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Advisory Board

Advisory Board

Advisory Board

Advisory Board

Advisory Board

Advisory Board

Contents

Research

167 **Evaluation of Protective Effects of Folinic Acid and Gonadotropin-Releasing Hormone Agonist and Antagonist Against Methotrexate Toxicity in Rats**
Folinik Asit, GnRH Agonist veya Antagonist Tedavilerinin Sıçanlarda Metotreksat Toksisitesi Üzerine Koruyucu Etkilerinin Değerlendirilmesi
Tolga Atakul, Gökalp Öner, Özgür Deniz Turan, Serkan Yaşar Çelik, Mustafa Yılmaz, Hasan Yüksel, Buket Demirci

173 **Effect of Comorbidities and Choice of Fixation on the Onset of Bone Healing Time on Surgically Treated Intertrochanteric Femoral Fractures**
Cerrahi Olarak Tedavi Edilen İntertrokanterik Femur Kırıklarında Eşlik Eden Durumların ve Fiksasyon Seçiminin Kemik İyileşme Süresinin Başlangıcına Etkisi
Alkan Bayrak, Altuğ Duramaz

179 **Comparing Surgical Results of Lower Eyelid Retractor Advancement and Retractor Advancement with Lateral Tarsal Strip for Involutional Entropion**
İnvolusyonel Entropionların Cerrahi Tedavisinde Lateral Kantal Askılama ile Kombine Edilen Alt Kapak Retraktör İlerletilmesi Yapılan Olguların Sadece Retraktör İlerletilmesi Yapılan Olgularla Karşılaştırılması
Ayşe Burcu Dirim, İbrahim Çağrı Türker, Ceylan Uslu Doğan, Sümeyra Keleş Yeşiltaş

184 **Diagnostic Value of Fine-Needle Aspiration Biopsy in Major Salivary Gland Masses**
Majör Tükrük Bezi Kitlelerinde İnce İğne Aspirasyon Biyopsisinin Tanısal Değeri
Filiz Gülüstan, Mehmet Akif Abakay, Ercan Atasoy, Zahide Mine Yazıcı, İbrahim Sayın

190 **Cognitive Dysfunction in Older Patients Undergoing Orthopedic Surgery: Analysis of Demographic, Clinical, and Intraoperative Risk Factors**
Ortopedik Cerrahi Geçiren Yaşlı Hastalarda Bilişsel İşlev Bozukluğu: Demografik, Klinik ve Intraoperatif Risk Faktörlerinin Analizi
Yasemin Tekdöş Şeker, Yaser Pektaş, Deniz Özel Bilgi, Gökhan Sertçakacılar

197 **Spinal Anesthesia in Vaginal Natural Orifice Transluminal Endoscopic Surgery: Experience of 12 Patients**
Vajinal Destekli Doğal Orifis Transluminal Endoskopik Cerrahide Spinal Anestezi: 12 Hasta Deneyimi
Güneş Özlem Yıldız, Gülsüm Oya Hergünsel, Gökhan Sertçakacılar, Aysun Fendal Tunca, Cihan Kaya, Zafer Çukurova

203 **Management of Phyllodes Tumors of the Breast: Our Clinical Experience**
Memenin Filloid Tümörlerinin Yönetimi ve Klinik Deneyimlerimiz
Osman Erdoğan, Alper Parlakgümüş, Uğur Topal, Zeynel Abidin Taş, Oktay İrkorucu

209 **The Relationship Between Warfarin Resistance and CYP2C9*2 and CYP2C9*3 Variatıons**
Varfarin Direnci CYP2C9*2 ve CYP2C9*3 Varyasyonlarıyla İlişkili Olabilir
Deniz Kıraç, Aysun Erdem Yaman, Tansu Doran, Elif Çiğdem Altunok

214 **Does High-Density Lipoprotein Have a Role in Dysregulated Host Response in Sepsis?**
Sepsisteki Disregüle Konak Yanıtında HDL'nin Rolü Var Mı?
Sinan Aşar, Mehmet Süleyman Sabaz, Evrim Kucur Tülübaş, Özlem Acicbe, Zafer Çukurova

Advisory Board

221 **The Roles of Thwaites' and Marais' Diagnostic Scoring Indexes and a Clinical Prediction Model in the Diagnosis of Tuberculous Meningitis**
Tüberküloz Menenjit Tanısında Thwaites ve Marais'in Tanı Skor İndeksleri ve Klinik Bir Tahmin Modelinin Rolü
Şemsi Nur Karabela, Gülten Ünlü, Sevtap Şenoğlu, Esra Canbolat Ünlü, Ramazan Korkusuz, Kadriye Kart Yaşar

227 **Pulmonary to Systemic Flow Ratio in Precapillary Pulmonary Hypertension Without Left-to-Right Shunts: A Prognostic Implication of the Fick Method**
Soldan Sağa Şantı Olmayan Prekapiller Pulmoner Hipertansiyonda Pulmoner/Sistemik Akım Oranı: Fick Yönteminin Prognostik Bir Sonucu
Bihter Şentürk, Bahri Akdeniz, Mehmet Birhan Yılmaz, Ebru Özpelit, Dilek Sezgin, Buse Özcan Kahraman, Burak Acar, Kemal Can Tertemiz, Merih Birlik, Can Sevinç

233 **Identification of Factors Associated with Prolonged Stay in the Intensive Care Unit**
Yoğun Bakım Ünitesinde Uzun Süre Kalış ile İlişkili Faktörlerin Belirlenmesi
Mehmet Süleyman Sabaz, Sinan Aşar

243 **Do Histopathological Findings of Kidney Biopsies Performed in Patients with Acute Kidney Injury Differ with Age?**
Akut Böbrek Hasarı Nedeniyle Yapılan Böbrek Biyopsilerinin Histopatolojik Bulguları Yaşla Değişir Mi?
Arzu Özdemir, Fatma Sibel Koçak Yücel, Kamile Gülçin Eken, Mürvet Yılmaz

248 **Evaluation of Serum Amyloid A Levels in Celiac Disease**
Çölyak Hastalığında Serum Amiloid A Düzeylerinin Değerlendirilmesi
Refika Yorulmaz Çakmak, Nergiz Ekmen, Mustafa Kavutcu, Özlem Gülbahar, Mehmet Arhan

254 **Intensive Care Unit Admission Parameters for Patients with COVID-19**
COVID-19 Hastalarında Yoğun Bakım Kabulünü Belirleyen Parametreler
Mehmet Süleyman Sabaz, Fatma Özdemir, Oğuz Özakin, Ülkü Aygen Türkmen, Veysel Dinç

262 **The Effect of PEEP Adjusted by Automatic Recruitment Maneuver on Total and Dynamic Mechanical Power in Bariatric Surgery**
Bariyatrik Cerrahide Otomatik Rekruitment Manevrası ile Ayarlanan PEEP Düzeyinin Total ve Dinamik Güç Üzerine Etkisi
Sinan Aşar, Evrim Kucur Tülübaş, Mehmet Süleyman Sabaz, Özlem Acicbe

268 **Clinical Profile and Predictors of Severe Malaria: A Study from Two Tertiary Care Centers in İstanbul**
Şiddetli Sıtmanın Klinik Profili ve Göstergeleri: İstanbul'da İki Üçüncü Basamak Hastaneden Çalışma
Ramazan Korkusuz, Şemsi Nur Karabela, Nagehan Didem Sarı, Sevtap Şenoğlu, Esra Canbolat Ünlü, Kadriye Kart Yaşar

274 **Low-Density Lipoprotein Cholesterol Levels and Disease Severity in COVID-19 Pneumonia**
COVID-19 Pnömonisinde Düşük Yoğunluklu Lipoprotein Kolesterol Düzeyleri ve Hastalık Şiddeti İlişkisi
Müge Bilge, Işıl Kibar Akıllı

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Research

Evaluation of Protective Effects of Folinic Acid and Gonadotropin-Releasing Hormone Agonist and Antagonist Against Methotrexate Toxicity in Rats

Folinik Asit, GnRH Agonist veya Antagonist Tedavilerinin Sıçanlarda Metotreksat Toksisitesi Üzerine Koruyucu Etkilerinin Değerlendirilmesi

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ABSTRACT

Objective: A single dose of the folic acid antagonist methotrexate (MTX) is commonly used in ectopic pregnancy. However, the safety of MTX therapy on the ovarian reserve is still controversial. This study aimed to evaluate the use of folinic acid, a gonadotropin-releasing hormone (GnRH) antagonist, and a GnRH agonist on single-dose MTX-induced rat ovarian toxicity.

Methods: A total of 40 Wistar albino rats were randomly divided into five equal groups. Then, all rats were administered MTX intramuscularly. While only physiological saline solution was administered to the control group, only MTX was administered to the MTX group. After 24 h of MTX administration, the MTX + leucovorin group received leucovorin. The MTX + GnRHa group received triptorelin acetate and MTX simultaneously. The MTX + GnRHant group received cetrorelix acetate with MTX.

Results: The anti-mullerian hormone (AMH) level was similar in the control, MTX, MTX + GnRHa, MTX + GnRHant, and MTX + folinic acid groups. The number of primordial follicles was lower in the MTX group than in the control group (p=0.004), whereas this number in the other groups was similar to that in the control group. AMH levels in the MTX + folinic acid (p=0.001) and MTX + GnRHa (p=0.002) groups were higher than those in the MTX group. The number of primordial, primary, secondary and tertiary follicles was significantly higher in the MTX + folinic acid, MTX + GnRHa, and MTX + GnRHant groups than that in the MTX group.

