used the insulin pen for less than 1 month, had severe retinopathy affecting vision, had severe neuropathy or motor deficits that could interfere with the use of the insulin pen, were unable to self-inject the insulin pen and were assisted by their relatives, and used needle tips of different brands and sizes. During this period, patients with a T2DM diagnosis using an insulin pen were evaluated consecutively, and patients who met the inclusion and exclusion criteria were included in the study. Care was taken to ensure that half of the patients enrolled in the study used disposable pens (with a prefilled insulin cartridge) and the other half used conventional insulin pens (with a replaceable insulin cartridge). During this 6-month period, a total of 126 patients were enrolled in the study. Patients had provided written informed consent. All patients underwent a physical examination and biochemical evaluation. Patients were asked questions about their demographic characteristics, disease characteristics, insulin treatment details, and ratings of the insulin pen they used. The presence of diabetic neuropathy was determined by the signs and symptoms of peripheral neuropathy, including numbness in the hands and/or feet, dysesthesia and/or paresthesia, hypersensitivity to touch, burning pain, neuropathic foot ulcers, and decrease or loss of deep tendon reflexes. Patients' renal functions were evaluated by measurements of serum creatinine, microalbuminuria, and creatinine clearance. After exclusion of hematuria and urinary tract infections, values of albumin/creatinine ratio greater than 30 mg/g in the spot urine collected at least twice were accepted as diabetic nephropathy. Diabetic retinopathy was diagnosed by an ophthalmologist after an ophthalmologic examination. A 10-cm visual analogue scale (VAS) was used to assess pain during injection, starting with horizontal "No pain" and ending with "Unbearable pain" (13). Fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) levels were recorded. For HbA1c analysis, cation exchange high performance liquid chromatography (HPLC) was used and for FBG and other biochemical tests, spectrophotometric method on Beckman coulter AU5800 (Beckman coulter Inc. CA, USA) autoanalyzer was used.

Patients' ratings of insulin pens in terms of ergonomics, ease of needle tip replacement, readability of the dosage scale, dose selection, and ease of use of the pen were assessed. Patients were asked their opinion on ergonomics with one of 5 different levels from very poor to excellent (very poor, poor, moderate, good, excellent). And they were asked their opinions on the ease of insulin pen use and the other three features with one of 5 different grades, from very difficult to very easy (very difficult, difficult, moderate, easy, very easy). The HbA1c values based on these 5 assessment groups were compared.

The SPSS 16 (Statistical Package for Social Sciences) program was used for statistical analysis of the results of the study. Results were expressed as mean±standard deviation (mean±SD). Descriptive statistics were performed for all variables. One-way ANOVA was used to determine if there were statistically significant differences between groups. Tukey's post-hoc test was used to determine where the difference existed between the groups. Shapiro-Wilk test was used to determine whether the distribution of the data was normal or not. Before correlation analysis, patient satisfaction status was graded. It was graded as very poor or very difficult, 1; poor or difficult, 2; moderately, 3; good or easy, 4; excellent or very easy, 5, and correlation analysis was performed between numeric parameters, not categorical. Pearson's correlation coefficient is preferred when the data are normally distributed, and Spearman Rank's correlation coefficient is preferred when the data are not normally distributed. The significance was evaluated at the $p < 0.05$ level.

RESULTS

One hundred twenty-six patients with a mean age of 55.3±11.1 years were included. 89 of the subjects (71%) were female. The mean duration of diabetes was 11.8±7.3 years and the mean duration of insulin use was 4.7±4.8 years. **Table 1** shows the demographic characteristics, microvascular complications, insülin pen types and laboratory findings of the subjects. Twenty-three (18.2%) patients had previously used other insulin pens. The remainder were either using an insulin pen for the first time or had previously used the same type of insulin pen. All patients used the same brand and size of needle tip (32 G - 6 mm). Only 53.2% of the patients used the needle tip once as recommended, while the others used it at least twice. The mean number of uses of the same needle tip was 1.76±1.1, and the mean intensity of needle pain was 2.8± 2.2. The number of needle repetitions of patients and the severity of needle pain according to the VAS scale were presented in **Tables 2** and **3**. The severity of needle pain and the number of uses of the same needle tip were compared. There was no correlation between the number of reuses of the same needle tip and the severity of pain (p=0.2; r=0.1).

Table 1. Demographic characteristics, microvascular complications and laboratory findings of the subjects included in the study

Age (year)	55.3±11.1
Gender (Male/Female) n (%)	37/89 (29/71)
Duration of diabetes (year)	11.8±7.3
Duration of insulin use (year)	4.7±4.8
Type of insulin pen n (%)	
Conventional pen	63 (50%)
Novopen TM	34 (54%)*
Humapen TM	29 (46%)*
Disposable pen	63 (50%)
Flexpen TM	12 (19%)**
Solostar TM	17 (27%)**
Kwikpen TM	34 (54%)**
Insulin regimen n (%)	
Premix insulin	68 (54%)
Basal insulin	29 (23%)
Basal-bolus insulin	29 (23%)
Insulin dose (IU)	39.6±22.9
Number of using the same needle tip	1.76± 1.1
Intensity of needle pain	2.87±2.2
Educational status n (%)	
Primary School	65 (51.6%)
High School	25 (19.8%)
University	24 (19%)
Illiterate	12 (9.5%)
FBG (mg/dl)	177.7±72.6
HbA1c (%)	8.4±1.7
Retinopathy n (%)	42 (33.3%)
Nephropathy n (%)	24 (19%)
Neuropathy n (%)	36 (28.6%)

All parameters are given as mean±standard deviation unless otherwise stated. FBG; fasting blood glucose, HbA1c; glycated hemoglobin. * The percentage in the conventional pen group is given. ** The percentage in the disposable pen group is given.

Table 2. Number of patients' reuse of the same needle tip

Number of needle tip reuse	Total n (%)
1	67 (53.2)
2	39 (31)
3	11 (8.7)
4	4 (3.2)
5	3 (2.4)
6	1 (0.8)
7	1 (0.8)

Table 3. Intensity of needle pain according to VAS score

Intensity of needle pain (cm)	total n
0	-
1	18
2	26
3	21
4	17
5	5
6	26
7	5
8	2
9	5
10	1

VAS: Visual Analogue Scale

Ergonomics and HbA1c

There were no patients who found the insulin pen ergonomically 'very poor'. 14.3% of the patients found the insulin pen ergonomically 'excellent'. According to the one-way ANOVA test, HbA1c values differed significantly between the four ergonomic evaluation groups (p=0.04). A post hoc analysis was performed to determine from which groups the difference originated. HbA1c values were significantly different in all groups. HbA1c values in the 'excellent' group were significantly lower than those in the other groups and HbA1c values in the 'moderate' group were significantly higher than those in the other groups (**Table 4**). Correlation analysis revealed a negative correlation between HbA1c and ergonomic satisfaction. When ergonomic satisfaction improves, HbA1c decreases (p=0.01; r=-0.2). A negative correlation was also found between FBG and ergonomic satisfaction (p=0.01; r=-0.2). A statistically significant difference was found between insulin regimens according to the ergonomic evaluation groups (p=0.0001). The rate of basal-bolus insulin regimens was significantly lower in the "excellent" group than in the other groups.

Table 4. Comparison of HbA1c values based on ergonomic assessment groups (mean±SD)

	Ergonomics				
	Excellent (n=18)	Good (n=77)	Moderate (n=24)	Poor (n=7)	p
HbA1c	8.0±1.4	8.3±1.6	9.3±1.9	8.8±1.3	0.04

SD: standard deviation; HbA1c: glycated hemoglobin

Ease of Needle Tip Replacement and HbA1c

In our study, no patient found needle tip replacement "very difficult." 34.9% of patients found it "very easy." According to the one-way ANOVA test, HbA1c values differed significantly between the four assessment groups (p=0.04). In a post hoc analysis, HbA1c values were significantly different in all groups. HbA1c values in the 'difficult' group were significantly higher than those in the other groups (**Table 5**). HbA1c values in the 'very easy' group were significantly lower than those of the other groups. In correlation analysis, a negative correlation was found between the ease of needle tip change and HbA1c level. The HbA1c value decreased with increasing ease of needle tip change (p=0.006; r=-0.2). When analyzing the correlation between the ease of needle tip change and FBG, a negative correlation was observed (p=0.001; r: -0.3). There was no significant difference between the groups with respect to the insulin regimen used (p=0.08).

Table 5. Comparison of HbA1c values based on ease of needle tip change assessment groups (mean±SD)

	Ease of needle tip change				p
	Very easy (n=44)	Easy (n=64)	Moderate (n=13)	Difficult (n=5)	
HbA1c	8.0±1.6	8.4±1.6	9.3±1.6	9.6±2.2	0.04

SD: standard deviation; HbA1c: glycated hemoglobin.

Ease of Reading the Dose Scale and HbA1c

7.1% of patients found the readability of the dose scale 'very difficult' and 21.4% of patients found it 'very easy'. No statistically significant relationship was found between the readability of the dose scale and HbA1c level. However, the group that perceived the readability of the dosage scale as 'easy' and 'very easy' had the lowest mean HbA1c value (8.3±1.6% and 8.3±1.7%, respectively) (p=0.53) (**Table 6**). No correlation was observed between the ease of reading the dosage scale and the HbA1c (p=0.21; r=-0.09) or FBG (p=0.12; r=-0.1). There was no significant difference between groups with respect to the insulin regimen used (p=0.08).

Table 6. Comparison of HbA1c values based on ease of readability of the dosage scale assessment groups (mean±SD)

	Ease of readability of the dosage scale					p
	Very easy (n=27)	Easy (n=57)	Moderate (n=22)	Difficult (n=11)	Very difficult (n=9)	
HbA1c	8.3±1.7	8.3±1.6	8.5±1.4	9.3±2.5	8.5±1.5	0.53

SD: standard deviation; HbA1c: glycated hemoglobin

Ease of Dose Selection and HbA1c

2.4% of patients found dose selection 'very difficult'. 27% found it 'very easy'. One-way ANOVA test showed that HbA1c values were significantly different between the five evaluation groups (p=0.02). In the post-hoc analysis, HbA1c values in the 'difficult' group were significantly higher than the other groups, and HbA1c values in the 'very easy' group were significantly lower than the other 4 groups (**Table 7**). A negative correlation was observed between the ease of dose selection and HbA1c (p=0.01; r=-0.2) and FBG (p=0.001; r=-0.3). There was no significant difference between groups with respect to the insulin regimen used (p=0.35).

Table 7. Comparison of HbA1c values based on ease of dose selection assessment groups (mean±SD)

	Ease of dose selection					p
	Very easy (n=34)	Easy (n=65)	Moderate (n=17)	Difficult (n=7)	Very Difficult (n=3)	
HbA1c	8.1±1.7	8.3±1.5	8.6±1.8	10.5±1.7	8.4±1.4	0.02

SD: standard deviation; HbA1c: glycated hemoglobin

Ease of the Insulin Pen Use and HbA1c

One patient (0.8%) rated the use of the pen as 'very difficult'. 27.8% of patients rated the use of the pen as 'very easy'. One-way ANOVA test showed that HbA1c values differed significantly between the five assessment groups (p=0.01). Post hoc analyzes showed that HbA1c values in the 'difficult' group were significantly higher than those in the other groups (**Table 8**). HbA1c levels were lower in the 'very easy' group than in the 'difficult' (p=0.001), 'very difficult' (p=0.04) and 'moderate' groups (p=0.01). There was no significant difference between the 'easy' group and the 'very easy' group (p=0.09). There was a negative correlation between the ease of use of the pen and HbA1c level (p=0.02; r=-0.2). No correlation was found between ease of use and FBG value (p=0.08; r=-0.2). There was no significant difference between groups in terms of insulin regimen used (p=0.16).

Tablo 8. Comparison of HbA1c values based on ease of insulin pen use assessment groups (mean±SD)

	Ease of insulin pen use					p
	Very easy (n=35)	Easy (n=75)	Moderate (n=13)	Difficult (n=2)	Very Difficult (n=1)	
HbA1c	8.2±1.4	8.3±1.7	9.0±1.7	12.2±1.6	8.7±1.3	0.01

SD: standard deviation; HbA1c: glycated hemoglobin

DISCUSSION

Studies have shown that adherence to insulin therapy is low (5,6). In a study of 1099 subjects, the average adherence to insulin was 71% (5). In a systematic review of type 2 diabetics receiving insulin, adherence was 63% (6). The type of injection and patient satisfaction with therapy are important factors influencing adherence and thus glycemic control (7). After insulin pens were shown to facilitate insulin injection and increase adherence, insulin pens with different features and brands were

developed to facilitate patient use. Another issue with insulin therapy and injection is that mistakes can have serious consequences. Overdose of insulin can lead to severe hypoglycemia and coma, and underdose of insulin can lead to hyperglycemia and sometimes ketoacidosis. Considering the importance of injecting at appropriate doses and patient compliance, it is now observed that insulin pen technology is gradually devoloping and different types of pens are being manufactured. While some pens have a half-unit option and a memory option, others offer different color options, an 80-IU dose option, a clearly audible click sound, and low injection effort. Patient preference, availability of the pen on the market, the insulin formulation it contains, price, and physician choice determine the type of pen used (14). Studies have shown that patients prefer some insulin pens to others, and insulin pen preferences may vary from person to person (15-18). Using the insulin pen that the patient prefers and is satisfied with may positively influence treatment outcome. We confirmed this hypothesis in our study and found that the more comfortable patients are with the method of insulin injection, the more likely they are to adapt to insulin therapy, which may contribute to a decrease in HbA1c. This observation is consistent with other studies that have shown that glycemic control improves as patient satisfaction increases (12,19,20). Nicolucci A et al. (19) used the WHO -Diabetes Treatment Satisfaction Questionnaire (DTSQ) to measure satisfaction with the diabetes treatment regimen. The DTSQ score was inversely related to HbA1c levels and diabetic complications. A large cohort study of 4513 type 2 patients treated with insulin DM showed that proper pen selection and professional education resulted in higher patient satisfaction and better glycemic control. This study also showed that both better glycemic control and satisfaction with treatment can reduce body mass index (12). In the study by WK Redekop et al. (20), diabetic patients with higher HbA1c levels were less satisfied with treatment than other patients. In this study, the presence of complications was found to be associated with lower satisfaction. In our study, treatment satisfaction had a significant effect on HbA1c levels. It is well known that proper use of insulin injection techniques is important to optimize treatment efficacy and achieve better glycemic control. If the patient finds the pen to be very ergonomic, this may indicate that there are no obvious problems in holding and gripping the pen and therefore they can inject more accurately. The fact that patients find it very easy to choose a dose may have a positive impact on glycemic control by reducing the possibility of errors in dose adjustment and making dosing more accurate. If it is easy to change the needle tip while using the pen, the injection time will be shorter and patients will need less time to inject insulin. This can reduce repetitive use of the needle tip. The ease of changing the needle tip likely contributes to treatment adherence and good glycemic control through correct injection. Correct injection is critical to achieving blood glucose goals. Failure to inject correctly will result in undesirable outcomes such as inability to deliver an adequate insulin dose, increasing pain, and tissue damage at the injection site. One of the biggest mistakes in insulin injection is the reuse of the needle tip. In our study, it was observed that about half of the patients (53.2%) discarded the needle tip after the single use as recommended. In the literature, reuse of the same needle tip for insulin injection as in our study is very common. In a study conducted in Moscow, single use of the needle was never observed, 7% of patients changed the needle every 2-3 days, 46% changed it once a week, and 23% changed it once a month (21). In the European epidemiological study of insulin technique, Strauss K et al. (22) found that a needle tip was used an average of 3.3 times. Injection pain increases when the needle tip is reused. In the study by Misnikova et al. (21) it was observed that injection site pain was more frequent when the needle tip was reused, and the risk of microbial contamination was 26.6%, even after the needle had been used once. In our study, there was a weak positive correlation between the number of times the same needle tip was reused and the severity of pain.