Conclusions: To the best of our knowledge, this is the first experimental study that can be benefited to minimize the damaging impacts of single-dose MTX administration on ovarian reserve and AMH levels. Although the negative impact of single-dose MTX on ovarian reserve is known, our results show that this effect can be minimized by the concurrent administration of GnRHa, GnRHant, or folinic acid. The findings of the present study need to be confirmed with more extensive laboratory studies as well as with randomized controlled clinical studies

Keywords: Methotrexate, ovarian reserve, anti-mullerian hormone, gonadotropin-releasing hormone, folinic acid

ÖZ

Amaç: Ektopik gebeliğin tedavisinde sıklıkla tek bir doz folik asit antagonisti olan metotreksat (MTX) kullanılır. Bununla birlikte, over rezervi üzerinde MTX tedavisinin güvenliği tartışmalıdır. Bu çalışmanın amacı, tek doz MTX kaynaklı sıçan over toksisitesi üzerinde folinik asit, gonadotropin salgılatıcı hormon (GnRH) agonisti ve GnRH antagonisti etkilerini değerlendirmektir.

Gereç ve Yöntem: Toplam 40 Wistar albino sıçan, rastgele beş eşit gruba ayrıldı. Kontrol grubu dışındaki tüm hayvanlara kas içine MTX enjeksiyonu uygulandı. Kontrol grubuna sadece fizyolojik salin çözeltisi verilirken, MTX grubuna MTX dışında bir tedavi uygulanmadı. MTX +

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lökovorin grubuna MTX uygulamasının ardından, 24 saat sonra lökovorin tedavisi uygulandı. MTX + GnRHa grubuna MTX ile eşzamanlı olarak triptorelin asetat ve MTX tedavisi uygulandı. MTX + GnRHant grubuna MTX ile setrorelix asetat tedavisi verildi.

Bulgular: Anti-müllerian hormon (AMH) düzeyi açısından kontrol grubu ile MTX, MTX + GnRHa, MTX + GnRHant ve MTX + folinik asit grupları benzer bulundu. Primordiyal folikül sayısı açısından ise sadece MTX grubunun folikül sayısının kontrol grubuna göre daha düşük olduğu (p=0,004), diğer grupların ise kontrol grubu ile benzer olduğu bulundu. MTX + folinik asit (p=0,001) ve MTX + GnRHa'nın (p=0,002) AMH düzeyinin sadece MTX ile tedavi edilen gruba göre daha yüksek olduğu bulundu. Primordiyal, primer, sekonder ve tersiyer folikül sayıları MTX'in yanına folinik asit veya GnRHa veya GnRHant ilave edilen gruplarda, sadece MTX ile tedavi edilen gruba göre anlamlı ölçüde daha yüksekti.

Sonuç: Bildiğimiz kadarıyla, bu çalışma, tek doz MTX uygulamasının over rezervi ve AMH seviyeleri üzerindeki zararlı etkilerini azaltmak için kullanılabilecek ilk deneysel araştırmadır. Tek doz MTX uygulamasının yumurtalık rezervi üzerindeki olumsuz etkisi bilinmesine rağmen, bu çalışmanın sonuçları bu etkinin eşzamanlı GnRHa, GnRHant veya folinik asit uygulamasıyla azaltılabileceğini göstermektedir. Bu sonuçları doğrulamak için, daha kapsamlı laboratuvar çalışmalarına ve randomize kontrollü klinik araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Metotreksat, yumurtalık rezervi, anti-müllerian hormon, gonadotropin salgılayan hormon, folinik asit

INTRODUCTION

Along with the early diagnosis, various chemotherapy options have been developed to treat ectopic pregnancies without surgical intervention. The most widely used agent in ectopic pregnancies is methotrexate (MTX) (1). With increasing experience in medical treatment, single-dose regimens of MTX have emerged to simplify treatment, improve compliance, and reduce side effects and costs. (2,3).

Serum anti-mullerian hormone (AMH) correlates best with the number of overleaf follicles. AMH, a member of the TGF-β family, is secreted during the development of the primary follicle and can be measured at any time. Its level does not alter much during the menstrual cycle and is almost similar to FSH level. AMH also plays a significant role in maintaining the ovarian reserve by inhibiting the development of primordial follicles (4). In recent years, it has been used clinically for predicting fertility and is frequently used as an adjunct to assisted-reproduction methods (5,6).

Several studies assessing the safety of single-dose MTX have reported controversial results (7,8). Notably, single-dose MTX has a harmful effect on ovaries (9-11). In a study using cisplatin, another drug widely used in cancer treatment, gonadotropin-releasing hormone (GnRH) agonists and antagonists were used to protect against cisplatin-induced ovarian toxicity (12). GnRH is a short-acting decapeptide secreted by the hypothalamus that can cause the pituitary gland to secrete luteinizing hormone and FSH (13). GnRHa is formed by a modification of amino acids 6 and 10 of GnRH, and it has higher biological activity than natural GnRH. It has been reported that the suppression of the female gonadal axis by GnRHa can minimize the impairment to primordial follicles induced by chemotherapeutic drugs (14). In a prospective experimental study conducted on animals, GnRHa preserved the ovarian reserve by reducing the number of primordial follicles lost during chemotherapy (15). Studies have also reported that GnRH agonists cause a hypogonadotropic condition, which lowers the amount of primitive follicles entering the differentiation stage, which are more vulnerable to chemotherapy (16). As per a hypothesis, the predicted rise in FSH concentration can be inhibited by GnRHa, and thus saving follicles from accelerated atresia (17). However, the use of GnRHa is limited owing to the initial aggravation impact of its therapy, which occurs in the first week (17). The protective impact of GnRHant on the ovarian function is manifested through a stronger and faster inhibition of the female gonadal axis (14,18).

The current study aimed to evaluate the histopathological and biochemical effects of a single dose of folinic acid, GnRH agonist, or GnRH antagonist in reducing the harmful effects of MTX on rat ovaries.

METHODS

This experimental study was approved by the Aydın Adnan Menderes University Animal Ethics Committee and followed the principles of good clinical practice and other international guidelines during the entire study period (approval number: HADYEK 64583101/2015/135).

Animals

In total, 40 female rats (220-270 g) were housed in a room controlled at 24±2 °C under a 12-h dark-light cycle and sufficient ventilation. Food and water were freely available to the animals. All rats had regular menstrual cycles and similar features.

Drugs

The 40 rats were randomly grouped into five groups (n=8 each). The control group was only administered 0.1 mL saline intramuscularly. In previous studies assessing single-dose MTX, a dose of 50 mg/m^2 was preferred; accordingly, we used 50 mg/m^2 of MTX in our study (Methotrexate®, Koçak Farma, İstanbul, Turkey) (19-21). The MTX group received no additional therapy; however, the MTX + leucovorin group received 0.1 mg/kg folinic acid (leucovorin) intramuscularly

24 h after MTX administration. The MTX + GnRHa group was administered the GnRH agonist triptorelin acetate (Decapeptyl® Depot 3.75 mg, Ferring Ilac San. ve Tic. Ltd. Sti, İstanbul, Turkey) subcutaneously at a dose of 1 mg/kg simultaneously with MTX. The MTX + GnRHant group was subcutaneously administered the GnRH antagonist cetrorelix (Cetrotide® 0.25 mg, Merck Serono, İstanbul, Turkey) at a dose of 1 mg/kg concurrent with MTX (9).

To eliminate any direct toxic and sclerosing effects on the ovaries, drugs were not administered intraperitoneally. As a result, treatment effect was assessed using the parenteral route. Following drug administration, a regular cycle schedule for MTX half-life and anti-metabolic effect was devised. Intracardiac blood samples were collected after a 1-month follow-up, and cervical dislocation and oophorectomy were conducted for rats.

AMH Measurement

The serum AMH level was determined using enzyme-linked immunosorbent assay (ELISA) kits (rat ELISA kit, 201-11-1246, Baoshan District, Shanghai, Chinese). These kits had a sensitivity of 0.101 ng/mL with a coefficient variation of <5%. The processes were conducted in accordance with the manufacturer's guideline.

Histopathology

Rat ovaries were extracted bilaterally and placed in a buffered 10% formaldehyde solution overnight and then fixed in paraffin blocks. Subsequently, sections of 5 µm thickness were cut and were then deparaffinized and rehydrated before staining with hematoxylin and eosin. Two expert histopathologists categorized oocyte-containing follicles according to developmental phases, and antral follicles in 12 regions of each slice were counted. Follicles were counted and classified in line with the criteria developed by Oktay et al. (22).

Follicles were classified as follows: primordial, primary, secondary, and tertiary (mature) follicles. A primordial follicle is characterized by the presence of a flattened granulosa layer surrounding some or all of the oocyte. A primary follicle is characterized by a single layer of cuboidal granulosa cells covering the oocyte periphery. The secondary follicle has a layer of multiple cuboidal granulosa with a few or non-selectable antral gaps. The oocyte is contiguous with a single large antral area in the tertiary follicle.

Statistical Analysis

All statistical analyses were performed using SPSS v22.0. Normality check was conducted using the Shapiro-Wilk test. Continuous variables were expressed as means ± standard deviations, whereas categorical variables were expressed as frequencies and percentages. The ANOVA test was performed to compare the means of groups. Fischer and Games-Howell tests were used in pairwise comparisons. Results with two-tailed $p<0.05$ were considered significant.

RESULTS

Assessment of Plasma AMH Levels

AMH levels did not differ among the groups. The AMH level in MTX + leucovorin group ($p=0.001$) and MTX + triptorelin group ($p=0.002$) was higher than that in the MTX group. However, this level in the MTX + cetrorelix group was lower than that in the MTX + leucovorin ($p=0.018$) and MTX + triptorelin ($p=0.026$) groups. In terms of AMH levels, no statistically significant difference was found in the comparisons of other groups (Table 1, Figure 1).

Evaluation of Ovarian Follicle Count

The number of primordial ($p=0.004$), primary ($p<0.001$), secondary ($p<0.001$), and tertiary follicle ($p<0.001$) in the MTX group was lower than that in the control group. The number of primary ($p=0.033$) and tertiary follicles ($p=0.025$) in the MTX + leucovorin group was lower than that in the control group. The number of primary ($p=0.009$), secondary ($p=0.005$), and tertiary ($p<0.001$) follicles in the MTX + cetrorelix group was lower than that in the control group. Primary ($p=0.004$), secondary ($p<0.001$), and tertiary ($p<0.001$) follicle counts in the MTX + triptorelin group were lower than those in the control group. Primordial ($p=0.011$), primary ($p<0.001$), secondary ($p<0.001$), and tertiary ($p<0.001$) follicle counts were lower in the MTX group than in the MTX + leucovorin group. Primordial ($p=0.001$), primary ($p=0.002$), secondary ($p=0.001$), and tertiary ($p=0.001$) follicle counts in the MTX group were lower than those in the MTX + cetrorelix group. In the MTX + triptorelin group, the number of primordial ($p=0.032$), primary ($p=0.009$), secondary ($p=0.009$), and tertiary ($p=0.002$) follicles was lower than that in the MTX group. The tertiary follicle count was higher in the MTX + leucovorin group than in the MTX + cetrorelix group ($p=0.019$). Secondary ($p=0.007$) and tertiary ($p=0.011$) follicle counts were higher in the MTX + leucovorin group than in the MTX + triptorelin group (Table 1, Figure 1).

DISCUSSION

MTX reverses and blocks the dihydrofolate reductase enzyme, which prevents the conversion of folic acid to tetrahydrofolic acid. Inhibiting the formation of tetrafolate preventing the synthesis of purine bases (adenine and

Table 1. Evaluation results of all rat groups

Groups			Anti-mullerian hormone	Primordial follicle	Primary follicle	Secondary follicle	Tertiary follicle
			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Control	**(n=8)**	**=I**	4.39 (1.34)	65.63 (10.13)	57.63 (8.50)	49.38 (7.07)	17.63 (2.50)
MTX	**(n=8)**	**=II**	3.53 (1.18)	45.50 (3.51)	34.50 (3.55)	33.63 (4.03)	8.75 (1.49)
MTX-leucovorin	**(n=8)**	**=III**	5.45 (1.38)	55.38 (5.78)	45.75 (4.17)	46.88 (4.19)	15.13 (2.53)
MTX-cetrorelix	**(n=8)**	**=IV**	4.10 (0.67)	54.00 (3.16)	42.88 (3.14)	42.38 (4.27)	12.50 (2.51)
MTX-triptorelin	**(n=8)**	**=V**	5.36 (0.54)	54.13 (6.06)	40.75 (2.25)	40.13 (2.70)	12.25 (1.28)
p			**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Pairwise comparison	III		**>0.05**	**0.004**	**<0.001**	**<0.001**	**<0.001**
	I-III		>0.05	>0.05	**0.033**	>0.05	**0.025**
	I-IV		>0.05	>0.05	**0.009**	**0.005**	**<0.001**
	I-V		>0.05	>0.05	**0.004**	**<0.001**	**<0.001**
	II-III		**0.001**	**0.011**	**<0.001**	**<0.001**	**<0.001**
	II-IV		**>0.05**	**0.001**	**0.002**	**0.001**	**0.001**
	II-V		**0.002**	**0.032**	**0.009**	**0.009**	**0.002**
	III-IV		**0.018**	>0.05	>0.05	>0.05	**0.019**
	III-V		**>0.05**	**>0.05**	>0.05	**0.007**	**0.011**
	IV-V		**0.026**	**>0.05**	>0.05	>0.05	**>0.05**

MTX: Methotrexate, SD: Standard deviation, Statistically significant are indicated in bold

guanine) that are necessary for DNA, RNA, and ATP synthesis and protein synthesis disruption. This consequently limits the conversion to deoxyuridylatedimidilate, which is necessary for DNA synthesis and cell regeneration (23). Single-dose MTX is the gold standard treatment for unruptured ectopic pregnancy. The success rate of single-dose MTX in ectopic pregnancy varies between 64% and 94% (24). Although this treatment option significantly reduces the need for surgical intervention, especially in patients with early onset and without obvious clinical symptoms, it has recently been reported to have adverse effects on the ovaries and to reduce ovarian reserve (10,11). In this study, we aimed to assess the protective effects of folinic acid, GnRHa, and GnRHant against the harmful effects of MTX on the ovarian reserve.

In this study, AMH levels were measured and follicles were enumerated to accurately identify the ovarian reserve and to histopathologically distribute and count the follicular series. There was no significant difference between the control group and other groups in terms of AMH levels. The number of primordial follicles of the MTX group was lower than that of the control group, whereas this number in the other groups was similar to that in the control group.

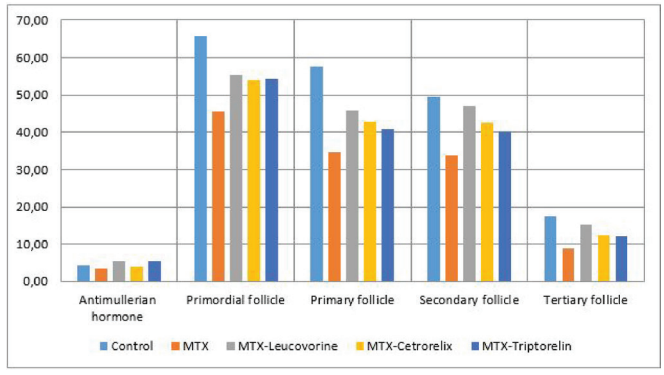

Figure 1. In the control group, there is a significant increase in the number of all follicles compared with in the other groups. The MTX group has a significantly decreased number of all follicular series and AMH levels compared with the other groups. All follicle groups and AMH levels were normal in the MTX + leucovorin group compared to other groups

MTX: Methotrexate

In addition, we found that the AMH level was higher in the folinic acid and GnRHa groups treated with MTX than that in the MTX group. In addition, the number of primordial, primary, secondary, and tertiary follicles were significantly higher in the groups treated with MTX and folinic acid, GnRHa, or GnRHant than the group treated with only MTX.

Recent studies on the use of MTX for ectopic pregnancy have shown that MTX treatment does not change the oocyte count in women undergoing ovarian stimulation for assisted-reproductive technology. However, it is interesting that very few of these studies evaluated antral follicle count (7,25). In a study evaluating 7 ectopic pregnancy cases of 329 cases who received assisted-reproductive technology treatment, MTX used for ectopic pregnancy did not affect the number of follicles and fertility (26). In the study by Uyar et al. (9) single-dose MTX treatment in ectopic pregnancy cases did not have a negative effect on the ovarian reserve, as assessed by basal FSH, E2, basal antral follicle number, and ovarian volume. Shirazi et al. (27) reported no adverse effect of MTX on the ovarian reserve as assessed by AMH level. Oriol et al. (28) reported that single dose-MTX did not have a negative effect on the ovarian reserve, since AMH levels did not differ in patients with ectopic pregnancy. A meta-analysis evaluating ectopic pregnancy cases reported no significant difference in oocyte counts before and after MTX treatment (29). In another study conducted on rats, MTX significantly reduced the AMH level and the total number of follicles (30). In our study, AMH level, one of the ovarian reserve markers, was found to be similar in the MTX group and the control group; however, the follicle counts in the MTX group were lower than those in the control group. It should be noted that single-dose MTX, which is used in ectopic pregnancy and is a part of routine practice, is not as safe as previously thought.

Yuan et al. (31) evaluated the effects of cyclophosphamide on the ovaries of rats treated with GnRHa and found that FSH, estradiol, and follicle numbers were significantly higher in the GnRHa group. Moreover, GnRHa reduced ovarian damage after cyclophosphamide treatment (31). In the study conducted by Peng et al. (32) in rats, GnRHa was effective in preventing ovarian function damage caused by cyclophosphamide, but GnRHant was not effective. In another study conducted by Parlakgumus et al. (33) in rats, neither GnRHa nor GnRHant provided protection against cyclophosphamide-induced damage and GnRHant reduced the number of primordial follicles. In our study, GnRHa and GnRHant had a protective effect on the ovarian reserve affected by MTX in terms of follicle numbers; however, only GnRHa was protective in terms of AMH. The AMH level in the GnRHa group was higher than that in the GnRHant group. Therefore, it can be said that GnRHa is a slightly better than MTX in protecting against the harmful effects on the ovary.

Li et al. (12) reported that the combined use of GnRH agonists and antagonists helped reduce cisplatin-induced ovarian toxicity in rats. In our study, the effect of GnRHa in combination with GnRHant was not assessed. This is one of the limitations of the research. As a result of not assessing the combining the effects of GnRHa and GnRHant, the expected protective effect may have been reduced owing to the exacerbation effect.

Study Limitations

Another important limitation of this study is that the long-term effects of single-dose MTX on ovarian reserve were not evaluated. Despite these limitations, this work is, to our knowledge, the pioneer study of agents that can be used to reduce the detrimental effects MTX on follicle number and AMH levels.

Based on our analyzes on the negative effects on ovarian reserve following MTX treatment, it can be said that folinic acid, GnRHa, and GnRHant provide protection against altered follicle numbers. Folinic acid and GnRHa are better as they significant increase the AMH level.

CONCLUSION

Our results may help guide treatment with single dose-MTX. The results found can be confirmed by more extensive laboratory studies and randomized controlled trials.

ETHICS

Ethics Committee Approval: This experimental study was approved by the Aydın Adnan Menderes University Ethics Committee and followed the principles of good clinical practice and other international guidelines during the entire study period (approval number: HADYEK 64583101/2015/135).

Informed Consent: Not applicable.