Our study had some limitations. This study did not compare the superiority of one insulin pen over another. In addition, the effect of patient ratings of each insulin pen group on glycemic parameters was not examined because there were not enough patients in each insulin pen group to make comparisons. Only whether patients' evaluations about the insulin pen they used affected HbA1c levels was examined. The study population was a heterogeneous group of participants that included patients who used premix as well as patients who used basal and basal-bolus insulin only. Apart from evaluating patients on treatment, other factors that may influence HbA1c levels and FBG, such as insulin dose used, number of insulin injections, diet, and physical activity, could not be studied. Although there were no patients with severe neuropathy and retinopathy in the study population, patients with neuropathy and retinopathy were not excluded when calculating the Visual Analogue Scale (VAS) score and assessing the ease of pen use and other factors. These complications may affect patients' assessment of the pen.

CONCLUSION

Our study showed that in T2DM, patients' opinions of insulin pen injection may affect the glycemic control. If patients perceive the insulin pen as easy to use and ergonomically good, this may lead to better glycemic

control. However, we did not investigate whether this situation is related to the appropriate dose of insulin injection or adherence to therapy. However, we think this could lead to new studies that involve more patients and investigate patient satisfaction and the importance of appropriate injection in insulin therapy. To demonstrate the influence of patients' opinions about the insulin pen on HbA1c levels, more comprehensive studies are needed that examine all parameters that may influence HbA1c levels.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of the Kırıkkale University (Date: 17.07.2013, Decision No: 14/01).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Examination of patients admitted to a university hospital with methanol intoxication

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ABSTRACT

Objective: The aim of this study is to evaluate the demographic data, clinical features and laboratory findings of patients followed up with methanol poisoning in our internal medicine clinic. In addition, to examine the data of the patients followed in our intensive care unit and to contribute to the literature in this direction.

Material and Method: In this study, 21 patients diagnosed with methanol intoxication who were hospitalized in the internal medicine clinic of our hospital between 01.01.2019 and 01.04.2022 were included. Demographic information of the patients, initial complaints, accompanying symptoms, laboratory results, blood gas values, intensive care unit requirements, mechanical ventilation needs, length of hospital stay and whether they received hemodialysis treatment were recorded from the hospital automation system.

Results: 21 patients were included in the study. The mean time for patients to apply to the hospital after drinking alcohol was calculated as 31.42±4.27 hours. The mean hospital stay was 3.0±1.02 days. While 12 patients were followed up in the intensive care unit, it was found that 6 patients needed mechanical ventilation and 9 patients needed hemodialysis. Glucose, creatinine, acetyl aminotransferase (AST), partial carbon dioxide pressure ($PaCO_2$), lactate, anion gap and base gap were found to be statistically significantly higher in the group treated in the intensive care unit ($p<0.05$). When the blood gas parameters at the time of admission were compared between the groups who received and did not receive hemodialysis treatment of the patients who presented with methanol intoxication, pH, lactate, anion gap and base deficit were found to be statistically significantly higher ($p=0.001$).

Conclusion: Hyperglycemia, increased serum creatinine value and metabolic acidosis were found to be significantly different in patients hospitalized in the intensive care unit

Keywords: Methanol, intensive care unit, hemodialysis

INTRODUCTION

Methanol, CH_3OH, is a clear, colorless, volatile liquid with a distinct odor that tastes the same as ethanol. Methanol intoxication may result from accidental exposure, overconsumption of compounds containing methanol with suicidal intent, or following consumption of distilled and contaminated alcoholic beverages(1). Toxicity of methanol is related to the production of toxic metabolites by the enzyme alcohol dehydrogenase (ADH), which can lead to metabolic acidosis, blindness (in methanol poisoning) and death(2). The initial acidic metabolites lead to metabolic acidosis, whereas the end metabolites mediate organ damage. Methanol is metabolized to formic acid, which produces acidosis as well as retinal and optic nerve damage leading to blindness observed in methanol poisoning (3). Although methanol poisoning can occur as an isolated ingestion, it is infamous for being involved in numerous epidemics. In outbreaks, methanol poisoning usually results from consumption of alcoholic beverages that have been spiked with methanol due to its low cost. These epidemics occur world-wide, often with high mortality rates(4-9).

Management of intoxicated patients starts with decontamination and supportive measurements besides the corrective metabolic therapy. Antidotal therapy with fomepizole or ethanol is a cornerstone, as it helps to inhibit toxic metabolites formation. Hemodialysis is an essential treatment for enhancing toxic metabolite removal. The time interval between methanol exposure and receiving treatment is closely related to the outcomes.

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The identification of at-risk patients requiring admission to the intensive care unit (ICU) and prompt treatment may prevent complications and long-term deaths(10,11).

The aim of this study is to evaluate the demographic data, clinical features and laboratory findings of patients followed up with methanol poisoning in our internal medicine clinic. In addition, to examine the data of the patients followed in our intensive care unit and to contribute to the literature in this direction.

MATERIAL AND METHOD

This study was planned retrospectively. The study was carried out with the permission of Hitit University Faculty of Medicine Non-Interventional Clinical Researches Ethics Committee (Date: 30.03.2022, Decision No: 2022-07). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In this study, 21 patients diagnosed with methanol intoxication who were hospitalized in the internal medicine clinic of our hospital between 01.01.2019 and 01.04.2022 were included. Our study was planned retrospectively. Since methanol level was not measured in our hospital, patients whose ethyl alcohol level was measured in the hospital file system (<10 mg/dl) and who had a history/clinic of suspected alcohol intake were included in our study. Fifteen patients with an ethyl alcohol level below 10 mg/dl and 6 patients with an ethyl alcohol level above 10 mg/dl who consumed unlabeled and homemade alcohol in their anamnesis, had visual impairment, changes in consciousness, and had a high anion gap in blood gas analysis were also included. Demographic information of the patients, initial complaints, accompanying symptoms, laboratory results, blood gas values, intensive care unit requirements, mechanical ventilation needs, length of hospital stay and whether they received hemodialysis treatment were recorded from the hospital automation system.

Statistical Analysis

SPSS (version 26.0) software was used for statistical analysis. Frequency and percentage values were used for categorical data, and mean±standard deviation values were used for continuous variables. If the continuous variables in our study were in a normal distribution, the "t-test in independent groups" was used when comparing independent groups, and the "Mann Whitney U" test was used when comparing continuous variables that did not fit the normal distribution in two groups. In the evaluation of categorical variables, "Pearson chi-square" and "Fisher's exact probability" tests were used. Statistically significant value was accepted as $p<0.05$.

RESULTS

The mean age of the patients was 35.52±11.22. The mean time for patients to apply to the hospital after drinking alcohol was calculated as 31.42±4.27 hours. The mean hospital stay was 3.0±1.02 days. When evaluated according to the symptoms of admission; It was determined that 6 patients had blurred vision, 6 patients had nausea, 6 patients lost consciousness, 1 patient had loss of vision, and 2 patients had no complaints, but the patients who had taken alcohol together had symptoms. Among the accompanying spmtoms; There were fatigue in 17 patients, nausea in 15 patients, vomiting in 12 patients, dizziness in 13 patients, and blurred vision in 15 patients. While 12 patients were followed up in the intensive care unit, it was found that 6 patients needed mechanical ventilation and 9 patients needed hemodialysis (**Table 1**).

Table 1. Demographic and clinical data of patients

Sex (n)	20 male / 1 Female
Age (Mean±SD)	35.52±11.22
Time to Admission to the Hospital (Mean±SD)	31.42±4.27 hours
Length of Stay in Hospital (Mean±SD)	3.0±1.02 days
Follow-up in the Intensive Care Unit (n)	12
Hemodialysis Treatment (n)	9
Mechanical Ventilation Requirement (n)	6
Presenting symptom (n)	
Blurred vision	6
Nausea	6
Vision loss	1
Loss of consciousness	6
No symptoms	2

The hemogram, biochemistry and blood gas analysis results of the patients who received and did not receive treatment in the intensive care unit are given in **Table 2**. Glucose, creatinine, acetyl aminotransferase (AST), pH, partial pressure of carbon dioxide ($PaCO_2$), bicarbonate (HCO_3), lactate, anion gap and base deficit were found to be statistically different in the group receiving treatment in the intensive care unit ($p<0.05$).

When the blood gas parameters at the time of admission were compared between the groups who received and did not receive hemodialysis treatment of the patients who presented with methanol intoxication, pH, $PaCO_2$, HCO_3, lactate, anion gap and base deficit were found to be statistically significantly different ($p=0.001$) (**Table 3**).

Table 2. Comparison of hemogram, biochemistry and blood gas parameters of patients treated and no treated in the intensive care unit

	Treated in the intensive care unit group	No treated in the intensive care unit group	p value
White blood cell (10^9/L)	10.12±4.74	8.96±3.63	0.072
Hemoglobin (g/dL)	17.09±2.1	17.01±3.02	0.896
MPV(fL)	9.98±1.67	9.96±1.76	0.671
Platelet(10^9/L)	247.45±90.72	227.06±91.72	0.328
Neutrophil leumphocyte ratio	5.34±4.37	4.46±2.63	0.186
Glucose (mg/dL)	199.43±74.95	138.33±52.76	0.013
Urea	26.07±9.75	27.19±10.83	0.234
Creatinine	1.09±0.41	0.96±026	0.041
Sodium	133.07±5.26	133.58±6.34	0.532
Chlorine	101.46±4.27	101.23±4.39	0.841
Potassium	4.76±0.92	4.52±0.87	0.547
Acetyl aminotransferase	82.90±61.06	65.23±27.41	0.001
Alanine aminotransferase	47.38±25.43	42.67±24.12	0.253
pH	6.91±0.13	7.31±0.19	0.001
$PaCO_2$	58.02±13.15	32.78±11.62	0.001
HCO_3	7.14±3.93	10.07±3.45	0.002
Lactate	8.02±3.96	4.63±2.73	0.019
Anion gap	29.09±4.78	21.23±6.43	0.043
Base deficit	-25.69±4.96	-16.45±5.32	0.001

Table 3. Comparison of initial blood gas values of patients receiving and not receiving hemodialysis treatment

	Hemodialysis group	Non-hemodialysis group	P value
pH	6.95±0.11	7.34±0.21	0.001
$PaCO_2$	65.73±11.93	28.85±10.34	0.001
HCO_3	6.34±2.45	11.85±3.42	0.001
Lactate	12.83±3.75	3.63±1.86	0.001
Anion gap	28.55±3.96	19.36±4.61	0.001
Base deficit	-24.05±4.13	-15.31±5.8	0.001

DISCUSSION

The current study established male predominance over females (20 male, 1 female), which was thoroughly reported (12,13). The mean age of patients involved in the current study was 35.52±11.22 years, which is consistent with multiple case reports in various settings (14,15). On the other hand, Ahmed et al. (13) reported slightly higher age (mean 36.2±8.6 years) (12). Furthermore, Kurtas et al. indicated that individuals aged 41–50 years are more exposed. Rulisek et al. (16) reported an increased incidence of methanol intoxication in the elderly aged 50.9±2.6 years. The noticed age variation indicates the prevalence of methanol exposure in all age groups, especially during outbreaks.

In this study reported in presentation to the hospital (mean 31.42±4.27 hours) and hospital stay of 3 days. These results are in line with a previous study conducted in the USA, in which patients intoxicated with methanol spent approximately 4.0±6.1 days. Prolonged hospitalization places a noticeable burden on health care providing services (17). In a study by Md Noor et al. (10). reported the time to hospital admission as 24-96 hours

The current research revealed that nausea, blurred vision, vision loss and loss of consciousness were the most common presentations. Similarly, Ahmed et al. (12). reported that about half of the presented patients suffered from blurred vision Md Noor et al. (10). reported in a study that approximately one-third of patients presented with vomiting, blurred vision, and altered consciousness level

While 12 of the patients included in our study were admitted to the intensive care unit, it was determined that 9 of these patients received hemodialysis treatment and 6 patients needed mechanical ventilation. In the study of Sharif et al. (18), it was reported that 9 out of 37 patients admitted with methanol intoxication were admitted to the intensive care unit. 51.4% of the included patients treated with supportive measures without requiring hemodialysis or antidotal therapy, 21.6% received fomepizole, 13.5% underwent hemodialysis, 10.8% underwent hemodialysis, and received fomepizole, while 2.7% only received ethanol

In our study, no significant difference was found in hemoglobin, white blood cells, NLR and platelet parameters between the patients who were followed up and those who were not followed up in the intensive care unit. These findings corroborate those of other studies (18,19).

Hyperglycemia and serum creatinine has been shown to be a poor prognostic factor in methanol intoxications in studies (10,11,20). In our current study, glucose and serum creatinine levels were found to be significantly higher in patients followed in the intensive care unit, which supports the studies in the literature.

In our study, pH and HCO_3 levels were found to be low in the blood gas analysis results of the patients followed in the intensive care unit, while lactate, $PaCO_2$ and anion gap were found to be high. There was no death due to methanol intoxication among our patients. In 1998, Liu et al. (21). reported that 18 of 50 (36%) patients at the Toronto Hospital died of methanol poisoning. Coma or seizure on presentation and severe metabolic acidosis (pH <7) were indicators of poor prognosisIn the study by Meyer et al. (22) the strongest predictor of death was a blood pH of <7.0. An analysis by Hovda et al. (4). of a methanol outbreak in Norway between 2002 and 2004 revealed that respiratory arrest, coma, and severe metabolic acidosis (pH<6.9 and base deficit >28 mmol/L) were strong predictors of poor outcome

Coulter et al. (23). analyzed the literature data of 119 patients with methanol poisoning and concluded that large osmolal gap, anion gap, and low pH (pH <7.22) were associated with increased mortality and that pH has the highest predictive value. Another study reported the significant function for the anion gap as an unfavorable outcome predictor, which agrees with other studies. The association between methanol toxicity and high anion gap metabolic acidosis is due to formic acid formation. The parallel decrease in HCO_3 and elevated serum formic acid in patients with unfavorable outcomes supports the crucial role of formic acid in methanol-induced acidosis. Acidosis accelerates the toxicity by enhancing more formic acid diffusion into the cells (18). Finally, it was suggested in a multicenter study that low pH (pH <7), coma (GCS score <8), and inadequate hyperventilation (pCO_2 ≥3.1 kilopascal (kPa) in spite of a pH <7) on admission were the strongest predictors of poor outcome after methanol poisoning (5).

Hemodialysis is a commonly used reliable management procedure. Hemodialysis removes methanol and its toxic metabolite formic acid from the blood. Indications for hemodialysis include a serum methanol concentration of 50 mg/dL (15.6 mmol/L) or more, the presence of metabolic acidosis, and visual disturbances(24,25). In our study, the pH value of the patients who received hemodialysis was 6.95±0.11 and this value was found to be significantly lower than the group that did not receive hemodialysis.

Study limitations: This study has some limitations. Retrospective planning, the small number of patients, and the fact that we do not know how much and what type of methanol alcohol the patients consume are among these limitations.

CONCLUSION

In summary, hyperglycemia, increased serum creatinine value and metabolic acidosis were found to be significantly different in patients hospitalized in the intensive care unit. However, the retrospective nature of the study, lack of control of a retrospective cohort, small patient population and absence of methanol, formic acid or ALDH2 measurements limit the certainty of our conclusions. In addition, since our study is planned retrospectively, post-discharge sequelae could not be evaluated in patients.

Ensuring high quality-controlled production and distribution of alcoholic beverages. Establishing a tracking system to limit illegal alcohol production. Increasing orientation of populations about hazards of industrial alcohol and illegal alcoholic beverages.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Faculty of Medicine Non-Interventional Clinical Researches Ethics Committee (Date: 30.03.2022, Decision No: 2022-07).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Can *Saccharomyces boulardii* treat and eradicate *Helicobacter pylori* among children instead of bismuth?

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ABSTRACT

Aim: The objective of this study is to compare the use of *Saccharomyces boulardii* (*S. boulardii*) in eradicating *Helicobacter pylori* (*H. pylori*) in children as an alternative to bismuth, which has a limited scope of application due to its side effects.

Material and Method: Included in the study were 220 pediatric patients with symptomatic *H. pylori* gastritis. The patients were randomized into three treatment groups. Patients who received bismuth or *S. boulardii* in addition to the standard triple therapy for 14 days were compared with the control group who received only triple therapy.

Results: Analysis of the bismuth, *S. boulardii*, and control groups' treatment success showed that the *H. pylori* eradication rate was highest among study participants who received bismuth (95.2%), followed by patients who received *S. boulardii* (92.4%). The most frequent side effects were observed in the patient group that received bismuth (17.5%).

Conclusion: Although bismuth continues to be successful in eradicating *H. pylori*, alternative treatment protocols are necessary because of its side effects and limited use in pediatric patients. *S. boulardii* can be administered instead when bismuth can not serve as alternative due to its side effects.

Keywords: Eradication, Helicobacter, *S. boulardii*, side effects, triple therapy

INTRODUCTION

Helicobacter pylori (*H. pylori*) may lead to superficial gastritis, peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma (1). While the worldwide rate of *H. pylori*–infected individuals is 50%, the corresponding rate in Turkey is 65% (2). Moreover, *H. pylori*'s prevalence rate has been reported at around 40% among children with symptoms (3). Various diagnostic methods and treatment models have been developed for the early detection of *H. pylori* infection and the prevention of chronic complications resulting from the infection. A study conducted in Turkey reported that *H. pylori*'s antimicrobial resistance to clarithromycin was 24.86%, while its resistance to metronidazole, levoflaxacin, amoxicillin, and tetracycline was 33.75%, 23.77%, 0.97%, and 3.51%, respectively (4). In another study carried out in Turkey, H pylori resistance to clarithromycin and fluoroquinolones were 27% and 15%, respectively (5). Since 2000, the success rate of triple therapy has fallen below 80% (50–79%) due to *H. pylori* resistance to metronidazole and clarithromycin (6). In cases of robust antibiotic resistance, bismuth, PPI, and two antibiotics are recommended. If bismuth is not available, quadruple treatments of three antibiotics and PPI are advised.

Recently, *H. pylori* eradication failure has increased to 20% in quadruple treatments comprising bismuth (7). Since bismuth treatments have high side effects, alternative treatment methods are necessary. Recently, probiotic treatment alternatives are receiving emphasis to improve both treatment compliance and success. *Saccharomyces boulardii* (*S. boulardii*)'s mechanism of action in the eradication of *H. pylori* is not clear yet. It is thought that it prevents the colonization of *H. pylori* and other bacteria and it strengthens the mucosal defense (8). Moreover, it is claimed that it can increase patient compliance due to fewer side effects relative to bismuth. There is not enough research on this subject. Randomized controlled studies in pediatric patients are insufficient in particular. This study compares the treatment efficacy and side effects of bismuth and *S. boulardii* in children with symptomatic *H. pylori* gastritis with the control group.

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In this study, it was aimed to compare the use of *S. boulardii* in the eradication of *H. pylori* in children instead of bismuth, which has a limited application area due to its side effects.

MATERIAL AND METHOD

The study was carried out with the permission of Taksim Training and Researches Hospital Ethics Committee (Date: 25.12.2019, Decision No: 177). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The patients, or their parents or guardians, signed written statements of informed consent.

This research involved a prospective, parallel design, single-center study. It included 220 pediatric patients aged between 12 and 18 years old who applied to the hospital's pediatric gastroenterology outpatient clinic due to chronic dyspeptic complaints. All patients had dyspeptic complaints for at least 3 months and received neither diagnosis nor treatment for *H. pylori* previously. A total of 26 patients were taken out (i.e., 8 patients with drug allergies and 18 patients lost to follow-up). Patients were randomly assigned to three treatment groups by a physician who had no involvement with the study. Each patient was assigned a number. The patients were then followed up on treatment and side effects based on these numbers.

Patients with upper gastrointestinal bleeding, patients who had undergone stomach or intestinal surgery, patients with a history of antibiotic or probiotic use in the previous three months, patients taking aspirin, and patients suffering from bleeding-coagulation disorders were excluded from the study.

Upper-gastrointestinal-system endoscopy and biopsies were performed on all patients. Biopsy samples were evaluated for *H. pylori* according to Sydney classification (0=absent, 1=mild, 2=moderate, 3=severe) (9). Patients' *H. pylori* eradication rates were analyzed via a stool antigen test four to six weeks after treatment. Stool antigen test is a qualitative immunochromatography test (CERTEST BIOTEC SL, Spain).

According to the most recently published ESPGHAN/NASPGHAN guidelines, patients underwent a treatment period of 14 days, during which they were randomly assigned to treatment groups. Treatment for the control group included pantoprazole (1mg per kg per day) (max. 40 mg) twice a day, amoxicillin (50 mg per kg per day, twice a day), and metronidazole (15 mg per kg per day, twice a day) (10). The two other groups received bismuth subsalicylate or *S. boulardii* treatments, for the comparison of *H. pylori* eradication across the three groups. These treatments were administered simultaneously with the standard antibiotic treatment. The bismuth treatment group consisted of 63 children, whereas the *S. boulardii* group and the control group had 66 and 65 children, respectively (**Figure 1**). For the bismuth subsalicylate treatment, four 262 mg tablets were administered daily (Bizmopen, 262 mg tab, manufactured by Dincsa İlac san. ve tic. A.S., Istanbul, Turkey). Bismuth subsalicylate was prefered over other bismuth salts due to its lower absorption within gastrointestinal system and weaker interaction with patient diet. For the *S. boulardii* treatment, two 250 mg *Saccharomyces boulardii* tablets (Reflor, manufactured by Sanofi-Synthelabo Ilac A.S., Istanbul, Turkey) were administered for 14 days.

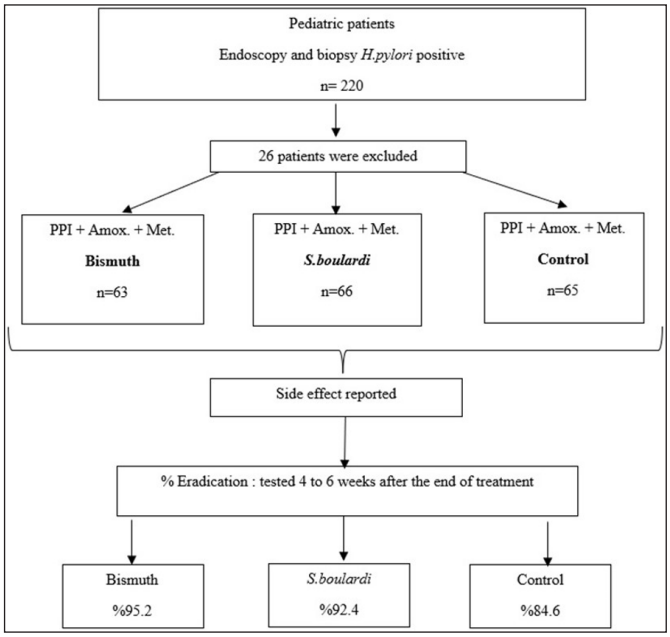

Figure 1. Flow chart of the current study

Treatment side effects were analyzed by an independent and blinded pediatrician during the 1st and 2nd weeks of treatment with open-ended questions and side-effect diaries of the patients.

Statistical Analysis

Statistical analysis was performed with SPSS software for Windows version 22.0 (SPSS Inc., Chicago, IL, USA). The normality condition in quantitative variables' was checked with a Kolmogorov-Smirnov test. Kruskal-Wallis H test was conducted to examine whether any difference in quantitative variables had occurred between the independent groups. The independence hypothesis between qualitative variables was tested using chi-square analysis. Since the quantitative variables did not resemble a normal distribution, their descriptive statistics were explained with the median (25th to 75th percentiles), and the descriptive statistics of the qualitative variables were expressed in terms of frequency (%). P values below 0.05 ($p<0.05$) were considered statistically significant.

RESULTS

Table 1 compares the control, bismuth, and *S. boulardii* groups in terms of age, gender, family history, *H. pylori* activity, and complaint duration. The three treatment groups did not differ from one another with respect to age, family history, gender, *H. pylori* activity, or complaint duration (p>0.05).

Table 1. Comparison of the general characteristics of the treatment groups

	Treatment			p
	Control	Bismuth*	*S. boulardii**	
Age (year)	14 (13-16)	15 (14-16)	14.50 (13-16)	0.745[a]
Family history				
Yes	35 (53.8)	31 (49.2)	35 (53)	0.855[b]
No	30 (46.2)	32 (50.8)	31 (47)	
Gender				
Male	33 (50.8)	22 (34.9)	30 (45.5)	0.185[b]
Female	32 (49.2)	41 (65.1)	36 (54.5)	
H. pylori activity	2 (1-2)	2 (1-2)	2 (1-2)	0.947[a]
Complaint duration (month)	10 (7-12)	10 (8-12)	10 (9-13)	0.199[a]

a: Kruskal Wallis H test ; b: Pearson Chi Square test, *: In addition to standard triple treatment

Table 2 shows the frequency and percentage distributions of the study's endoscopic findings by treatment group. The most common endoscopic finding across all three treatment groups was antral hyperemia. No statistically significant difference in endoscopic findings was observed between treatment groups (p=0.960). Among the control, bismuth, and *S. boulardii* groups, the most common pathological diagnosis was antral gastritis. A comparison of the treatment groups based on biopsy diagnosis revealed that the groups shared similar diagnosis distributions, with no difference between them (p=0.175).

Table 2. Comparison of endoscopic findings and biopsy diagnosis according to treatment groups

	Treatment			p
	Control	Bismuth	*S. boulardii*	
Endoscopic Findings				0.966[c]
Normal	8 (12.3)	4 (6.3)	4 (6.1)	
Atrophy	3 (4.6)	2 (3.2)	3 (4.5)	
Erosion	5 (7.7)	4 (6.3)	5 (7.6)	
Hyperemia	33 (50.8)	38 (60.3)	41 (62.1)	
Nodularity	13 (20)	13 (20.6)	11 (16.7)	
Ulcer	3 (4.6)	2 (3.2)	2 (3)	
Biopsy diagnosis				0.175[b]
Antral gastritis	30 (46.2)	31 (49.2)	42 (63.6)	
Chronic gastritis	15 (23.1)	9 (14.3)	9 (13.6)	
Pangastrit	20 (30.8)	23 (36.5)	15 (22.7)	

b: Pearson Chi Square test ; c: Fisher-Freeman-Halton test

As **Table 3** shows, an evaluation of the control, bismuth, and *S. boulardii* treatment groups' *H. pylori* stool antigen tests pointed out that *H. pylori* eradication success was highest among the bismuth group at 95.2%. The patients who received *S. boulardii* treatment followed, with a 92.4% eradication rate, and the difference between these two groups was not statistically significant. Among the control group, the eradication rate was 84.6%. This rate is near the acceptable level recommended for *H. pylori* eradication.

Table 3. Comparison of eradication rates of treatment groups

		Treatment			p
		Control	Bismuth	*S. boulardii*	
Stool antigen test	Negative	55 (84.6)	60 (95.2)	61 (92.4)	0.099[b]
	Pozitive	10 (15.4)	3 (4.8)	5 (7.6)	

b: Pearson Chi Square test

Table 4 exhibits the frequency of side effects—such as headache, nausea, diarrhea, constipation, abdominal pain, and taste disturbances—across the treatment groups. While abdominal pain and taste disturbances were not present in the control group, headache and diarrhea were not reported in the *S. boulardii* group. A comparison of the treatment groups' side effects revealed that the most common side effects were prevalent in the bismuth group (17.5%). However, the three treatment groups experienced a similar frequency of side effects, and no statistically significant difference was observed (p=0.123).