Authorship Contributions

Surgical and Medical Practices: T.A., G.Ö., Ö.D.T., Concept: T.A., S.Y.Ç., M.Y., Design: T.A., B.D., Data Collection or Processing: G.Ö., Ö.D.T., S.Y.Ç., Analysis or Interpretation: T.A., M.Y., H.Y., Literature Search: T.A., G.Ö., S.Y.Ç., Writing: T.A., G.Ö., Ö.D.T., S.Y.Ç., M.Y., H.S.Y., B.D.

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Research

Effect of Comorbidities and Choice of Fixation on the Onset of Bone Healing Time on Surgically Treated Intertrochanteric Femoral Fractures

Cerrahi Olarak Tedavi Edilen İntertrokanterik Femur Kırıklarında Eşlik Eden Durumların ve Fiksasyon Seçiminin Kemik İyileşme Süresinin Başlangıcına Etkisi

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ABSTRACT

Objective: This study aimed to evaluate whether comorbidities affect the onset of bone healing time (BHT) in surgically treated intertrochanteric femoral fractures (IFFs).

Methods: The study comprised 55 patients (12 male and 43 female) who underwent surgical treatment of IFFs. The mean age of patients was 79.29±81.13 years. The fractures were classified according to the AO Classification. Twenty-one patients were treated with dynamic hip screw, 15 with an external fixator, and 19 with proximal femoral nail. Thirty-one patients had comorbidities such as diabetes and hypertension.

Results: Patients were divided into three groups according to the BHT. Group 1 had BHT<30 days (G1), group 2 had BHT 30-60 days (G2), and group 3 had BHT >60 days (G3). There were no statistically significant differences among the groups in terms of age, sex, additional disease, and the fixation method. There were statistically significant differences among the groups in terms of receiving intensive care unit (ICU) treatment. The rates of ICU referral in G3 were significantly higher than those in G1, statistically close to being meaningfully higher than those in G2. Discharge duration was close to being meaningful in patients with more than one comorbidity.

Conclusion: Fixation type, age, and comorbidities did not affect BHT. Patients with more than one comorbidities had long hospitalization time owing to their prolonged preoperative surgical preparation time and postoperative evaluation of comorbidities.

Keywords: Intertrochanteric femoral fractures, fracture healing, fixation method, proximal femoral nail, dynamic hip screw

ÖZ

Amaç: Bu çalışmanın amacı intertrokanterik femur kırıkları (IFK) olan hastaların cerrahi tedavisinde komorbiditelerin ve fiksasyon yöntemlerinin, kaynamaya başlama süresini (KBS) etkileyip etkilemediğini değerlendirmektir.

Gereç ve Yöntem: Çalışmaya IFK nedeniyle cerrahi tedavi uygulanan 55 hasta (12 erkek ve 43 kadın) alındı. Hastaların yaş ortalaması 79,29±81,13 yıl (61,6 ile 91,5 yıl arasında değişmekteydi). Kırıklar AO sınıflamasına göre sınıflandırıldı. Yirmi bir hasta dinamik kalça vidası (DHS) ile tedavi edildi, 15 hasta eksternal fiksatör (EF) ile tedavi edildi, 19 hasta proksimal femoral çivi (PFN) ile tedavi edildi. Otuz bir hastanın diyabet, hipertansiyon vb. gibi ek hastalıkları vardı.

Bulgular: Hastalar kaynamaya başlama süresine göre üç gruba ayrıldı. KBS'si <30 gün grup 1 (G1), 30-60 gün grup 2 (G2),>60 gün grup 3 (G3) olarak değerlendirildi. Gruplar arasında (G1, G2, G3) yaş, cinsiyet, ek hastalık ve fiksasyon yöntemi açısından istatistiksel olarak anlamlı bir fark saptanmadı. Gruplar arasında postoperatif dönemde yoğun bakım ünitesine (YBÜ) refere edilme oranı açısından istatistiksel olarak anlamlı farklar vardı. G3'te YBÜ refere edilme oranları G1'den anlamlı olarak yüksekti, istatistiksel olarak G2'den anlamlılığa yakın yüksekti. Birden fazla komorbiditesi olan hastaların hastanede yatış süresi diğer hastalardan anlamlılığa yakın derecede yüksekti.

Sonuç: Fiksasyon tipi, yaş ve komorbiditelerin KBS'sini etkilemediği gözlenmiştir. Birden fazla komorbiditesi olan hastaların, uzun preoperatif cerrahi hazırlık süresi ve ek hastalıkların postoperatif dönemde kontrol edilmesi nedeniyle hastanede yatma sürelerinin daha uzun olduğu gözlendi.

Anahtar Kelimeler: İntertrokanterik femur kırıkları, kırık iyileşmesi, fiksasyon tekniği, proksimal femoral çivi, kayan kalça vidası

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INTRODUCTION

Intertrochanteric femur fractures (IFFs) are common in elderly patients, especially in post-menopausal women, usually due to low-energy trauma such as simple falls (1). However, these can also occur in young patients following high-energy trauma, such as vehicle injuries (2). In the near future, the geriatric population will probably increase, and the incidence of osteoporotic bone fractures will be seen in orthopedic practice. In 1990, 26% of all hip fractures occurring in Asia were IFFs; however, this incidence is expected to reach 37% in 2025 and 45% in 2050 (3). The goal of treating IFFs is to ensure stable fixation for early mobilization and return to pre-fracture activity levels. Early mobilization is important for preventing complications, such as deep vein thromboembolism and decubitus ulcers, as well as for improving patient functions (4).

Patients with IFFs are at a risk of significant morbidity and high mortality (5,6). In elderly patients, IFFs are usually associated with comorbidities such as diabetes; hypertension; pulmonary, renal, and cardiac conditions (7). Early reduction and stable surgical fixation of these fractures prevent complications such as avascular necrosis and non-union as well as allows early mobilization (8). Comorbidities increase the risk of surgery in these patients.

Previous studies on IFFs usually examined the effect of fixation techniques on aspects such as union, stabilization, and weight-bearing time (7,8). This retrospective study aimed to evaluate whether comorbidities have an effect on the onset of bone healing time after the surgical treatment of IFFs. In addition, we hypothesized that patients with comorbidities have a delayed onset of healing time.

METHODS

All patients who underwent surgical correction for IFFs between January 2014 and January 2015 were retrospectively investigated after the University of Health Sciences Turkey, Bakirköy Dr. Sadi Konuk Training and Research Hospital Approval of the Local Ethics Committee (IRB approval no: 2015/16/10). Data were collected both from patient files and electronic medical records. Age, sex, length of hospital stay, concomitant disease, discharge disposition, intensive care requirement, and fixation type were evaluated based on patient medical records (Table 1). Inclusion criteria were as follows: age >60 years; closed fractures; closed reduced fractures; AO-31-A1, A2, and B3 fracture types; unilateral fractures; anatomic or near anatomic reduction; and no accompanying lower limp fracture. Exclusion criteria were as follows: age <60 years; undergone hemiarthroplasty; pathological fractures; AO-31-A3, B1-2 type fractures; open fractures; accompanying lower limp fracture; non-anatomic reduction; and reoperated patients. Standard preoperative planning was conducted. Radiographs of the pelvis with both hips anteroposterior and the lateral view were obtained to confirm the diagnosis. Dynamic hip screw (DHS), proximal femoral nail (PFN), or external fixation (EF) were the commonly used methods for IFF fixation. Materials for use during surgery were selected according to the surgeons' preference. All three fixation materials are frequently used in our clinic and daily orthopedic practice. No specific selection criteria were used. All IFFs were performed by closed reduction using traction table and C-arm fluoroscopy. The reduction criteria were based on the study by Fogagnolo et al. (9). Patients were mobilized on the postoperative day 2. The PFN group was subjected to full weight-bearing during the early postoperative period. The DHS and EF groups were subjected to only partial weight-bearing. Sutures were removed on the 14th or 15th day. X-rays were obtained in the 2nd week and 1st, 2nd, 3rd, 6th, and 12th month postoperatively. Three

Table 1. Distribution of demographic characteristics

		Min-Max	Mean ± SD
Age (years)		61.6-91.5	79.29±81.13
Hospitalization (days)		2-28	15±6.74
		n	%
Sex	Female	43	78.2
	Male	12	21.8
AO classification	31.A1.2	30	54.5
	31.A1.3	9	16.3
	31.A2.2	10	18.2
	31.B3	6	11
Comorbidities	Hypertension	16	29.1
	Diabetes	7	12.7
	Chronic renal failure	4	7.2
	Cardiac disease	4	7.2
	Cancer	3	5.5
	COPD	2	3.6
	Alzheimer's disease	3	5.5
ASA score	1	4	7.2
	2	45	82
	3	5	9
	4	1	1.8

ASA: American society of anesthesiology, COPD: Chronic obstructive pulmonary disease, SD: Standard deviation, Min-Max: Minumum-maximum

orthopedic surgeons (with at least 10 years' experience in trauma surgery) reviewed preoperative and postoperative anteroposterior and lateral X-ray reports of each patient. The surgeons consensually decided the onset of healing time, according to callus formation on anteroposterior and frog-leg hip X-rays. The union times of IFFs were evaluated radiologically and clinically. The surgeons noted the healing time. The callus formation on three cortices was used for determining healing time. Clinical findings such as joint motion and pain with weight-bearing were collected from patient medical records.

Patients were divided into three groups according to the onset of union time. The postoperative radiographs of patients were evaluated, and the onset of union time was recorded. Radiographic evaluations included callus formation on the fracture side, shortening of the femoral neck length, lateral migration of the helical screw, and cortical thickening of the fracture site. The radiological finding of fracture healing was first observed at <30 days in the first group (G1), 30-60 days in the second group (G2), and >60 days in the third group (G3).

Statistical Analysis

Statistical analysis was performed using the NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA). Many-Whitney U test was performed for descriptive statistical method evaluation (average, standard deviation, median, frequency, ratio, minimum, and maximum) and for comparing non-normally distributed data. Kruskal-Wallis test was used for comparing non-normally distributed quantitative data of three and upper groups. Bonferroni correction Dunn's test for conducted for determining the diversity group. Pearson ki-square test, Fisher-Freeman-Halton test, Fisher's Exact test, and Yates were used for qualitative data comparison. P-values were considered statistically significant when $p<0.01$ and $p<0.05$.

RESULTS

The demographic features of study patients are presented in Table 1. Thirteen patients had one comorbidity and 18 had more than one comorbidity. Twenty-one patients were operated with DHS, 15 with EF, and 19 with PFN. Four patients were referred to the critical care unit. There were no statistically significant differences among the groups (G1, G2, G3) in terms of age, sex, additional disease, and the fixation method (p=0.377, p=0.373, and p=0.792, respectively). Interobserver There were statistically significant differences among the groups in terms of receiving intensive care (p=0.021). According to post-hoc binary comparisons made to identify the group that was responsible for the difference, the rates of intensive care referral in G3 were significantly higher than those in G1 (p=0.026), statistically close to being meaningfully higher than those in G2 (p=0.055; p>0.05), and no difference in G1 and G2 (p=1.000) (Table 2). According to the type of fixation and additional disease, there were no statistically significant differences in the rate of referral to the intensive care unit (ICU) (p=0.183 and p=0.123, respectively) (Table 3). The time interval between the operation to discharge day and ICU referral showed a statistically significant difference in terms of the onset of bone healing (p=0.0021 and p=0.041, respectively) (Table 4). There was no statistically significant relationship of the number of comorbid diseases with the onset of healing and ICU referral. The duration of discharge had close to statistically significant difference between patients who have only one and more than one comorbidities (p=0.053) (Table 5).

Table 2. Post-hoc binary comparisons in terms of intensive care referral and discharge day

	<30 days 30-60 days	<30 days >60 days	30-60 days >60 days
[aa]Discharge day (day)	1.000	0.084	0.048*
[bb]Intensive care referral	1.000	**0.026***	0.055

[aa]Bonferroni corrected dunn's test, [bb]Fisher's Exact test, *p<0.05, statistically significant p values were marked bold

Table 3. The relationship between referral to intensive care unit and comorbidities and types of fixation

		Intensive care		p
		Not referred n (%)	Referred n (%)	
Comorbidities	No	24 (100)	0 (0)	[d]0.123
	Yes	27 (87.1)	4 (12.9)	
Type of fixation	DHS	21 (100)	0 (0)	[b]0.185
	EF	13 (86.7)	2 (13.3)	
	PFN	17 (89.5)	2 (10.5)	

[b]Fisher-Freeman-Halton test, [d]Fisher's Exact test, DHS: Dynamic hip screw, EF: External fixation, PFN: Proximal femoral nail

DISCUSSION

The key question we raised in the introduction of this study was whether there were any effects of comorbidities on the healing IFFs. We hypothesized that a delayed onset of callus formation occurs in patients with comorbidities. In the three groups, there were no statistical differences at the beginning of the callus formation. We did not observe

Table 4. The relationship between the onset of bone healing and the type of fixation duration of intensive care referral and discharge day

<30 days (n=33)		Onset of bone healing			p
		30-60 days (n=13)	>60 days (n=9)		
Type of fixation	DHS	13 (39.4)	7 (53.8)	1 (11.1)	[b]0.332
	EF	8 (24.2)	3 (23.1)	4 (44.4)	
	PFN	12 (36.4)	3 (23.1)	4 (44.4)	
Intensive care unit	Not referred	32 (97.0)	13 (100)	6 (66.7)	**[b]0.021***
	Referred	1 (3.0)	0 (0)	3 (33.3)	
Time interval between operation to discharge day	Min-Max (median)	1-25 (4)	1-10 (5)	0-9 (2)	**[a]0.041***
	Mean ± SD	6.00±6.28	5.23±2.98	2.44±2.79	
Time interval between admission and discharge day	Min-Max (median)	0-28 (12)	2-27 (11)	10-26 (17)	[a]0.102
	Mean ± SD	12.73±6.48	13.69±8.76	17.67±4.64	

[a]Kruskal-Wallis H test, [b]Fisher-Freeman-Halton test, *p<0.05, statistically significant p values were marked bold, DHS: Dynamic hip screw, EF: External fixation, PFN: Proximal femoral nail, SD: Standard deviation, Min-max: Minumum-maximum

Table 5. Comparison of time of bone healing. duration of intensive care, and discharge period according to the count of the comorbid disease

		Count of the comorbid disease		p
		1 disease (n=13)	>1 disease (n=18)	
Beginning of healing (day)	<30 days	7 (53.8)	11 (61.1)	[b]0.423
	30-60 days	2 (15.4)	5 (27.8)	
	>60 days	4 (30.8)	2 (11.1)	
Intensive care	Not referred	10 (76.9)	17 (94.4)	[d]0.284
	Referred	3 (23.1)	1 (5.6)	
Duration of discharge (day)	Min-max (median)	0-9 (3)	2-25 (4)	[e]0.053
	Mean ± SD	3.31±2.81	7.11±6.60	

[b]Fisher-Freeman-Halton test, [d]Fisher's Exact test, [e]Mann-Whitney U test, SD: Standard deviation, Min-max: Minumum-maximum

any effects of additional diseases such as diabetes mellitus, coronary artery disease, and chronic renal failure. These comorbidities affect the duration of hospitalization. There was no statistical relationship between comorbidities and ICU referral, but all patients referred to ICU had comorbidities. Patients who had more than one additional disease had a longer hospital duration than the others. Bennett et al. (10) emphasized that hospitalization time was delayed in older patients who had an additional disease and proximal femoral fracture. The duration of admission time to surgical time is delayed in these patients because they require additional disease management such glucose regulation and waiting for lack of bed in the ICU (2).

DHS has been used for a long time for ensuring stable extra-capsular intertrochanteric femoral fractures (11). Reportedly, DHS has increased the failure rate of unstable fractures and reverse obliquity fractures (12,13). PFN is also used for treating proximal femoral fractures. PFN was developed by AO/ASIF for proximal femoral fractures for preventing gamma nail complications (14). A 6.5 mm anti-rotation hip screw decreases the incidence of implant cut-out, and a smaller diameter and fluting of the tip of the nail reduces the distal forces, which can prevent distal femoral fractures (15). EF is a fast and minimally invasive method for IFF stabilization (16). EF is used for reducing surgery duration and intraoperative bleeding. Edipoğlu et al. (17) reported that EF reduces surgery time and intraoperative bleeding compared to PFN and DHS. DHS, PFN, and EF are minimally invasive methods for fixation of proximal femur fractures, and these methods do not dramatically increase surgical damage (18). In our study, there was no statistical relationship between the onset of callus formation and fixation type. We believe that there was no difference between the onset of union time because the three fixation types provide similar and essential stability for bone healing. In addition, ICU referral was not related to the type of fixation. Patients in the three groups did not differ in terms of age, sex, and mean duration of hospitalization stay. As the three fixation types used are minimally invasive surgical procedures, their effect on the medical status of the patients and the referral of ICU was similar.

Reportedly, comorbidities decreased soft tissue nutrition (19). In our study of older patients with comorbidities, we observed that this decreased soft tissue nutrition did not affect the onset of healing because the hip joint was

covered with sufficiently thick soft tissue and because the fixation types were stable. We noted delayed union in patients who were admitted to the ICU. Patients who were referred to ICU have limitations for mobility, and we believe this causes delayed union. However, we could not observe any relationship between the admission of patients with multiple comorbidities to the ICU. Multiple comorbid diseases result in higher ASA classifications. ASA 3 and 4 patients require longer hospitalization time from admission to surgery (20).

In the current study, patients with comorbidities had longer hospitalization stays. Additional diseases and older age result in long-term preparation of patients, especially before surgery (21,22). In patients with comorbidities, the risk of referral to ICU is increased; similarly, massive blood loss is noted in the postoperative period (22-24). The present study also showed that patients with comorbidities were hospitalized for longer periods due to preoperative preparation and postoperative care period. We consider that a multidisciplinary evaluation is necessary for such patients, which ultimately prolongs hospitalization.

Study Limitations

The limitations of the study were its retrospective design, a relatively small number of patients, and no randomization. In contrast, the strength of the study is in its contribution to the limited number of studies investigating the effect of comorbid diseases on fracture healing with different fixation techniques.

CONCLUSION

There is no relationship between the onset time of union and comorbidities. However, we detected a delay of the onset of union time in patients who were referred to the ICU and noted a longer hospital stay in patients with more than one comorbidities.

ETHICS

Ethics Committee Approval: The study were approved by the Local Ethics Committee of University of Health Sciences Turkey, Bakirköy Dr. Sadi Konuk Training and Research Hospital (IRB approval no: 2015/16/06).

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Authorship Contributions

Surgical and Medical Practices: A.B., A.D., Concept: A.B., A.D., Design: A.B., A.D., Data Collection or Processing: A.B., A.D., Analysis or Interpretation: A.B., A.D., Literature Search: A.B., A.D., Writing: A.B., A.D.

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Research

Comparing Surgical Results of Lower Eyelid Retractor Advancement and Retractor Advancement with Lateral Tarsal Strip for Involutional Entropion

İnvolusyonel Entropionların Cerrahi Tedavisinde Lateral Kantal Askılama ile Kombine Edilen Alt Kapak Retraktör İlerletilmesi Yapılan Olguların Sadece Retraktör İlerletilmesi Yapılan Olgularla Karşılaştırılması

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ABSTRACT

Objective: This study aimed to examine the efficiency of lower eyelid retractor (LER) advancement alone and LER advancement with a lateral tarsal strip (LTS) procedure for involutional lower eyelid entropion treatment.