Table 4. Distribution of side effects by treatment groups

	Treatment			p
	Control	Bismuth	*S. boulardii*	
Side effects				0.123[b]
Headache	1 (1.5)	2 (3.2)	0 (0)	
Nausea	2 (3.1)	2 (3.2)	1 (1.5)	
Diarrhea	2 (3.1)	2 (3.2)	0 (0)	
Constipation	2 (3.1)	1 (1.6)	1 (1.5)	
Abdominal pain	0 (0)	2 (3.2)	1 (1.5)	
Taste disturbance	0 (0)	2 (3.2)	1 (1.5)	

b: Pearson Chi Square test

DISCUSSION

H. pylori remains a serious public health problem due to its high prevalence in the population, and it continues to increase the number of symptomatic pediatric patients as well as antibiotic resistance (11,12). Treatment regimens containing bismuth are emphasized because of rising eradication rates. In a pediatric study carried out in Turkey, a bismuth containing sequential treatment has achieved *H. pylori* eradication rate over 90% (13). Reports by the Fifth National China Consensus and the Maastricht-V Consensus have also recommended quadruple therapy containing bismuth. Nevertheless,

bismuth is not preferable because of its side effects and its unavailability in some countries (14,15). Limitations also affect the use of bismuth in pediatric patients. Therefore, a search for alternative *H. pylori* eradication treatment in pediatric patients is ongoing.

Upper gastrointestinal endoscopy and biopsy remain the gold standard in diagnosing *H. pylori*. An antral nodularity appearance in endoscopy is associated with *H. pylori* (16). Antral nodularity (93%) was the most common endoscopy finding in a study on children less than 20 years of age. Moreover, other endoscopy findings related to *H. pylori* such as mucosal edema, diffuse rash, and antral hyperemia were also observed in these patients. It is acknowledged that *H. pylori* positive children may present various endoscopy findings besides antral nodularity (17). In our study, the most common endoscopic finding was antral hyperemia, followed by antral nodularity.

The most common method for evaluating *H. pylori* eradication success is the ^{13}C urea breath test or stool antigen test for *H. pylori* (18). In our study, an evaluation of the control, bismuth, and *S. boulardii* treatment groups' *H. pylori* eradication success via stool antigen tests identified the highest success rate in bismuth treatment (95.2%). Liu et al. (19) reported the eradication rate of quadruple therapy containing bismuth at 98.8%. The eradication rate for 10-day sequential treatment reached 92.4% in their study. In another pediatric study conducted in Turkey, the eradication rate with bismuth-containing quadruple therapy was 92% (13). We found the similar success rate among patients who received *S. boulardii* in our study. However, with sequential treatment, varying eradication rates have been reported in Asian countries. For instance, Korea has 81.9% eradication rates, China 82.6%, and Taiwan 90.5%. Researchers have associated these different eradication rates with poor patient compliance in sequential therapy. A recent study conducted in Taiwan showed that extending sequential therapy from 10 days to 14 days increased eradication rates by 3-4% (20). Therefore, we decided to prescribe a 14-day combined treatment for our patients. As a result, we detected an eradication rate of 84.6% for our patients in the control group. This rate is near the acceptable level recommended for *H. pylori* eradication.

Lee et al. (21) reported epigastric pain (23.6 %) and diarrhea (16.7%) as the most common side effects in a 14-day quadruple therapy containing bismuth. This rate was more common among the patient group who received bismuth, in parallel with our study. In our study, the rate of side effects was 17.5%, and nausea and abdominal pain were among the most frequent side effects. In conventional *H. pylori* eradication treatments, side effects are observed at a rate of 5–30%, which reduce treatment success. The frequency of side effects was 6.7% for patients who received quadruple bismuth treatment for 10 days, versus 11.7% for patients who received quadruple therapy containing bismuth for 14 days (22). In a study conducted on children in China, side effects were reported in 15.3% of patients whose treatments included bismuth (23). This situation affects treatment compliance and success. Therefore, the use of probiotics to increase eradication success among children with *H. pylori* gastritis is an important topic of discussion. Therefore, some studies have involved yeast as probiotics, such as *S. boulardii* (24). A study by Zhou et al. (25) found that the administration of *S. boulardii* decreased nausea, vomiting, diarrhea, and abdominal distention compared to the control group; however, no significant difference was detected between the two groups. Another study in children showed that *S. boulardii* increased treatment compliance by reducing diarrhea during eradication treatment. Furthermore, a slight increase was detected in the *H. pylori* eradication rate relative to the control group (26). Similarly, in our study, the side effects decreased, and the eradication rate increased compared to the control group. Unlike other studies, children receiving *S. boulardii* treatment and bismuth treatment were put into comparison. The eradication rate in children on *S. boulardii* treatment was close to those who received bismuth treatment.

In conclusion, the use of probiotics in the treatment of gastrointestinal problems has been popular in recent years. Probiotic bacteria and *S. boulardii* fungi have been used for *H. pylori* eradication in adult studies. Their positive contributions to treatment have been noted. Therefore, they may be used to increase eradication success, particularly among pediatric patients when bismuth is not a feasible option. With fewer side effects, *S. boulardii* increases treatment compliance and success for pediatric patients, a sensitive group that needs additional consideration for treatment. The most important limitation of this study is that a *H. pylori* culture could not be obtained. Moreover, antibiotic resistance could have been checked, and the treatment groups could have been randomized accordingly.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Taksim Training and Researches Hospital Ethics Committee (Date: 25.12.2019, Decision No: 177).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The relationship between white coat hypertension and the index of cardiac electrophysiological balance (ICEB)

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ABSTRACT

Aim: The index of cardiac electrophysiological balance (ICEB) is a new marker that can show the potential for ventricular arrhythmia and indicate the balancebetween ventricular depolarization and repolarization. A tendency toward ventricular arrhythmia has been shown innon-dipper hypertension and prehypertensive patients in various studies. White coat hypertension (WCH) has been shown to be associated with target organ damage and the actual development of hypertension. In this study, we aimed to evaluate the effect of dipper and non-dipper patterns on the ICEB in patients diagnosed with WCH.

Material and Method: A total of 108 patients were included in this study. Patients were divided into two groups as dipper and non-dipper patterns according to ABPM. QT/QRS (ICEB) and cQT/QRS (ICEBc) were recorded with computerized interpretation of the electrocardiogram.

Results: While electrocardiographic parameters including heart rate, PR interval, QT interval, cQT interval, and ICEB were similar in both groups (p>.05), in the non-dipper group, QRS duration was lower (p=.017) and ICEBc was higher (p=.001).

Conclusion: ICEBc may predict asusceptibility to ventricular arrhythmias in WCH patients. Therefore, non-dipper WCH patients with a high ICEBc should be followed for arrhythmia outcomes in addition to hypertensive outcomes.

Keywords: White coat hypertension, non-dipper pattern, the index of cardiac electrophysiological balance, electrocardiography, ventricular arrhythmia, QT interval

INTRODUCTION

White coat hypertension (WCH) is a condition in which home blood pressure (BP) or ambulatory BP measurements (ABPM) are normal, but outpatient measurements are high (1). Although it appears to be a benign condition, it has been shown to be associated with target organ damage and the actual development of hypertension (2,3). Normal blood pressure shows a circadian rhythm. At night, blood pressure decreases by more than 10% compared to the average daytime value; this is called a dipping pattern. A non-dipping pattern is a reduction in blood pressure of less than 10% at night and is associated with increased cardiovascular risk and target organ damage (1,4,5). Supraventricular and ventricular arrhythmias are causes of cardiovascular morbidity and mortality. Predicting these dysrhythmias and initiating early treatment or avoiding the causative conditions can reduce morbidity and mortality. Many studies have shown a predisposition to ventricular arrhythmias in patients with non-dipper hypertension and left ventricular hypertrophy (6–8). A tendency toward ventricular arrhythmia in patients with prehypertensive non-dipping pattern is demonstrated by a fragmented QRS (9). The predictive value of the frontal QRS axis, which demonstrate myocardial repolarization, has been demonstrated in patients with non-dipper hypertensionand without left ventricular hypertrophy (10). In the evaluation of arrhythmia, electrocardiographic (ECG) findings such as QT dispersion, QTc, Tp-e (Tp-e=peak-to-end interval of the T wave), Tp-e/QT have been evaluated in many studies (11–14). The index of cardiac electrophysiological balance (ICEB) is the ratio of QT to QRS (QT/QRS), which is a new marker that can show the potential for ventricular arrhythmia and is easily calculated on asurface ECG (15). It has been demonstrated that ICEB can be used in place of cardiac wavelengthλ (λ= effective refractory period [ERP] x conduction velocity [CV]), which is measured in anelectrophysiology laboratory and can

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indicate the balance between ventricular depolarization and repolarization (16). An imbalance may predispose one to Torsades de Pointes (TdP) and other ventricular arrhythmias. In this study,we aimed to evaluate the effect of dipper and non-dipper patterns on ICEB in patients diagnosed with WCH.

MATERIAL AND METHOD

This retrospective study was carried out with the permission of Ankara Keçiören Training and Research Hospital, Non-Invasive Clinical Researches Ethics Committee (Date: 09.11.2021, Decision No: 2012-KAEK-15/2424). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patient Selection

In our study, patients with a systolic blood pressure (SBP) of ≥140 mmHg and/or a diastolic blood pressure (DBP) of ≥90 mmHg in a physical examination and who underwent ABPM with suspected WCH (the blood pressure readings in the hospital were higher than they are in other settings, such as the home) were evaluated from May 2020 to July 2021. In accordance with the 2018 European Society of Cardiology (ESC) / European Society of Hypertension (ESH) guidelines (1), 151 patients were diagnosed with WCH. A detailed physical examination was performed on the patients and medical history was taken. Demographic and laboratory data were obtained from the automated hospital system and recorded. Diagnoses of hypertension on ABPM records, diabetes mellitus, chronic kidney disease, coronary artery disease, left ventricular systolic dysfunction (EF<50%) and moderate-severe valve disease in echocardiography, endocrine diseases (hyperthyroidism, anemia), obstructive sleep apnea syndrome, chronic obstructive pulmonary disease, pregnancy, drug use affecting the autonomic nervous system, branch block, atrial fibrillation, and atrioventricular block on an ECG were determined as exclusion criteria. Forty-three patients with one or more of the exclusion criteria were excluded from the study.

ABPM Recordings

Twenty-four-hour ambulatory blood pressure recordings were obtained using the oscillometric system (Altgasse 68 6341 Baar, Switzerland). Daytime recordings were made between 06:00 and 22:00, nighttime recordings were madebetween 22:00 and 06:00. Blood pressure values were taken every 30 minutes for daytime recordings and were taken every hour at nighttime. Information about sleep duration was obtained from the patients, and no adverse events related to the device were reported during the day or at night. It was required that the false measurement rate be less than 20%. Daytime, nighttime, and 24-hour systolic, diastolic, and mean blood pressures were obtained from ABMP recordings. A 24-hour mean of ≥130/80 mmHg, daytime mean of ≥135/85 mmHg, and nighttime mean of≥120/70 mmHg were accepted as hypertension, and clinical blood pressure measurements were SBP ≥140 mmHg and/or DBP ≥90 mmHg; those with normal ABPM values were considered WCH (1). A dipper pattern was determined as a ≥10% decrease in mean blood pressure during the nighttime, according to the mean blood pressure value during the daytime, and a ≥10% decrease in mean blood pressure was a non-dipper pattern (1). The patients diagnosed with WCH were divided into two groups, dipper and non-dipper, according to ABPM records.

Electrocardiography and Echocardiography

A 12-lead surface ECG was obtained from all patients, in the supine position, after resting for at least 5 minutes. Heart rate, PR, QRS, QT, and cQT intervals were obtained from computerized interpretation of the electrocardiogram results (Nihon Kohden, Tokyo, Japan). Also, QT/QRS(ICEB) and corrected QT (QTc)/ QRS (ICEBc) values were calculated from these ECG data for every patient. Transthoracic echocardiography was performed on all patients with GE Healthcare Vivid S60N. Left ventricular diastolic diameter, interventricular septum, and posterior wall measurements were taken on the parasternal long axis just below the mitral valve tips in diastole to calculate the left ventricular mass and index (17). The left ventricular ejection fraction was measured by using the modified Simpson's method (17). Left ventricular hypertrophy was evaluated according to the LV (left ventricle) mass index. LV mass was calculated with the following formula: Lv mass=0,8x(1,04x((LVEDD (left ventricle end diastolic diameter)+IVSd (Interventricular septum distance)+PWd (posterior Wall distance))3-LVEDD3)))+0,6. LV mass index was calculated using the following formula: LV mass index=LV mass/Body surface area (BSA) (17). BSA was calculated with the Mosteller Formula: BSA= ([height(cm)x weight(kg)]/3600)1/2. Values >95 g/m^2 for women and >115 g/m^2 for men were accepted as left ventricular hypertrophy.

Statistical Analysis

Numerical data were expressed as mean standard deviation, and non-numeric data as a percentage (%). Data were saved in the SPSS version 23.0 statistics program. Continuous variables with normal distribution were compared with the Student t-test. The Pearson method was used in the correlation analysis of the independent variables in each population studied.

RESULTS

The mean age of the entire population in our study was 52.8, and 52% of the dipper group and 50% of the non-dipper group were women. There was no difference between the two groups in terms of body-mass index and smoking, respectively (p=.426,p=.882). Transthoracic echocardiographic parameters, left ventricular ejection fraction (LVEF), left ventricle end-diastolic diameter (LVEDD), interventricular septum thickness (IVST), and posterior wall thickness (PWT) were examined, and no significant difference was observed in both groups (p>.05). In addition, there was no statistically significant difference between the two groups in terms of left ventricular hypertrophy (p>.05) (**Table 1**). There was no difference between the two groups in terms of hemoglobin and electrolyte values (p>.05). While forthe ABPM records, the 24-h mean SBP was not statistically different between the two groups (p>.05), the 24-h mean DBP was significantly higher in the dipper group (p<.001). Daytime SBP and DBP were significantly higher in the dipper group (p=.024 ,p<.001), and nighttime SBP was significantly higher in the non-dipper group (p=.002)(**Table 2**). While electrocardiographic parameters including heart rate, PR interval, QT interval, cQT interval, and ICEB were similar in both groups (p>.05), in the non-dipper group, QRS duration was lower (p=.017) and ICEBc was higher (p=.001) (**Table 3**).

Tablo 1. Demographic laboratory and echocardiographic features

Variable	Dippers (n=50)	Non-dippers (n=58)	P value
Age, years	51.06±9.7	52.97±9.5	0.310
Gender			
Male, n (%)	24 (48%)	29 (50%)	0.838
Female, n (%)	26 (52%)	29 (50%)	
Body-mass index, kg/m^2	28.15±3.7	28.74±3.9	0.426
Smoking, n (%)	26 (52%)	31 (53.4%)	0.882
LVEF, %	60.64±2.8	59.8±2.7	0.132
LV mass (g)	146.06±51.2	155.53±25.1	0.216
LV mass index (g/m^2)	74.28±9.6	81.46±11.6	0.001
LVEDD (cm)	4.68±0.36	4.68±0.29	0.976
IVST (cm)	0.87±0.06	1.08±1.06	0.172
PWT (cm)	1.03±1.00	0.96±0.08	0.604
Hemoglobin (g/dl)	14.1±1.26	16.16±1.51	0.339
Na (mEq/L)	140.1±2.3	139.1±2.8	0.114
K+ (mEq/L)	4.10±0.26	4.19±0.42	0.199
Ca (mEq/L)	9.54±057	9.52±0.44	0.980

LVEF:left ventricular ejection fraction. LV:left venricul. SBP:systolic blood pressure. DBP:diastolic blood pressure ,LVEDD:left ventricle end-diastolicdiameter IVST:interventricular septum thicknessPWT:posterior wall thickness. P value in bold :statistical significanse

Tablo 2. ABPM records

Variable	Dippers (n=50)	Non-dippers (n=58)	P value
24 h mean SBP, mmHg	118.34±8.9	116.72±8.7	0.345
24 h mean DBP, mmHg	72.38±5.58	67.26±5.77	<0.001
Daytime SBP, mmHg	122.88±9.44	119.02±8.07	0.024
Daytime DBP, mmHg	76.4±5.98	70.34±5.76	<0.001
Night-time SBP, mmHg	107.36±8.56	112.16±7.46	0.002
Night-time DBP, mmHg	63.28±5.09	63.59±5.44	0.765

SBP: systolic blood pressures DBP: diastolic blood pressures. P value in bold :statistical significanse

Tablo 3. Electrocardiographic findings

Variable	Dippers (n=50)	Non-dippers (n=58)	P value
Heart rate, beats/min	81.02±14.15	83.6±16.49	0.379
PR interval, msec	146.5±14.51	152.8±19.4	0.061
QRS duration, msec	85.30±7.82	81.38±8.84	0.017
QT interval, msec	369.80±34.99	369.31±37.75	0.945
QTc interval, msec	405.5±22.24	410.76±19.71	0.196
ICEB	4.38±0.52	4.55±0.58	0.119
ICEBc	4.78±0.45	5.09±0.64	0.001

ICEB: index of cardiac electrophysiological balance, ICEBc: index of cardiac electrophysiological balance with heart rate correction. P value in bold :statistical significanse

DISCUSSION

Hypertension is one of the most common diseases in clinical practice (1). Identifying conditions predisposing individuals to hypertension, taking the necessary precautions, and treating them contribute to areduction of morbidity and mortality (1). In addition, predicting arrhythmias that may be associated with hypertension and taking the necessary precautions may have a positive effect on outcomes in cardiovascular system-related conditions. Although WCH seems to be benign, Mancia et al. (18) showed that hypertension developed in 42.6% of WCH patients in a 10-year follow-up. In another study, it was shown that the development of hypertension was approximately three times more common compared to people with normal blood pressure (19). Not only hypertension, but also WCH has been found to be associated with cardiovascular diseases. Therefore, WCH is a relevant clinical condition. Recent studies have shown that left ventricular hypertrophy and carotid atherosclerosis are more common as target organ damage in patients with WCH than in normotensive patients (20,21). In addition, it has been shown that cardiovascular mortality is higher in patients with WCH than in normotensive or prehypertensive patients (22).

A Non-dipping pattern is a reduction in blood pressure of less than 10% at nightcompared to the average daytime value and is associated with increased cardiovascular risk and target organ damage (1,4,5). In many studies, it has been shown that the non-dipper

pattern predisposes for VA (ventricular tachicardia and/or ventricula fibrillation) in patients with hypertension (6–14). Therefore, WCH may be a predisposing factor for cardiovascular diseases as well as for arrhythmia. By identifying the WCH population with a predisposition to ventricular arrhythmia, it may be possible to ensure that the agents to be selectedas antihypertensives in the future have antiarrhythmic properties or to avoid proarrhythmic agents in different treatment options. LVH has been shown to have a proarrhythmic effect in hypertensive and prehypertensive patients in studies (6–8,10). In our study population, there was no statistically significant difference between the groups in terms of LVH. LVH had no effect on our study population in terms of both ECG findings and arrhythmia. Therefore, the importance of the ICEB value as a proarrhythmic indicator was increased in our study group.

ICEB is a relatively new marker that indicatesventricular arrhythmogenesis and is obtained by dividing QT/QRS in ECG (15). Lu et al. (16) suggested that a significant increase in ICEB may cause TdP-induced VA, while a decrease may cause non-TdP-induced VA. ICEBc is the QTc/QRS ratio, and in healthy chronic smokers it may predicta tendency toward TdP-induced VA (23). It has also been shown that ICEB can predict cannabinoid-induced VA (24). In our study, ICEB values were higher in the non-dipper group, and ICEBc was found to be significantly higher. The shorter QRS duration on the depolarization side and the longer QTc interval in the direction of repolarization may explain the higher ICEBc values in the non-dipper group. It is known that non-dipper status increases the frequency of left ventricular hypertrophy and causes cardiac conduction problemsandautonomic dysfunctionand heterogeneity in ventricular repolarization (25,26). Sympathetic activity has been shown to increase in WCH patients compared to normatensives, and, in another study, anincrease in sympathetic activity and decrease in vagal activity have been shown to prolong QTc (27,28). In addition, an increase in nocturnal sympathetic activity and a decrease in vagal activity were shown in patients with the non-dipper pattern (29,30). The non-dipper state and increased adrenergic effect may cause deterioration in ventricular homogeneity, and this may be related to the ICEBc results in our study. Ultimately, non-dipper statusmay lead to increased susceptibility to ventricular arrhythmia in WCH patients.

The main limitations of our study were the small number of patients and its retrospective nature. ABPM could not be used in normotensive patients due to theretrospective nature of the study. Prospective follow-up periods were not performed for the evaluation of arrhythmia in the patients.

CONCLUSION

ICEBc was found to be significantly higher in patients with WCH and non-dipper patterns compared to those with dipper patterns. ICEBc may predict asusceptibility to ventricular arrhythmias in WCH patients. Therefore, non-dipper WCH patients with a high ICEBc should be followed for arrhythmia outcomes besides hypertensive outcomes.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Keçiören Training and Research Hospital, Non-Invasive Clinical Researches Ethics Committee (Date: 09.11.2021, Decision No: 2012-KAEK-15/2424).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Original Article

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Clinical, sonographical and cytological comparison of toxic and non-toxic thyroid nodules

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ABSTRACT

Aim: To compare patients with toxic and non-toxic nodular/multinodular goiter in terms of clinical, sonographical and cytological features.

Material and Method: The medical data of 326 patients were reviewed retrospectively. Clinical and sonographic features were examined. Four hundred and eighty-one nodules were compared sonographically and cytologically. One hundred twenty-four patients had toxic nodular goiter and 202 of them had non-toxic nodular goiter.

Results: The toxic nodular goiter group was older, they had more male sex, more multi-nodularity, larger thyroid glands and nodules with more sonographically suspicious features ($p<0.05$). One hundred sixty-five of 481 nodules belonged to the toxic group. Nodule size was > 40 mm in 13.9% of the nodules in the toxic group and 5.4% of those in the non-toxic group ($p= 0.003$). Central vascularization ($p <0.0001$) and hypoechogenicity ($p =0.005$) were higher in nodules of the toxic group. The two groups were similar in terms of fine needle aspiration biopsy (FNAB) results.

Conclusion: Toxic nodules can have sonographically suspicious features like non-toxic nodules, and their evaluation with FNAB should not be avoided or postponed, thus ensuring more adequate treatment and follow-up of toxic nodular thyroid disease.

Keywords: Thyroid nodule, toxic, sonography, biopsy, malignancy risk

INTRODUCTION

Hyperthyroidism is characterized by increased thyroid hormone synthesis and release in the thyroid gland. Toxic adenoma (TA) and toxic multinodular goiter (TMNG) are the most common causes of hyperthyroidism after Graves' disease and their incidence increases with age (1).

The treatment of TA and TMNG is radioactive iodine or surgery (2). However, there is an opinion that, because thyroid stimulating hormone (TSH) is suppressed in hyperthyroidism, oncogenesis is also inhibited and thus hyperthyroidism has a protective effect against thyroid cancer (3). While thyroid cancer affects 7-15% of the population, early publications showing that the incidence of cancer is lower in hyperthyroidism (4). Therefore, cytological evaluation for toxic (or hot) nodules has not been considered. Previously, the estimated cancer risk of non-functioning nodules is around 10-20%, while this rate is very low for hot nodules (5,6). However, there are more recent studies showing that the cancer risk is higher in hyperthyroidism (7).

In a recent study investigating the risk of malignancy in hot nodules, a similar risk of malignancy was found between hot nodules and cold nodules. The risk in hot nodules was determined as 4.3% (8).

According to American Thyroid Association (ATA) 2015 guideline, fine needle aspiration biopsy (FNAB) is not recommended for toxic nodules as they are rarely considered malignant (9). However, as mentioned above, hot nodules may not be as benign as it seems. In this study, we compared hot nodules with non-toxic nodules in clinical, sonographic and cytological terms. And so, we tried to determine the malignancy risk of toxic nodules.

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MATERIAL AND METHOD

Ethical approval was obtained from Amasya University Hospital Non-Invasive Ethics Committee (Date: 07.01.2021, Decision No: 18). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This is a multicenter study. It included 326 patients who were diagnosed as TA, TMNG and non-toxic nodular goiter between 2018-2020.

Patients with toxic nodular goiter (TNG) were diagnosed according to the following diagnostic criteria: suppressed serum TSH levels with elevated serum free thyroid hormones and increased uptake on thyroid scan. Patients with non-toxic nodular goiter (non-TNG), had 1 or more nodules and their thyroid functions were euthyroid or hypothyroid.

Patients who were<18 years of age or pregnant or had active infections, chronic organ failure and/or malignancy were excluded.

Age, gender, TSH, thyroid ultrasonography (USG) and FNABs were examined. Thyroid USG (first center's USG is Aloka, Mollsfeld, Meerbusch, Germany, second is Toshiba TA700, Japan) and FNABs were performed by two endocrinologists educated from the same center. Thyroid gland and thyroid nodules were reported in three dimensions as antero-posterior, transverse and longitudinal. Thyroid volume was calculated with this formula: Anterior-posterior diameter x transverse diameter x longitudinal diameter x0.52. USGs were reported according to ATA guideline (9). 99mTc pertechnetate scintigraphy was performed to separate hot-cold nodules. Size (>1 cm), content (solid-mix), echogenicity (hypoechoic), vascularization (central), calcification (microcalcification), margin structure (irregular edge), anteroposterior/transverse diameter ratio (>1) of the nodules were evaluated with USG. FNAB was applied by aspirating method to 481 nodules with at least 1 suspicious feature. Thyroid functions of the patients in the toxic group were in the subclinical hyperthyroid and euthyroid stages while FNABs were performed. FNABs were reported as benign, malignant, suspicious for malignancy, follicular neoplasia, atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (AUFL) according to the Bethesda classification (10). Performing sonography and biopsy procedures by endocrinologists following the patients is an important difference of this study.

Statistical Analysis

Statistical analysis were made by IBM SPSS for Windows Version 25.0. Numerical variables were summarized with mean±standard deviation or median (min-max). Categorical variables were indicated by number and percentage. The Pearson chi-square test, Continuity Correction or Fisher's exact test were used to determine whether there was any difference between the groups in terms of categorical variables. The Kolmogorov Smirnov test, mean/standard deviation ratio, Skewness/Kurtosis, histogram and detrended normal graphics were used to determine whether the normal variables showed normal distribution, and the homogeneity of the variance was examined by the Levene test. Differences between two independent groups in terms of numerical variables; t-test and Mann Whitney U were used. Post-hoc analysis was used to determine which subgroup made the difference. The significance level was taken as $p < 0.05$.

RESULTS

The patients with TNG consisted of 124 individuals and 202 of the patients had non-TNG. General characteristics of the participants are given in **Table 1**. Age 65 and over was defined as geriatric age. The TNG group was older, they had more male sex, more multi-nodularity, larger thyroid glands, nodules with more suspicious features and, as expected, lower TSHs. (**Table 1**).

Binary logistic regression analysis was performed for the effect of these parameters on toxicity; Only TSH and age were significantly different between two groups (**Table 2**), no meaningful results were reached for other parameters (these were not shown in the table).

Table 1. Comparisons of two groups according to individual characteristics

Parameter	Toxic goiter (n=124)	Non-toxic goiter (n=202)	P
Age, year	58.49±10.906	50.79±11.282	<0.0001*
Geriatric age, n (%)	40 (32.3)	21 (10.4)	<0.0001*
Male gender, n (%)	36 (29)	34 (16.8)	0.009*
Subgroup (according to nodule number), n (%)			
NG	19 (15.3)	54 (26.7)	
MNG	105 (84.7)	148 (73.3)	0.016*
Right thyroid volume, mm^3	23332.49±16128.059	17873.14±13342.562	0.001*
Left thyroid volume, mm^3	21244.21±14253.341	15811.78±12649.67	<0.0001*
Number of nodules with suspicious features	1.78±1.079	1.42±0.703	0.003*
TSH	0.10 (0.001-7.66)	1.30 (0.01-9.44)	<0.0001*

NG: nodular goiter, MNG: multinodular goiter, TSH: thyroid stimulating hormone *: statistically significant p value

Table 2. Binary logistic regression analysis for parameters that differ significantly in Table 1

Parameter	β	P	Exp(B)	95% CI for Exp(B)	
				Lower	Upper
Geriatric age	-1.230	0.018*	0.292	0.105	0.810
TSH	1.740	<0.0001*	5.697	3.468	9.358

*: statistically significant p value

A total of 481 FNABs were received. TNG group had 165 nodules. The comparison of the two groups in terms of the nodules undergoing FNAB was given in **Table 3**. Chi square analysis was performed for categorical variables; Which subvariables caused the difference was determined by post-hoc analysis and these were marked with letters. Accordingly, it was found that the nodules in the TNG group were larger, had more smooth edges, more calcification, worse blood flow patterns (central, central + peripheral), and more hypoechoic pattern. There was no difference in biopsy results and nodule content (**Table 3**).