Methods: In this study, 26 eyelids of 14 patients who underwent LER advancement + LTS (group 1) and 26 eyelids of 24 patients who underwent LER advancement alone (group 2) were retrospectively examined. Patients with or without horizontal laxity were included. The surgery was considered successful with a completely corrected normal eyelid position.

Results: A total of 52 eyelids of 38 patients were included. No relapse was noted in group 1, whereas seven eyelids (26.9%) in group 2 had a relapse, as noted in the 12-month postoperative follow-up examinations (p=0.004). One patient in group 2 had ectropion after 2 months. A significant difference was found between the surgical failure rates of the LER advancement + LTS and LER advancement alone.

Conclusion: LER advancement + LTS was entirely successful. The study emphasized the significance of assessing the horizontal eyelid laxity before surgery to avoid recurrence. Those who underwent LER advancement + LTS, rather than LER advancement alone, had higher rates of successful outcomes.

Keywords: Lower eyelid, involutional entropion, horizontal eyelid laxity, lower eyelid retractor advancement, tarsal strip surgery

ÖZ

Amaç: İnvolusyonel entropion cerrahisinde lateral kantal askılama (LKA) ile birlikte alt kapak retraktör ilerletilmesi yapılan olgularla sadece alt kapak retraktör (AKR) ilerletilmesi yapılan olguların karşılaştırılması amaçlandı.

Gereç ve Yöntem: Bu retrospektif çalışmaya Temmuz 2018-Ağustos 2019 tarihleri arasında alt kapak involusyonel entropionu olan 38 hastanın 52 gözü dahil edildi. On dört hastanın 26 gözüne LKA ile birlikte AKR ilerletilmesi (1. grup); 24 hastanın 26 gözüne ise sadece AKR ilerletilmesi yapıldı (2. grup). Yatay kapak gevşekliği olan veya olmayan involusyonel entropiyonlu hastalar çalışmaya dahil edildi. Ameliyat sonrası normal kapak pozisyonunun elde edilmesi klinik başarı olarak kabul edildi. Fonksiyonel başarı kapak malpozisyonuna bağlı epifora, oküler irritasyon gibi şikayetlerin azalması olarak değerlendirildi.

Bulgular: Hastaların 23'ü erkek, 15'i kadın olup yaş ortalaması 74,4±7,6 idi. Birinci grupta nüks görülmezken, ikinci gruptaki 24 hastanın 7'sinde (%26,9) ameliyat sonrası 12 aylık takiplerde nüks görüldü. İkinci grupta 1 hastada ameliyattan 2 ay sonra ektropion gelişti. İki grup arasında istatistiksel olarak anlamlı fark saptandı.

Sonuç: İkinci gruptaki hastaların hepsinde cerrahi başarı sağlandı. LKA ile kombine edilen retraktör güçlendirmelerinde başarı oranı sadece retraktör cerrahisi yapılanlara göre daha yüksek bulundu. Çalışmamız nüksü önlemek için ameliyattan önce yatay göz kapağı gevşekliğini değerlendirmenin önemini vurguladı.

Anahtar Kelimeler: Alt göz kapağı, involusyonel entropiyon, horizontal göz kapağı gevşekliği, alt göz kapağı retraktörlerinin ilerletilmesi, tarsal şerit cerrahisi

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INTRODUCTION

Involutional entropion is an eyelid malposition frequently seen in older people. Epiphora, corneal irritation, and keratitis are also seen due to ocular surface distress resulting from the contact of eyelashes to the ocular surface (1,2). Horizontal and vertical eyelid laxity, tarsal atrophy, senile orbital fat tissue atrophy, preseptal orbicular muscle laxity, and lower eyelid retractor (LER) weakness lead to this condition. The involutional form of this pathology commonly occurs in the lower eyelid; however, the cicatricial form is commonly seen in the upper eyelid (3-5).

Quickert everting sutures are useful to decrease patient distress until the operation, and primary treatments include Jones retractor advancement, Wies repair with or without eyelid shortening, lateral tarsal strip (LTS), and variations of these procedures (6-12). Patients frequently experience recurrence when an accurate technique or an appropriate combination of operations is not performed. Accurate repositioning of LER and sufficient rectification of horizontal eyelid laxity are required during operation to decrease the recurrence possibility (13-20).

This study assessed the efficiency of LER advancement + LTS compared to LER advancement alone for involutional entropion treatment.

METHODS

This retrospective and comparative study included 52 eyelids of 38 patients with involutional entropion, and it was carried out between July 2018 and August 2019. The study was approved by the Medical Ethics Committee of University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, Turkey. Horizontal lower eyelid laxity before surgery was assessed using a snap backtest (21). Patients with a follow-up duration of <12 months or with a history of any involutional surgery were excluded from the study. The surgery was considered successful with a completely corrected normal eyelid position.

Patients, with or without horizontal lid laxity, were randomly divided into two groups according to the surgical technique. A total of 26 eyelids of 14 patients undergoing LER advancement + LTS (group 1) and 26 eyelids of 24 patients undergoing LER advancement alone (group 2) were analyzed.

Surgical Technique

All surgeries were carried out under local anesthesia using 2 mL of 1% lidocaine with 1:100.000 volume of epinephrine. LER advancement application (Jones procedure): An incision was made on the skin (length 15 mm) 3 mm under the cilia. The layer below the orbicularis oculi muscle was cut through the inferior border of the tarsus of the lower eyelid and dissected toward the cilia to reveal the lower tarsus. The posterior layer of the LER was separated from the conjunctiva. The orbital septum was transversely incised to reveal the sheet-like anterior layer of the LER. Following this, the edge of the posterior layer LER was fixated to the inferior border of the tarsus using three 6.0 double-armed polyglactin sutures. In addition, a strip of preseptal orbicularis was excised to decrease the inward rotation of the lower eyelid. Later on, the pretarsal orbicularis and the lower edge of the tarsus were affixed at three points. Lastly, the skin was closed using a 6.0 polyglactin suture.

LER advancement + LTS: Following the LER advancement procedure, a lateral canthotomy and inferior cantholisis were carried out first. The tarsal strip preparation was performed by detaching the anterior and posterior lamellae of the lateral lower eyelid and the removal of the anterior lamellae and tarsal conjunctiva through an incision along the inferior tarsal border. The level of horizontal shortening was identified by pulling the strip superolaterally. The tarsal strip was connected to the internal orbital portion of the lateral orbital rim periosteum using 5-0 polypropylene sutures. The orbicularis and skin were then closed up using the 6-0 polyglactin and polypropylene sutures, respectively.

Patients were evaluated on postoperative days 1 and 15. Follow-up visits were planned on the first, third, sixth, and twelfth months. The operation was considered successful if the normal eyelid position was completely corrected and entropion-related symptoms were absent.

Statistical Analysis

Mean, standard deviation, median, minimum and maximum value frequency, and percentage were used for descriptive statistics. The distribution of variables was analyzed with the Kolmogorov-Smirnov test. Quantitative data were compared using the Mann-Whitney U test. Data were analyzed using the SPSS software package program version 26.0.

RESULTS

A total of 38 patients underwent involutional entropion repair during the one-year study period, wherein 23 were males and 15 were females. Group 1 included 26 eyelids of 14 patients, whereas group 2 included 26 eyelids of 24 patients. Both groups were similar in age and sex distribution (Table 1). The mean age was 75.3±8.2 years (median 76.5) in group 1 and 74.0±7.4 in group 2 (Table 2). All operations were carried out by a single surgeon. In

group 2, seven treated eyelids had a lower eyelid entropion recurrence after 12 months (Table 2), whereas group 1 had no recurrence till the end of the 12-month follow-up period (Figure 1). A statistically significant difference was found between the surgical failure rates in groups 1 and 2 (p=0.004). No serious complications were observed in either group. Postoperative secondary ectropion was observed in a patient, two months after undergoing LER advancement.

DISCUSSION

Involutional entropion is usually considered to occur with a combination of horizontal laxity, LER dehiscence, and orbicularis override (22,23).

Entropion is corrected using several surgical techniques. Anatomical and functional success can be enhanced with the use of the correct surgical technique or surgical combinations (24). Entropion is frequently caused by multifactorial reasons, thus the primary aim of the operation is to handle the right pathological mechanism. As revealed by previous studies, if at least two pathological mechanisms are corrected, the surgical success increases. At least two of these pathological mechanisms should be corrected to have a higher rate of long-term success (25-27). The recurrence rate ranges from 0% to 17%, with variable lengths of follow-up. Techniques addressing all three factors responsible for involutional entropion have a lower recurrence rate (0%-5%) (28).

Table 1. Demographic characteristics and recurrence rates of patients

		Min-Max	Median	Mean ± SD/n-%
Age		54.0-89.0	75.5	74.4±7.6
Gender	Famale	-	15	39.5%
	Male		23	60.5%
Recurrence	(-)		45	86.5%
	(+)		7	13.5%

SD: Standart deviation, Min-Max: Minimum-maximum

In recent years, loosening the LERs is one of the most important causes of involutional entropion, thus retractor repairs became the most effective approach (Jones procedure). This procedure does not address the issue of orbicularis override; however, the skin incision into the anterior lamellae creates a cicatricial barrier between the lamellae that minimizes the vertical overriding of the preseptal orbicularis (29). Horizontal eyelid laxity is a crucial problem that leads to entropion. LTS is one of the most significant procedures for amending a loose horizontal eyelid (30). A case series of 15 patients who underwent LTS did not show entropion recurrence after an average 13 months of follow-up (31). In another series of 42 eyelids with involutional entropion without horizontal eyelid laxity, 8% recurrence was reported within 14 months after pentagonal excision (32).

The Wies procedure, with a full-thickness horizontal lid split with everting sutures, is used to turn the lid border outward, with a relatively high rate of recurrence (17%) (33). Wies reported a recurrence rate of 10% and a high rate of overcorrection, which is one of the most notable complications of this surgery (34,35).