Table 3. Comparison of two groups according to the biopsied nodules

Parameter	Toxic group (n=165)	Non-toxic group (n=316)	P
Biggest diameter	27.86±11.310	21.131±10.227	<0.0001*
Size, n (%)			0.003*
< 10 mm	4 (2.4)	15 (4.7)	
10.01-39.99 mm	138 (83.6)	284 (89.9)	
> 40 mm	23 (13.9)[a]	17 (5.4)[b]	
Content, n (%)			0.282
Solid	57 (34.5)	125 (39.6)	
Mixed	108 (65.5)	191 (60.4)	
Edge, n (%)			0.014*
Smooth	141 (85.5)[a]	236 (74.7)[b]	
Irregular	14 (8.5)[a]	57 (18)[b]	
Unclear	10 (6.1)	23 (7.3)	
Calcification, n (%)			0.038*
Absent	122 (73.9)[a]	268 (84.8)[b]	
Micro	11 (6.7)	11 (3.5)	
Macro	19 (11.5)	21 (6.6)	
Micro+macro	7 (4.2)	5 (1.6)	
Eggshell	6 (3.6)	11 (3.5)	
Vascularization, n (%)			<0.0001*
Absent	94 (57)	206 (65.2)	
Peripheral	43 (26.1)	91 (28.8)	
Central	7 (4.2)[a]	1 (0.3)[b]	
Peripheral+central	21 (12.7)[a]	18 (5.7)[b]	
Echogenicity, n (%)			0.005*
Isoechoic	31 (18.8)	70 (22.2)	
Hypoechoic	38 (23)[a]	47 (14.9)[b]	
Hyperechoic	0[a]	16 (5.1)[b]	
spongiform	96 (58.2)	183 (57.9)	
FNAB result, n (%)			0.152
Benign	122 (73.9)	197 (62.3)	
Malign	1 (0.6)	1 (0.3)	
Susp for malign	3 (1.8)	9 (2.8)	
FN/ Suspicious FN	0	2 (0.6)	
AUS / AUFL	18 (10.9)	51 (16.1)	
ND	21 (12.7)	56 (17.7)	

FN: follicular neoplasia, AUS / AUFL: unspecified atypia / indeterminate follicular lesion, *: statistically significant p value, a: There was a significant difference with compared non-toxic group in post-hoc comparison., b: There was a significant difference with compared toxic group in post-hoc comparison.

DISCUSSION

The results of the study: Patients with TNG were older, they had more male gender than patients with non-TNG, had larger thyroid glands and more nodules with suspicious features. The toxic nodules had larger size, more central blood supply, and hypoechogenicity rather than the other group. However FNAB results show similar cytological risk with the non-TNG. Since only 12% of the patients were thyroidectomy, the FNAB results could not be confirmed by pathological data. Undoubtedly, the Covid-19 pandemic had an unfavourable effect on this. In our country, as in many countries, elective hospital admissions and elective surgeries have decreased significantly due to the pandemic. The patients had to be followed conservatively with antithyroid drugs instead of primary treatment.

The frequency of TNG in areas of iodine deficiency increases up to 50% and is more common in the elderly (11). There is no distinct gender dominance in toxic nodular goiter (12). In our study, patients with toxic goiter were older and they had more geriatric age patients (>65 years) than non-TNG group. Female gender was higher in both groups, but individuals with male gender had a larger ratio in TNG. (29% vs 16.8, p=0.009, **Table 1**). Since women in our country apply to hospitals more than men, there may be such a difference with the literature in terms of gender. Besides, in logistic regression analysis, the high number of getriatric patients was significant, while no significant effect of gender was observed (**Table 2**).

Thyroid nodules are usually seen as multiple nodules but may also be single (13). In this study, MNG was higher in both groups, but the rate of multinodularity was higher in the toxic group (84.7% vs 73.3%, p=0.016). The reason for this is that the number of nodules increases with age and the TNG patients were older.

In the study, the TNG group's thyroid volumes were higher and they had more nodules with

suspicious features (**Table 1**). The development of toxic nodular/multinodular goiter depends on many factors. Chronic stimulation of thyroid gland causes diffuse enlargement of the gland, which progresses to goiter and nodule formation. In addition to the autonomic activity of nodules, toxic nodular/multinodular goiter develops with the effect of other factors such as genetic factors, iodine deficiency, stress, gender, and drugs (14). Therefore, the detection of larger thyroid gland and higher number of nodules in the toxic group seems compatible with the literature.

FNAB is the most appropriate method for preoperative evaluation of the malignancy risk for thyroid nodules. Performing it with USG increases its effectiveness. FNAB is recommended for nodules with suspicious features such as largest diameter > 1 cm, hypoechogenicity, poorly

defined margin, absence of halo, presence of micro-calcification, antero-posterior diameter/transverse diameter ratio > 1, and intranodular vascularization (9). In toxic nodular thyroid diseases, radionuclide scintigraphy is performed, in this procedure, non- or hypo-functioning nodules have high risk of malignancy. It is recommended to perform FNAB of these nodules, but if the nodule is hyperfunctioning, FNAB is not recommended (15). In practice, FNAB of hyperfuntioning nodules is not performed (16). In addition, if biopsy is performed on a hot nodule, it may show a follicular pattern, which is not associated with malignancy.

However, an increasing number of follicular cancers and follicular variant papillary thyroid cancers are detected in hot nodules. For this reason, the diagnosis of follicular neoplasia in the biopsy of hot nodules is not as reassuring as previously thought (17).

Therefore, if hot nodule has suspicious sonographic feature(s), it should be considered for FNAB. In our study, FNABs were performed for 481 suspicious nodules and the two groups were compared. The TNG group had suspicious sonographic features such as larger size, central blood supply, hypoechogenicity, and they had lower risk in terms of calcification and margin structure and are at similar risk with the other group for nodule content. Hence, it can be said that toxic nodules carry suspicious features like non-toxic nodules or more than them. All in all, FNAB results were similar in both groups. Interestingly, there is no result with follicular neoplasm in the toxic group. The meaningful result of our work is that, toxic nodules are not less risky than non-toxic.

Limitation

Essentially, the patients should be operated and histopathological diagnosis should be seen to tell the actual malignancy risk, but unfortunately, we could not confirm the FNAB results. Because of the pandemic, only 26 were operated, appropriate follow-up of most of the patients could not be performed.

CONCLUSION

In summary, toxic nodules have significant suspicious patterns like non-toxics, and their evaluation with FNAB should not be avoided, thus ensuring more adequate treatment and follow-up of toxic nodular thyroid disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethical approval was obtained from Amasya University Hospital Non-invasive Ethics Committee (Date: 07.01.2021, Decision No: 18).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Pneumococcal and influenza vaccination rates among patients with cardiovascular disease

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ABSTRACT

Introduction: Influenza virus infections and pneumococcal pneumonia are associated with significant increases in hospitalization and mortality rates in patients with cardiovascular diseases (CVD). In Turkey, pneumococcal and flu vaccines are recommended for patients with chronic heart disease, adults aged 65 and older and adults who are at risk of pneumonia.

Material and Method: This study was conducted between July 2019 and December 2019 among 240 CVD patients. The survey consisted of 29 questions aimed at evaluating the sociodemographic characteristics of the patients, the history of influenza and pneumococcal vaccines, and their knowledge and attitudes towards vaccines.

Results: Vaccination rates were low in CVD patients. Last year, only 10% of the participants had been previously vaccinated with the pneumococcal vaccine and 27.75% with the influenza vaccine. While the percentage of vaccination influenza vaccine among university graduates was significantly higher than all other groups, this high was not detected for pneumococcal vaccine.

Eighty percent of the participants visited their family physicians regularly in the previous year, while only 61.6% received information about the vaccination from the doctor or nurse. Influenza vaccine (39.2%) was recommended at a higher rate than pneumococcal pneumonia (12.1%) by the physicians or nurses of the participants during the visits. ($p < 0.05$)

Conclusion: Our results suggest that training programs on adult vaccination are needed for both healthcare professionals and patients at risk to achieve higher vaccination rates.

Keywords: Cardiovascular disease, influenza vaccine, pneumococcal vaccine, survey

INTRODUCTION

Background and Significance

Pneumococcal pneumonia and influenza are associated with excess mortality and morbidity in the elderly and are associated with acute myocardial infarction risk (1-2). While community-acquired pneumonia is associated with up to eightfold increases in the risk of myocardial infarction. Influenza virus infections are also associated with considerable increases in cardiovascular hospitalizations and mortality (3-4). Pneumococcal vaccination is recommended for at-risk populations, including cardiovascular diseases (CVD's) in the USA in 2015 and Europe in 2016 (5-6). In Turkey, pneumococcal and influenza vaccinations are recommended for adults >65 years of age and adults at risk of pneumonia, including patients with chronic heart disease (7). Despite the above recommendations, a large proportion of at-risk adults, are thought to be non-immunized.

Purpose

There is little information regarding cardiovascular patients' knowledge, attitudes, and behaviors with cardiovascular disease concerning pneumococcal and influenza vaccinations. The survey was conducted to evaluate the pneumococcal and influenza vaccine coverage among cardiovascular disease patients, their knowledge, and the factors influencing pneumococcal and influenza vaccinations.

MATERIAL AND METHOD

The study was carried out with the permission of the Selçuk University Faculty of Medicine Clinical Researches Ethics Committee (Date: 12.06.2019, Decision No: 2019/142). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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The questionnaire was prepared by the researchers based on previous literature (8). The questionnaire consisted of 29 questions aiming to assess patients' sociodemographic characteristics, influenza and pneumococcal vaccine history, and their knowledge and attitudes concerning the vaccines.

Participants were consecutive 240 adult patients (120 women and 120 men) who presented to the Department of Cardiology Outpatient Clinic who previously had a diagnosis of CVD. Patients with heart failure and cardiomyopathies, atherosclerotic heart disease, heart valve diseases, cyanotic congenital heart disease, pulmonary hypertension who were recommended to be vaccinated according to the expert opinion of Turkish Society of Cardiology were included in the study (9). The patients were examined in two different groups as those with coronary artery disease (CAD) and other cardiovascular diseases (non-CAD) recommended for vaccination. Heart failure was evaluated in the non-CAD group, regardless of etiology. Patients who were unable to conduct a face-to-face questionnaire and patients who refused to participate in the study were not included in the survey. Diagnoses of CVD, coronary artery disease (CAD), chronic diseases, and vaccination history were based on the participants' statements.

Statistical Analysis

The statistical power analysis tool "G*Power version 3.1" was used to calculate the sample size. Based on the preliminary study results, 240 participants were needed for the study based on β=86 power, α=0.05 margin of error and d=0.25 effect size.

All data were evaluated using SPSS 21.0 statistical package program. Number, percentage (%), and mean±standard deviation was used to evaluate the data. Chi-square test was used for categorical data. Kappa test was used to compare vaccination status and post-poll vaccination opinions. A p-value of <0.05 was considered significant.

RESULTS

A total of 240 participants were included in the study. The mean age was 65.90±11.56 years (min:31 max:93). The gender ratio was equal. The mean body mass index (BMI) was 28.58±4.59 (min: 15.40 max: 46.29).

Relation between sociodemographic characteristics and the participants' knowledge and opinion on pneumonia and influenza vaccines and their immunization levels are given in detail in **Tables 1** and **2**. The education and income levels were mainly considered as low. Participants were diagnosed with CVD for a median of 7.00 years (min: 1 max:42). 165 patients (68.75%) had a diagnosis of CAD (**Figure 1**). CAD was the leading heart disease, and majority of the participants had at least one chronic disease involving another system besides cardiovascular disease.

Table 1. Relation Between Demographic, Social and Health Status, Vaccination Status and Knowledge of Participants

All participants n=240		Vaccinated for		Knows need for vaccination	
Sex		influenza/	pneumococcus	influenza	pneumococcus
Female	n=120	25.8% (n=31)	10.8% (n=13)	30.8% (n=37)	10.0% (n=12)
Male	n=120	21.7% (n=26)	9.2% (n=11)	34.2% (n=41)	11.7% (n=14)
Education status					
Illiterate	n=13	0% (n=0)	7.7% (n=1)	0% (n=0)	0% (n=0)
Literate	n=49	24.5% (n =12)	10.2% (n=5)	30.6% (n=15)	6.1% (n=3
Primary	n=110	22.7% (n =25)	11.8% (n =13)	31.8% (n =35)	12.7% (n =14)
High School	n=45	15.6% (n=7)	8.9% (n=4)	33.3% (n=15)	11.1% (n=5)
University	n=23	56.5% (n=13)*	4.3% (n=1)	56.5% (n=13)*	17.4% (n=4)
Occupation					
Housewife	n=95	20.0% (n=19)	10.5% (n=10)	26.3% (n=25)	8.4% (n=8)
Public servant	n=15	40.0% (n=6)	6.7% (n=1)	60.0% (n=9)	20.0% (n=3)
Worker	n=9	33.3% (n=3)	11.1% (n=1)	11.1% (n=1)	0.0% (n=0)
Retired	n=81	23.5% (n=19)	8.6% (n=7)	39.5% (n=32)	14.8% (n=12)
Other	n=40	25.0% (n=10)	12.5% (n=5)	27.5% (n=11)	7.5% (n=3)
Smoking habit					
Yes	n=42	21.4% (n=9)	9.5% (n=4)	35.7% (n=15)	9.5% (n=4)
No	n=198	24.2% (n=48)	10.1% (n=20)	31.8% (n=63)	11.1% (n=22)
Alcohol consumption					
Yes	n=9	11.1% (n=1)	0.0% (n=0)	33.3% (n=3)	11.1% (n=1)
No	n =231	24.2% (n=56)	10.4% (n=24)	32.5% (n=75)	10.8% (n=25)
Chronic illness other than CVD					
Yes	n=211	24.6% (n=52)	10.4% (n=22)	33.2% (n=70)	10.0% (n=21)
No	n =29	17.2% (n=5)	6.9% (n=2)	27.6% (n=8)	17.2% (n=5)

CVD: Cardiovascular Disease. *p<0.05 compared to other education status

Table 2. Vaccination and Knowledge Status of the Participants			
All participants N=240			
		n	**%**
Dib you receive an influenza vaccination last year?	Yes	57	23.8
Are you vaccinated regularly for influenza every year?	Yes	54	22.5
How many influenza vaccines have you received in your life?			
	1	33	3.8
	2	30	12.5
	3	15	6.2
	4	54	22.5
	never	108	45
Do you know that heart patients should receive an influenza vaccine?	Yes	78	32.5
Has a doctor or nurse previously suggested you should be vaccinated for influenza?	Yes	94	39.2
Did you ever receive a pneumococcal vaccine?	Yes	24	10
Do you know that heart patients should receive the pneumococcus vaccine?	Yes	26	10.8
Has a doctor or nurse previously suggested you should be vaccinated for pneumonia?	Yes	29	12.1
Have you ever been diagnosed with pneumonia?	Yes	44	18.3
Were you hospitalized for pneumonia?	Yes	28	11.7
If you were vaccinated for pneumonia, which vaccine was it?			
	Polysaccharide	5	2
	Conjugated	0	0
	I don't know	19	8
	I haven't been vaccinated	216	90
Why are you not vaccinated for influenza? (more than one answer)			
	I don't trust it	13	5.4
	I don't believe in its protection.	53	22.1
	I don't need it	35	14.6
	It is expensive	1	0.4
	I didn't know I needed it	84	35
	I received the influenza vaccine	57	23.8
	I don't trust	11	4.6
		n	**%**
Why are you not vaccinated for pneumonia? (more than one answer)			
	I don't believe in its protection.	11	4.6
	It is expensive	35	14.6
	I didn't know I needed it	159	66.2
	I received the pneumonia vaccine	24	10.0
Are you considering getting an influenza vaccine this year	Yes	168	70.0
Are you considering getting a pneumonia vaccine this year?	Yes	151	62.9
Have you visited your family doctor in the last year?	Yes	192	80.0
From what source did you obtain information about vaccines			
	Doctor	124	51.6
	A nurse or other healthcare worker	24	10.0
	Internet or media	23	9.6
	Friends and environment	24	10.0
	I didn't need information	45	18.8

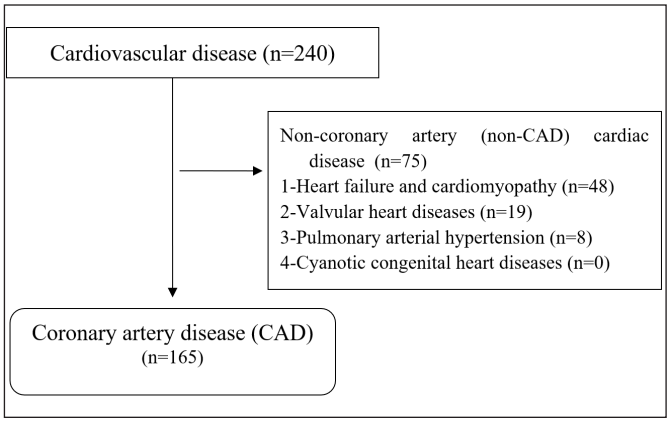

Figure 1. Cardiovascular Disease

Vaccination rates were low in CVD patients. Only 10% of the participants had been vaccinated ever before with pneumococcal vaccine and 27.75% with influenza vaccine last year.

There was no significant difference in vaccination status and knowledge levels when patients were classified and compared in two groups according to under and over 65 years of age, gender, and with or without a family physician examination in the previous year ($p>0.05$). Likewise, when the patients were grouped according to their profession, income, smoking and alcohol habits, and chronic diseases, there was no significant difference in vaccination status and knowledge levels ($p>0.05$).

Education status was of importance for influenza vaccine (**Table 1**). The percentage of university graduates who were vaccinated was significantly higher than all other groups, while illiterate participants had the least percentage ($p<0.05$). There was no significant difference between the other three groups ($p>0.05$). Similarly, higher percentage of university graduates know that they should receive the influenza vaccine than other groups ($p<0.05$). There was no significant difference between the other three groups ($p>0.05$). No relation was found between education level and pneumococcal vaccination. There was no significant difference regarding the participants' education level and the percent of participants who previously received pneumococcal vaccination ($p>0.05$). Similarly, there was no significant difference in education level and the percentage of participants who said they knew cardiovascular disease patients should be vaccinated against pneumococcal pneumonia ($p>0.05$). There was no significant difference between participants with heart failure and others in influenza and pneumococcal vaccination and information levels ($p>0.05$).

When asked if they would get vaccinated after the survey, significant number of participants who were not immunized with influenza or pneumococcal vaccine said they would consider getting vaccinated in the following days ($p<0.05$) (**Table 3**).

Comparison of CAD patients with non-coronary artery (non-CAD) cardiac disease patients are presented in **Table 5**. Greater percentage of participants with CAD had received the influenza vaccine in the last year and were vaccinated for pneumococcal disease and knew they should receive influenza vaccine. Also, significantly more CAD patients were informed by their doctors or nurses that they should receive an influenza vaccine ($p<0.05$).

While eighty percent of participants visited their family physician regularly during the previous year only 61.6% obtained information concerning vaccination from a doctor or a nurse. During the visits higher percentage of participants were suggested by their physician or their nurse to be vaccinated for influenza (39.2%) than pneumococcus pneumonia (12.1%) ($p<0.05$) (**Tables 1,2**). Participants with CAD were informed more than non-CAD patients (**Table 4**).

Table 4: Comparison of coronary artery disease (CAD) patients with non-coronary artery (non-CAD) cardiac disease patients. $p<0.05$ = significant

	All participants N = 240			
	CAD	Non-CAD	X2	p
Did you receive an influenza vaccine last year?				
Yes	27.3% (n = 45)	16.0% (n = 12)	3.812	0.039
Are you vaccinated regularly for influenza every year?				
Yes	4.8% (n = 41)	17.3% (n = 13)	1.670	0.196
Do you know that heart patients should receive an influenza vaccine?				
Yes	35.8% (n = 59)	25.3% (n = 19)	4.757	0.045
Has a doctor or nurse previously suggested you should be vaccinated?				
Yes	46.1% (n = 76)	24.0% (n = 18)	10.532	0.001
Did you ever receive a pneumococcal vaccine?				
Yes	13.3% (n = 22)	2.7% (n = 2)	6.519	0.011
Do you know that heart patients should receive the pneumococcus vaccine?				
Yes	10.9% (n = 18)	10.7% (n = 8)	0.003	0.955

DISCUSSION

This survey findings highlight the knowledge and immunization levels of our CVD patients about pneumococcal and influenza vaccines. Despite high rates of visits to family physicians and nurses, the survey results show low vaccine coverage in CVD patients at risk for bacterial and viral pneumonia. Patients with CAD have higher rates of vaccination against influenza and pneumococcal pneumonia compared to other CVD patients. The survey also displays a relation between the rate of influenza vaccination and the participants' education level.

CVD accounts for approximately 31% of all global mortality (10). Vaccination is a basic approach for the prevention of pneumonia and influenza. Immunization of patients with CVD (particularly congestive heart failure, ischemic heart disease, and stroke) with pneumococcal and influenza vaccine effectively reduces mortality (11). The influenza vaccine is also associated with a reduced risk of primary cardiac arrest and mortality (12). Despite the effectiveness of vaccination in reducing mortality in at-risk patients, vaccination coverage seems to be lower than desirable, even among CVD patients. One of the main reasons for the low rate of immunization in the elderly population and patients

Table 3. Comparison of Pre-Survey Vaccination Status with Post-Survey Tendency for Vaccination. $p<0.05$=significant

		All participants N=240 Are you considering getting an influenza vaccine this year?		
		Yes	No	
Did you receive an influenza vaccine last year?	Yes (n=57)	91.2% (n=52)	8.8% (n=5)	Kappa: 0.167
	No (n=183)	63.4% (n=116)	36.6% (n=67)	p<0.001
		Are you considering getting a pneumonia vaccine?		
		Yes	No	
Did you ever receive a pneumococcal vaccine?	Yes (n=24)	87.5% (n=21)	12.5% (n=3)	Kappa: 0.081
	No (n=216)	60.2% (n=130)	39.8% (n=86)	p:0.009

with CVD may be the low awareness of primary care physicians and nurses. According to a European survey on the pneumococcal vaccine, one of the main drivers for vaccination was healthcare professionals' recommendations. The same survey showed that only 50% of the 1300 primary care physicians were aware of invasive pneumococcal disease. The researchers concluded that to increase vaccination rates, primary care physicians should be aware of patients at risk of pneumococcal infections (13). In Turkey's healthcare system every citizen is assigned to a family physician (general practitioner) and a nurse and the cost is covered by the General Health Insurance Scheme (14). Previously Korkmaz et al. (15) examined the influenza vaccination rates of 818 individuals with chronic diseases in Turkey. Their results showed that only 12.6% of all participants and 19% of participants over the age of 65 were vaccinated annually. The most common reason for not getting vaccinated was not knowing the necessity of the vaccine. The most significant source of knowledge on vaccination was their physician. Kaya et al. (16) conducted a questionnaire on the influence of regular influenza vaccination on heart failure-related hospitalization and mortality rates in patients registered to the Turkish Research Team-HF (TREAT-HF) network. Although the heart failure-related hospitalization rate was lower in vaccinated patients, their results failed to show a difference between vaccinated and non-vaccinated heart failure patients in terms of mortality. Although our results show a high rate (80%) of a visit from our participants to their family physician during the last year; the vaccination rate was low. The pneumococcus and influenza vaccine rates were 10% and 27.75%, respectively, in our study. In the study of Korkmaz et al. (15), which included patients with all chronic diseases, the influenza vaccination rate was 12.6% in all age groups. This rate was twice as high in our study, probably because heart diseases are considered riskier by physicians and patients. Likewise, the vaccination rate was higher in the Korkmaz et al. (15) study in patients over 65 years of age, considered at higher risk for mortality. Examining hospitalization and mortality rates in the heart failure cohort only, Kaya et al. (16) determined the influenza vaccination rate in high-risk patients as 40%. These results imply that vaccination rates increase in diseases with high mortality risk.

Vaccination rates of our patients are well below the desired level of vaccine coverage. CVD patients who are at high risk should be endorsed by their family physician, nurse, or cardiologist to be immunized. We asked the participants if they were directly advised by their family physician or their nurse to be vaccinated against pneumococcal pneumonia and influenza. Their healthcare workers did not inform approximately forty percent of the participants. The present immunization rate points out that even healthcare workers are not well informed concerning the hazards of influenza pneumonia and especially pneumococcal pneumonia in CVD patients.

Educating both the patients and healthcare workers, and setting targets for vaccination coverage rates effectively increases vaccination rates. There were significant increases in vaccination coverage in the elderly population after a single day education program for family physicians in Denizli Province, Turkey (17). Before the education program, the pneumococcal vaccination rate among patients >65 years of age was 11.6%. By the 8th month after the program, vaccination coverage reached 59.5% cumulatively (17). Likewise, a prospective observational study conducted during routine follow-up visits showed a significant relationship between patients' knowledge and vaccination rates for influenza and pneumococcal pneumonia. Vaccination rates were 6.44% for pneumonia and 22% for influenza in elderly patients receiving healthcare at home (18). Even the present survey resulted in a remarkable increase in the number of our participants who decided to get vaccinated this year after being aware of the benefits of immunization during the survey.

Similar to our results, low vaccination rates were reported worldwide. A survey conducted in Japan in 2013 reported a 20.9% overall vaccination rate in persons aged >65 (19). Reports from Japan point out that public subsidies and patients' knowledge about pneumococcal vaccines are important for the lower vaccination rate (19-20). National free routine vaccination programs are effective in increasing vaccination rates (20). According to General Health Insurance legislation in Turkey, both pneumococcal and influenza vaccines are provided free of charge to naïve persons >65 years of age and at-risk patients regardless of age by family physicians. Vaccine administration requires the attention of all physicians and public health organizations (21). Unless all physicians and healthcare workers, most notable cardiologists are aware of the impact of vaccination in reducing mortality in CVD patients, free vaccine programs will not be effective.

Because of the social burden of influenza and pneumococcal disease, implementing standing order programs to improve adult vaccination coverage for these diseases should be a national public health priority. It is of foremost importance to raise the vaccination rates amongst patients with CVD. Advisory Committee on Immunization Practices (ACIP) recommends implementing standing orders programs alone or combined with other effective interventions that can help improve vaccination coverage by institutional providers (5).

CONCLUSION

Although patients with CVD are at increased risk for viral and bacterial pneumonia, our survey displays low vaccine coverage in this high-risk group of patients. Our results display significant relation between healthcare workers suggestions and vaccination rates of CVD patients. We are in the opinion that education programs for both healthcare workers and at-risk patients about adult immunization, and cheaper availability or free vaccination provision will provide higher vaccination rates. Further cohort studies are warranted to provide necessary information on vaccination rates to plan an education program to promote pneumococcal and influenza vaccination among patients with CVD.

Limitations

This study is only a cross-section from Konya Province, central Turkey; still, it demonstrates the need for better education of both the healthcare workers and at-risk patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Selçuk University Faculty of Medicine Clinical Researches Ethics Committee (Date: 12.06.2019, Decision No: 2019/142).

Informed Consent: All patients signed the free and informed consent form.

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The pathobiological harmony between the local pulmonary/bone marrow RAS and its management via tissue-RAS modulating agents in COVID-19

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ABSTRACT

Coronavirus disease 2019 (COVID-19) outbreak, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poses an unprecedented threat to public health and healthcare systems. It presents unusual pathophysiological effects mainly characterized by immune-inflammatory response and prothrombotic state causing acute respiratory distress syndrome and multiple organ failure. SARS-CoV-2 enters target cells after binding to the angiotensin-converting enzyme 2 (ACE2) receptor and therefore has a direct effect on the renin-angiotensin system (RAS). Apart from affecting numerous organs including lungs, heart, gastrointestinal system, spleen, brain and kidneys, the spike protein of SARS-CoV-2 could attack hematopoietic stem cells and hematopoietic progenitor cells in bone marrow (BM) microenvironment together with the precursor and mature blood cells. Within this hematopoietic viral spread context, it is crucial to search the clinicopathological correlations of COVID-19 in order to develop specific potential therapeutics against pleiotropic SARS-CoV-2 actions. Therefore, pharmacological disruption of the pathological cross-talk of local BM RAS and pulmonary RAS via administration of the tissue-RAS modulating agents such as soluble ACE2, angiotensin (1-7), TXA127 and MAS receptor agonists may prevent the clinical progression of the COVID-19 syndrome via reducing the hematopoietic virus propagation and systemic multi-organ spread.

Keywords: COVID-19, SARS-CoV-2, ACE2, renin-angiotensin system, bone marrow

INTRODUCTION

The genesis of immunoinflammatory prothrombotic COVID-19 syndrome takes place following the infection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1,2). COVID-19 can affect multiple organ systems, and in particular, its impact on the respiratory tract has led to an increase in morbidity and mortality worldwide (3). Systemic COVID-19 syndrome could affect numerous organs including lungs, heart, gastrointestinal (GI) system, brain, kidneys, and bone marrow (BM) with the exaggerated or disproportionate immunological activation. SARS-CoV-2 virus or its spike protein attacks on hematopoietic stem cells (HSC), hematopoietic progenitor cells (HPC), together with the precursor and mature blood cells (4-6). Within this hematopoietic viral spread context, it is crucial to search the clinicopathological correlations of COVID-19 in order to develop specific potential therapeutics against pleiotropic SARS-CoV-2 actions (2,4,7).

COVID-19 syndrome has three clinicopathological courses comprising initial, propagating and complicating phase (8). Each of these phases includes unique characteristics in relation to distinct immunogenomic mechanisms affecting critical tissue-based renin-angiotensin system (RAS) genes (9). The circulating RAS is usually considered as an endocrine system, however there are also numerous local tissue RASs within many organs, such as the BM, lung, kidney, heart, pancreas, brain, liver, GI tract and muscles (10,11). Being an important component of RAS, angiotensin-converting enzyme 2 (ACE2) which is a 40 kb gene positioned on chromosome Xp22 counteracts the adverse effects

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of angiotensin II. The ACE2 enzyme is found in high levels especially in lung alveolar tissue, nasopharyngeal tissue, enterocytes, vascular tissue, nervous tissue, and the kidney, nearly all points of potential viral entry for SARS-CoVID-2 (12). ACE2 transcription is enhanced during the initial phase of COVID-19. ACE2 molecule is the critical receptor of SARS-coronaviruses to enter the target cells (13). SARS-CoV-2 entry to human cells requires binding to the ACE2 receptor and utilizing a spike protein (S) for attachment. The viral S protein must be primed by transmembrane protease 2 (TMPRSS2) to facilitate interaction with ACE2 receptor and the subsequent fusion of viral and cellular membranes (14). ACE2 receptor is expressed on the surface of hematopoietic stem/ progenitor cells within the context of local BM RAS, which represents a target for the SARS-CoV-2 attack on BM hematopoiesis (5).