Everting (Quickert) sutures temporarily solve the problem of entropion. In this surgical technique, scar tissue is created

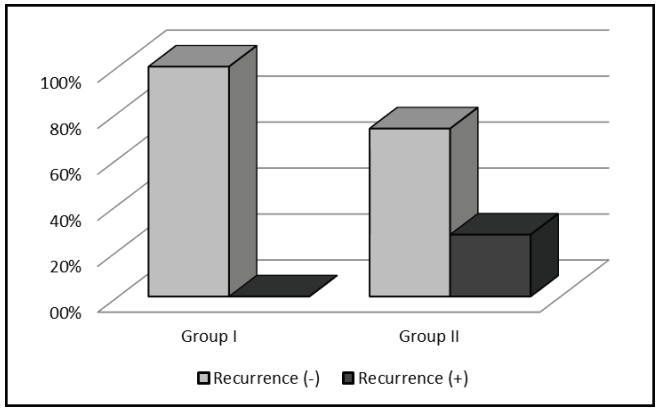

Figure 1. Recurrence rates in the compared groups.

Table 2. Demographic characteristics and recurrence rates of the groups

		Group I		Group II		p
		Mean ± SD/n-%	Median	Mean ± SD/n-%	Median	
Age		75.3± 8.2	76.5	74.0±7.4	75.5	0.544[m]
Gender	Famale	5	35.7%	10	41.7%	0.717[x2]
	Male	9	64.3%	14	58.3%	
Recurrence	(-)	26	100.0%	19	73.1%	0.004[x2]
	(+)	0	0.0%	7	26.9%	

[m]Mann-Whitney U test, [x2]Chi-square test, SD: Standart deviation

between the LERs, skin, and orbicularis (36). Jand et al. (37) reported a recurrence rate of >1% in six months, which increased to nearly 50% in two years.

All studies emphasized the significance of eyelid retractor tightening, orbicularis muscle resection, and horizontal eyelid shortening. In studies conducted by Lee et al. (38) and Erb et al. (39) combining tarsal strip with retractor advancement was more successful than retractor advancement alone, with a 96.7% success rate. Combined surgery is much more effective than LER advancement alone in cases of involutional entropion with horizontal eyelid laxity (38,39). Recently, Scheepers et al. (13) conducted a comparative study that demonstrated the superiority of the combined approach over simple lid retractor tightening with respect to the recurrence rates (38). In addition, Ranno et al. (40) showed that the combination of the Jones procedure and LTS resulted in lower recurrence rates (40).

Our study results were similar to those in the study by Lee et al. (38) Scheepers et al. (13) and Ranno et al. (40) which revealed that LER advancement + LTS was more effective than LER advancement alone for involutional entropion correction, with a lower recurrence rate. The recurrence rate was 0% in group 1 of our study. The most common complication of transcutaneous LER advancement is ectropion (41,42). One case of ectropion in group 2 was also observed.

Study Limitations

The limitation of this study is that LTS alone results were not investigated. Future studies comparing LTS alone as an effective method similar to LER advancement are necessary.

CONCLUSION

In conclusion, combination surgery had higher surgical success and a lower recurrence rate compared to LER advancement alone. Both techniques were safe without serious complications. A combination surgery is recommended to prevent recurrence of involutional lower eyelid entropion regardless of horizontal eyelid laxity.

ETHICS

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital of Local Ethics Committee (no: 2909, date: 11.08.2020).

Informed Consent: Informed consent was obtained from the patient.

Authorship Contributions

Surgical and Medical Practices: A.B.D., İ.Ç.T., Concept: A.B.D., İ.Ç.T., Design: C.U.D., S.K.Y., Data Collection or Processing: A.B.D., C.U.D., Analysis or Interpretation: C.U.D., S.K.Y., Literature Search: C.U.D., S.K.Y., Writing: A.B.D., İ.Ç.T.

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Research

Diagnostic Value of Fine-Needle Aspiration Biopsy in Major Salivary Gland Masses

Majör Tükrük Bezi Kitlelerinde İnce İğne Aspirasyon Biyopsisinin Tanısal Değeri

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ABSTRACT

Objective: To investigate the diagnostic efficacy and accuracy of fine-needle aspiration biopsy (FNAB) in solid and cystic tumors of the major salivary glands, examine the histopathologic distribution and malignancy rates of non-diagnostic cases, and investigate any significant difference between benign and malignant salivary gland tumors according to age and tumor size

Methods: Age, sex, tumor location, side, tumor size, preoperative diagnosis of FNAB, and final histopathological diagnosis of 182 patients with major salivary gland mass were retrospectively evaluated. Each lesion is categorized as solid and cystic. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and FNAB accuracy were separately calculated for parotid gland tumors, submandibular gland tumors, solid, and cystic tumors.

Results: Among the 182 performed FNABs, 153 were benign, 9 were malignant, and 20 were non-diagnostic. Malignancy was detected in 12.2% of the parotid gland, 25.9% of the submandibular gland, and 14.2% overall. Unlike the parotid gland, when the FNAB of the submandibular gland was non-diagnostic, the final histopathology was most likely to be malignant than benign. No difference was found in the risk of malignancy between the solid and cystic tumors (p=0.192). The sensitivity, specificity, PPV, NPV, and accuracy of the major salivary gland FNAB in this study were 42.1%, 99.3%, 88.8%, 92.8%, and 92.5%, respectively.

Conclusion: In the context of a non-diagnostic FNAB, high suspicion especially for the submandibular gland tumors is warranted for otorhinolaryngologists. Based on low sensitivity values, FNAB of the major salivary glands is limited as the only diagnostic tool.

Keywords: Fine-needle aspiration biopsy, malignancy, salivary gland, sensitivity, specificity

ÖZ

Amaç: Majör tükrük bezlerinin solid ve kistik tümörlerinde ince iğne aspirasyon biyopsisinin (İİAB) tanısal etkinliğinin ve doğruluğunun araştırılması, non-diagnostik olguların histopatolojik dağılımının ve malignite oranlarının incelenmesi, yaş ve tümör boyutuna göre benign ve malign tükrük bezi tümörleri arasındaki farkın araştırılması amaçlanmıştır.

Gereç ve Yöntem: Majör tükrük bezi kitlesi olan 182 hastanın yaşı, cinsiyeti, kitle lokalizasyonu, tarafı, kitlenin boyutu, preoperatif İİAB tanısı ve son histopatolojik tanısı retrospektif olarak değerlendirildi. Her bir lezyon solid ve kistik olarak kategorize edildi. Parotis bezi tümörleri, submandibuler bez tümörleri, solid ve kistik tümörler için duyarlılık, özgüllük, pozitif predediktif değeri (PPV), negatif prediktif değeri (NPV) ve doğruluğu ayrı ayrı hesaplandı.

Bulgular: Gerçekleştirilen 182 İİAB'den 153'ü benign, 9'u malign ve 20'si non-diagnostik idi. Parotis bezi kitlelerinin %12,2'si, submandibuler bez kitlelerinin %25,9'u ve tüm majör tükrük bezi kitlelerinin ise %14,2'si maligndi. Parotis bezinden farklı olarak, submandibuler bezin İİAB'si non-diagnostik olduğunda, histopatolojik tanı büyük oranda (%80) maligndi. Solid ve kistik tümörler arasında malignite riski açısından fark yoktu (p=0,192). Bu çalışmada majör tükrük bezi İİAB'nin duyarlılığı, özgüllüğü, PPV, NPV ve doğruluğu sırasıyla %42,1, 99,3, 88,8, 92,8 ve %92,5 olarak bulundu.

Sonuç: Özellikle submandibuler bez tümörleri için non-diagnostik bir İİAB varlığı, yüksek malignite şüphesi açısından kulak burun boğaz uzmanları için uyarıcı olabilir. Majör tükrük bezi tümörlerinde İİAB'nin düşük duyarlılık değerleri nedeniyle tek başına bir tanı aracı olarak kullanımı sınırlıdır.

Anahtar Kelimeler: İnce iğne aspirasyon biyopsisi, malignite, tükrük bezi, duyarlılık, özgüllük

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INTRODUCTION

Major salivary gland tumors account for 3-10% of the head and neck cancers and represent a various and heterogeneous group of neoplasms with complex clinicopathologic characteristics and different biological behavior. Their diversity makes the diagnosis challenging (1,2). Treatment of choice for salivary gland tumors is based on clinical evaluation and diagnostic tests: ultrasonography, fine-needle aspiration biopsy (FNAB), endoscopic ultrasound-guided fine-needle aspiration, biopsy, computed tomography, and magnetic resonance imaging (3). FNAB is a safe and reliable tool for salivary gland lesion diagnosis. It is simple, relatively painless, and easily repeated if another sample is needed. However, its diagnostic accuracy is still controversial despite being used for many years (4).

Several non-neoplastic lesions, benign neoplasms, and malignancies of the salivary gland present with a predominant or minor cystic component (5). Distinguishing these lesions from one another is important since patient management often differs among these groups. FNAB is often used to guide management decisions; however, it is frequently non-diagnostic in assessing cysts, as the aspirate may only capture cystic fluid. Despite non-diagnostic FNAB results, at least one-third of cystic salivary gland lesions are neoplastic (6,7).

This study aimed to investigate the diagnostic efficacy and accuracy of FNAB in solid and cystic tumors of the major salivary glands, examine the histopathologic distribution and malignancy rates of non-diagnostic cases, and investigate any significant difference between benign and malignant salivary gland tumors according to age and tumor size.