This review aims to demonstrate the pathobiological harmony between local pulmonary RAS and tissue hematopoietic BM RAS during the immunogenomic progression of the multi-systemic COVID-19 syndrome. Furthermore, the possible role of RAS modulating agents such as soluble ACE2, angiotensin (1-7), TXA127 and MAS receptor agonists will also be discusses as an alternative treatment alternatives against COVID-19 in order to reduce hematopoietic virus propagation and systemic spread.

Local Pulmonary RAS and COVID-19

Local tissue-based RAS significantly impacts on the injury/repair response within distinct organ/tissue systems. In this context, local pulmonary RAS is an already established regulator of the lung epithelial and endothelial cells (15,16). Through a combination of the circulating and tissue homeostatic systems, the local tissue RAS affects cellular biological events in the lungs. Local RAS activation within the pulmonary circulation and lung parenchyma could influence the pathogenesis of the lung injury via numerous mechanisms including a substantial increase in vascular permeability, vascular tonus and fibroblast activity, and by decreasing alveolar epithelial cell survival (15). Pulmonary ACE2 plays a pivotal role in protecting the lung from Ang II-AT1R induced inflammation because not only is there a local pulmonary RAS but the lung is also the major site for conversion of inactive Ang I to Ang II (17,18). Therefore, loss of the ACE2, following the binding of SARS-CoV-2, not only exposes the lung tissue epithelium to locally produced Ang II as well as to Ang II produced in the lung from circulating Ang I.

SARS-CoV which is very similar to SARS-CoV-2, and ACE2 interactions have been extensively studied in lung microenvironment (13,19-21). The first genetic proof whether ACE2 is indeed crucial for SARS-CoV infections in vivo was investigated by Kuba et al (13). It has been reported that SARS-CoV infections lead to the ACE2 down-regulation by the binding of the SARS-CoV spike protein to ACE2. Thus, the SARS-CoV family, including SARS-CoV-2, utilizes ACE2 as a critical receptor to enter target host cells. This loss of ACE2 expression resulted in severe acute respiratory failure. Recently in a study by Turk et al. (9) the whole-genome expression data of the lung epithelial cells infected with SARS-CoV for 12, 24, and 48 hours were analyzed, and a total of 15 RAS family and 29 immune genes were found to be highly associated with the exposure time to the virus in the studied groups. This finding strongly suggests the crucial role of the RAS genes on the initiation of the infections caused by coronavirus family members in the lung ecosystem.

Local Bone Marrow RAS, Hematopoietic Stem/ Progenitor Cells and COVID-19

Despite the obvious fact that the local pulmonary RAS is a major victim of the SARS-CoV-2 related immune dysregulation and cytokine release via ACE2, there is scarce evidence depicting the role of BM RAS in the progression of SARS-CoV-2 infection. Locally active RAS in the BM that affects the growth, production, proliferation, and differentiation of hematopoietic cells. The interactions of hematopoietic RAS with other tissue RASs are evident (10,11,22,23). All of the major RAS molecules including renin, angiotensinogen, angiotensin receptors and ACE are located within the BM microenvironment. Being the entry receptor for SARS-CoV-2, ACE2 expression has also been described in several types of cells including hematopoietic stem cells (HSCs) and endothelial progenitor cells (EPCs). In a recent study by Ratajczak et al (14), ACE2 and the entry-facilitating transmembrane protease TMPRSS2 are reported to be expressed on very small CD133+CD34+Lin-CD45- cells in human umbilical cord blood, which can be specified into functional HSCs and EPCs. Moreover, the authors demonstrated that in human small embryonic-like stem cells (VSELs) and HSCs, the interaction of the ACE2 receptor with the SARS-CoV-2 spike protein activates the Nlrp3 inflammasome, which if hyper-activated may cause to cell death by pyroptosis. Therefore, human VSELs residing in adult tissues could be damaged by SARS-CoV-2, with remote effects on tissue/organ regeneration.

Recently, Kucia and coworkers demonstrated that ACE2 receptor is expressed on the surface of Hematopoietic Stem/Progenitor Cells, for SARS-CoV-2 viral entry (5). In their experiments; CD34+CD133+lin–CD45–, CD34+Lin–CD45+ HSCs and CD34+ CD133+ KDR+ CD31+ EPC cells were phenotyped for the expression of ACE2 and the SARS-CoV-2 entry-facilitating transmembrane protease TMPRSS2 at the mRNA level

and by FACS at the protein level. They exposed those cells to the NCP-CoV (2019-nCoV) spike protein. The authors disclosed that the ACE2 receptor and SARS-CoV-2 entry-facilitating transmembrane protease TMPRSS2 are expressed by all types of hematopoietic stem cells (5).

Likewise, Ropa et al. also explored the expression of ACE2 in primitive and mature blood cells using RT-qPCR as well as western blotting that ACE2 is expressed at both the mRNA and protein levels. Moreover, they established that the SARS-CoV-2 spike protein could induce critical cellular alterations in the primitive and mature hematopoietic cells (6). They also observed ACE2 expression on the cell surface of small subpopulations of mature blood immune cells, including 1-2% of T-cells, 2-4% of B-cells, and <1% of NK cells and monocytes. In their study, ACE2 receptor was found to be expressed in 15-60% of the HSCs, 5-50% of the multipotent progenitor cells and 5-15% multipotent lymphoid progenitor cells. The exposure to viral S protein critically affected those cell populations. CD34+ hematopoietic stem cells exhibited 33% less expansion, granulocyte-monocyte progenitors 38% less expansion, and common myeloid progenitors/megakaryocyte-erythroid progenitors 15-30% significantly less expansion when exposed to the SARS-CoV-2 S protein. Thus, SARS-CoV-2 S protein significantly impacts hematopoiesis and myeloid differentiation based on their findings (6).

The data on the implications regarding the viral attack on hematopoiesis and immune response in COVID-19 syndrome are accumulating. Ihlow and colleagues suggested that severe lymphocyte depletion and over-activation of the adaptive immune system commonly observed during the COVID-19 progression are caused by the substantial loss of B-cells associated with viral SARS-CoV-2 burden (24). The authors demonstrated BM hypercellularity with increased myeloid/erythroid ratio, and left shift of erythropoiesis with leukoerythroblastic anemia blood picture. Prothrombotic state is associated with left shift of BM megakaryopoiesis. Their striking finding is that CD20+ B-cell and plasma cell depletion in both BM and spleen of the patients with COVID-19 associated with severe lymphocytopenia. Interestingly, there was a tendency towards higher pulmonary SARS-CoV-2 RNA load in COVID-19 patients with B-cell depletion in their study (24). In accordance with these findings, a recent dual center study from Deutsche COVID-19 OMICS initiative demonstrated elevation of HLA-DRhiCD11chi inflammatory monocytes with an interferon-stimulated gene signature in mild COVID-19, and dysfunctional mature neutrophils, HLA-DRlo monocytes and occurrence of neutrophil precursors as evidence of emergency myelopoiesis in severe COVID-19 patients (25). Thus, severe COVID-19 infection is associated with profound alterations in the myeloid cell compartment providing a detailed insight into the systemic immune response to SARS-CoV-2 infection.

All of those preliminary data cast further focus on the interactions of pulmonary RAS and hematopoietic BM RAS for the proper description of the COVID-19 pathogenesis and clinical management in the bedside. SARS-CoV-2 entry and damage to human cells expressing ACE2 receptor is a key factor in determining the tropism and influencing the severity of infection. Therefore, following the respiratory tract illness by regulating molecular pathways associated with ACE2 receptor in the lungs, the virus might affect local BM RAS, as well. Subsequently, BM hematopoietic stem and progenitor cells can trigger the spreading of the virus to different circulating and local angiotensin systems including local adipose tissue RAS, local cardiac RAS, local pancreatic RAS and local renal RAS. That pathobiologic sequence could further enhance to a multi-systemic immune dysfunction.

The Intimate Relationship Between Local Pulmonary RAS and Bone Marrow RAS

While the BM is the major site of hematopoiesis in the adult, spleen and liver also has important hematopoietic functions in some part of life. Although all of these systems are well known in respect to their roles in hematopoiesis, accumulating evidence suggests that lung is also a primary site for platelet biogenesis and reservoir for resident megakaryocytes (MK) and HPCs (26,27). Lefrançais et al. (27) demonstrated that the lung contains an array of hematopoietic progenitors including short term-HSCs, multipotent progenitors (MPP)2, MPP3/4, and myeloerythroid progenitor populations, which were morphologically indistinguishable from BM primitive HPCs. These cells were found to exist at lower numbers versus the BM and spleen, except for larger short term-HSCs in the lung versus spleen. In accordance with these findings, studies also showed that thrombopoietin (Tpo) stimulation can cause platelet release in the pulmonary vasculature (28). Haznedaroglu et al. (29) explored local Tpo concentrations inside the pulmonary artery and associated vessels in patients with and without pulmonary hypertension (PHT). Tpo concentration inside the pulmonary artery was found to be significantly higher than the Tpo concentrations in the right and left ventricles in patients with PHT. Authors suggested that lung vasculature holding the major regulatory thrombopoietic hormone, Tpo, may be an important place for megakaryocytopoiesis. Based on those data it is reasonable to suggest that BM and lung tissue work in a harmony in hematopoiesis under the direct control of tissue RASs. Meanwhile, pulmonary inflammation is the key event in the lung damage of COVID-19

(30). Localized inflammation in the SARS-CoV-2+ patients can cause decrements in the anticoagulant pathways. Likewise, the inflammatory process leads to the stimulation of endothelial cells expressing tissue factor, secreting molecules. Those pathobiological pro-thrombotic events are driven by the monocytes, platelets, neutrophils, platelet-leukocyte aggregates, all of which are of hematopoietic BM origin.

Preventing immunogenomic progression of COVID-19 syndrome by disrupting pathobiological harmony between local pulmonary RAS and bone marrow RAS with RAS modulating agents

ACE2 catalyses the hydrolysis of angiotensin II to its metabolite, angiotensin 1–7 and angiotensin I to angiotensin 1–9 to protect tissues from several types of injuries (31). It is highly expressed in several human organs and tissues at varying degrees, including lungs (on the surface of type II alveolar epithelial cells), BM, heart (on myocardial cells, coronary vascular endothelial cells, and vascular smooth muscle), kidney (on proximal tubule cells), and small intestine (on the enterocytes) (10,32). Therefore, in addition to efforts to synthesize direct viral inhibitors of replication, using RAS modulating agents for preventing immunogenomic progression of COVID-19 syndrome may be a reasonable option for blocking ACE2 which is the cellular target of SARS-CoV-2 (12). In this context, RAS modulating drugs including soluble ACE2, angiotensin (1-7), TXA127 and MAS receptor agonists might be useful by not only blocking the entry of SARS-CoV-2 into the human cells, as well as by blocking the spreading of the virus from local pulmonary RAS to other local tissue RAS systems including BM.

Soluble ACE2 is a novel compound under development with two actions of mechanisms against SARS-CoV-2. The first mechanism is its capability to bind to viral spike protein and thereby neutralising SARS-CoV-2, and the second action of mechanism is minimising injury to multiple organs, including the lungs, kidneys, and heart, because of unabated RAS hyperactivation and increased angiotensin II concentrations (33,34). Inhibition of SARS-CoV-2 infections in engineered human tissues using soluble ACE2 was first demonstrated by Monteil et al. (35) in which authors showed that soluble ACE2 can significantly block early stages of SARS-CoV-2 infections and clinical-grade recombinant human ACE2 can reduce SARS-CoV-2 infection in cells and in multiple human organoid models. Apart from this experimental study, the safety and efficiency of soluble ACE2 was also provided in a patient with severe COVID-19. In this case report, intravenous delivery of soluble ACE2 demonstrated a significant effect on blocking the systemic spread of the virus from the lung to other organs (34). Angiotensin-(1-7) is another key component of the RAS, which can counter-regulate several deleterious effects caused by angiotensin II. Intravenous infusion of angiotensin 1-7 theoretically activate RAS axis to prevent a further drop in blood pressure and the ACE level will increase and the ACE2 level will decrease owing to the accumulation of angiotensin 1-7 (36,37). This means that providing high levels of angiotensin 1-7 and ACE while reducing inflammatory bradykinin will be protective against ACE2, the entry site of the virus into the host cells (38). The clinical potential of this peptide as a therapeutic agent to treat several pathologies including tumoral conditions by inhibiting the growth of tumor cells and reducing local inflammation and angiogenesis is successfully demonstrated (39,40). Thus, there are also ongoing clinical studies available investigating the safety, efficacy and clinical impact of the infusion of angiotensin-(1-7) in COVID-19 patients with or without respiratory failure requiring mechanical ventilation (www.clinicaltrials.gov; NCT04332666, NCT04401423, NCT04375124). Apart from angiotensin-(1-7), the identification of Mas as a G protein-coupled receptor for Ang-(1-7) undoubtedly contributed to establish Ang-(1-7) as a biologically active component of the RAS (41). Experimental and clinical evidences supported the idea that Mas receptor activation is an important mechanism to fight the deleterious effects triggered by an inappropriate increase in Ang II/AT1 receptor in different diseases as demonstrated by Santos et al. (42). Moreover, the activation of the Mas receptor with Mas analogs can be important additive measures to control the inflammatory response mediated by SARS-CoV-2 (43,44).

CONCLUSION

There is a body of evidence now that demonstrates the critical role of local tissue RAS systems in COVID-19 pathophysiology. Tissue-based RAS genes are important at the initiation of the infections caused by coronavirus family members and may have a strong relationship with the exchange of immune genes in due course following the infection (9). Thus, the spread of the disease to other parts of the body through RAS activation seems to be responsible for the progression of the disease from respiratory viral illness (SARS-CoV-2) to a multisystemic immunoinflammatory pro-thrombotic syndrome. Therefore, it is crucial to acquire a greater knowledge on the biological role of the tissue RASs within different organs and in distinct physiological pathways since the RAS components have the ability of widespread tissue involvement in COVID-19. Among them, the pathobiological interactions of the local pulmonary RAS and tissue-based hematopoietic BM microenvironment RAS together with their pharmacological manipulation seems to be the rational fields for future experimental and clinical research studies.

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