METHODS

Between February 2010 and February 2020, 267 cases that undergone parotidectomy or submandibular gland resection due to major salivary gland tumors in the otorhinolaryngology department of our tertiary referral center were retrospectively examined. Patients who underwent open biopsy before surgery, patients with unavailable FNAB, patients with non-diagnostic cytology depend on insufficient cellularity, and patients with preoperative FNAB done at an outside hospital, except in the situation where the original slides were transferred to our center for review by our institution pathologists, were excluded. Age, sex, tumor location, side, tumor size as measured by ultrasound/computed tomography/magnetic resonance imaging (by measuring the largest diameter of tumor), preoperative FNAB diagnosis, and final histopathological diagnosis were noted. Major salivary gland lesions were categorized as solid and cystic (pure cystic or has a cystic component in radiologic imaging studies) based on radiology reports and from available ultrasound, computed tomography, and/or magnetic resonance imaging studies. In addition, patients without any imaging studies or those with insufficient imaging report details were excluded. The study protocol was approved by the Institutional Ethics Committee of University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital (date: 17.02.2020, no: 2020/77). The study was conducted by the principles of the Helsinki declaration.

All of the FNAB was performed under ultrasound guidance using a 25 gauge needle and standard aspiration technique without local anesthesia in the radiology department of our hospital. The needle is inserted from a single point and moved in 3-4 directions through the tumor. After obtaining enough samples, the needle is withdrawn and detached from the syringe. The aspirate is sprayed on at least 4 glass slides, smeared, fixed in alcohol for hematoxylin-eosin stain, and sent to the pathology laboratory. All specimens were examined in the Pathology Department of our hospital. Preoperative cytological findings of the FNAB were classified as benign, non-diagnostic, and malignant. The subtypes were noted if possible. Final histopathological diagnosis was grouped as benign and malignant, and typing was noted. When the FNAB is non-diagnostic, our usual practice in such cases is to perform a second FNAB, if the cytological diagnosis is still not obtained with the second FNAB, then it is accepted as non-diagnostic.

FNAB results were compared to the final surgical pathology. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and FNAB diagnostic accuracy was calculated, and patients with non-diagnostic cytology were excluded from this analysis. Sensitivity, specificity, PPV, NPV, and FNAB accuracy were separately calculated for parotid gland tumors, submandibular gland tumors, solid, and cystic tumors, and also patients with non-diagnostic cytology were excluded from this analysis. The Mann-Whitney U test was used to examine the relationship of age and tumor size with the final histopathological diagnosis. The risk of malignancy between solid and cystic tumors was examined with the chi-square test. The confidence interval was 95% and p-values of <0.05 were considered to be significant.

RESULTS

Of the 267 retrospectively investigated patients, 85 were excluded due to unavailable FNAB results and/or insufficient

details of imaging reports. A total of 182 patients were retrospectively examined, wherein 95 (52.2%) were male and 87 (47.8 %) were female, with an average age of 49.07±16.43 years (range: 10-82 years). The mean age of patients with benign final histopathology was 48.79±15.96 years (range: 10-80 years, median: 52 years), whereas 50.77±19.23 years (range: 14-82 years, median: 56 years) in malignant tumors, without any statistically significant difference (p=0.7). The mean size of the salivary gland tumors with benign final histopathology was 27.41±14.37 mm (range: 5-65 mm, median: 25 mm), whereas 30.61±16.33 mm (range: 16-120 mm, median: 27 mm) in malignant ones. No statistically significant difference was found between the benign and malignant tumors (p=0.348).

Samples were obtained from the parotid in 155 (85.2%, 76 right parotids, 79 left parotids) cases and submandibular in 27 (14.8%, 13 right submandibular, 14 left submandibular) cases. Among the 182 performed FNABs, 153 (84%) were benign, 9 (4.9%) were malignant, and 20 (10.9%) were non-diagnostic. FNAB distribution and final histopathologic results among the parotid and submandibular gland tumors are shown in Table 1. Malignancy was detected in 12.2% (n=19) of the parotid gland tumors, 25.9% (n=7) of the submandibular gland tumors, and 14.2% (n=26) overall based on final histopathology. The most common benign diagnosis among the parotid gland tumors were pleomorphic adenoma (n=69, 50.7%) and Warthin's tumor (n=50, 36.7%), whereas the most common malignant diagnoses were mucoepidermoid carcinoma (n=5, 26.3%) and acinic cell carcinoma (n=4, 21%; Table 2). The most common benign diagnosis among the submandibular gland tumors was pleomorphic adenoma (n=19, 95%) and the most common malignant diagnosis was adenoid cystic carcinoma (n=2, 28.5%; Table 3). When the FNAB of the parotid gland is non-diagnostic, the final histopathology was most likely to be benign (80%) than malignant (20%). In contrast, when the FNAB of the submandibular gland was non-diagnostic, the final histopathology was most likely to be malignant (80%) than benign (20%). The distribution of final histopathology in non-diagnostic FNAB cases was presented in Table 4. A total of 125 solid (68.6%) and 57 cystic tumors (31.3%) (19 pure cystic, 38 have cystic component) were found among the major salivary gland tumor cases. FNAB of solid tumors accounting for 14 (11.2 %) were non-diagnostic, whereas 6 (10.5%) FNAB of cystic tumors were non-diagnostic (Table 5). Malignancy was detected in 12% (n=15) of solid tumors and 19.2% (n=11) in cystic tumors based on final histopathology. A higher malignancy rate was

Table 1. Distribution of FNAB results and final histopathologic diagnosis among parotid and submandibular gland tumors

	Final histopathologic diagnosis, n (%)	
FNAB diagnosis	**Benign**	**Malignant**
Parotid gland		
Benign (n=132)	123 (93.1)	9 (6.8)
Malignant (n=8)	1 (12.5)	7 (87.5)
Non-diagnostic (n=15)	12 (80)	3 (20)
Submandibular gland		
Benign (n=21)	19 (90.4)	2 (9.5)
Malignant (n=1)	0 (0)	1 (100)
Non-diagnostic (n=5)	1 (20)	4 (80)

FNAB: Fine-needle aspiration biopsy

Table 2. Final histopathologic diagnosis of parotid gland lesions

	n	%
Benign (n=136)		
Pleomorphic adenoma	69	50.7
Warthin tumor	50	36.7
Granulomatous sialadenitis	2	1.4
Lymphoepithelial sialadenitis	2	1.4
Lymphoepithelial cyst	2	1.4
Lipoma	2	1.4
Oncocytoma	2	1.4
Basal cell adenoma	1	0.7
Castleman disease	1	0.7
Epidermoid cyst	1	0.7
İnt alymphoid epidermoid cyst	1	0.7
Cavernous hemangioma	1	0.7
Non-specific chronic sialadenitis	1	0.7
Parotid duct cyst	1	0.7
Malignant (n=19)		
Mucoepidermoid carcinoma	5	26.3
Acinic cell carcinoma	4	21
Ductal carcinoma	2	10.5
Myoepithelial carcinoma	2	10.5
Squamous cell carcinoma	2	10.5
Adenoid cystic carcinoma	1	5.2
B-cell non-hodgkin lymphoma	1	5.2
Carcinoma ex-pleomorphic adenoma	1	5.2
Cystadenocarcinoma	1	5.2

Table 3. Final histopathologic diagnosis of submandibular gland lesions

	n	%
Benign (n=20)		
Pleomorphic adenoma	19	95
Non-specific chronic sialadenitis	1	5
Malign (n=7)		
Adenoid cystic carcinoma	2	28.5
Hodgkin lymphoma	1	14.2
Myoepithelial carcinoma	1	14.2
Malign epithelial tumor	1	14.2
Solitary fibrous tumor	1	14.2
Low-grade mesenchymal tumor	1	14.2

Table 4. Distribution of final histopathology in non-diagnostic FNAB cases

Final Histopathology	n, (%)
Parotid gland (n=15)	
Benign	
Pleomorphic adenoma	3, (20)
Whatin tumor	3, (20)
Lymphoepithelial cyst	2, (13.3)
Epidermoid cyst	1, (6.6)
Granulomatous sialadenitis	1, (6.6)
Castleman disease	1, (6.6)
Cavernous hemangioma	1, (6.6)
Malignant	
B-cell non-Hodgkin lymphoma	1, (6.6)
Mucoepidermoid carcinoma	1, (6.6)
Myoepithelial carcinoma	1, (6.6)
Submandibular gland (n=5)	
Benign	
Pleomorphic adenoma	1, (20)
Malignant	
Low-grade mesenchymal tumor	1, (20)
Hodgkin lymphoma	1, (20)
Adenoid cystic carcinoma	1, (20)
Solitary fibrous tumor	1, (20)

FNAB: Fine-needle aspiration biopsy

found in cystic tumors; however, no difference was found in the risk of malignancy between solid and cystic tumors (p=0.192).

Table 5. FNAB of solid and cystic tumors

	FNAB			
	Benign n %	Malignant n %	Non-diagnostic n %	Total n %
Solid tumors	105, 84	6, 4.8	14, 11.2	125, 100
Cystic tumors	48, 84.2	3, 5.2	6, 10.5	57, 100
Total	153, 84	9, 4.9	20, 10.9	182, 100

FNAB: Fine-needle aspiration biopsy

A total of 142 true-negatives, 8 true-positives, 11 false-negatives, and 1 false-positive case was found among the major salivary gland tumors, and the sensitivity, specificity, PPV, NPV, and accuracy of the major salivary gland FNAB in this study were 42.1%, 99.3%, 88.8%, 92.8%, and 92.5 %, respectively. The final histopathology of the only false-positive case was the Warthin's tumor, which was located in the parotid gland. Diagnostic accuracy measures among solid/cystic tumors, parotid/submandibular gland, and overall were shown in Table 6.

DISCUSSION

Salivary gland tumors are rare, mostly benign, with an annual estimated global incidence is 0.4-13.5 per 100,000 people. More than 50% of all primary salivary gland tumors occur in the major salivary glands, which are mainly present in the parotid gland, with 80%-85% being benign. Overall, tumors are more common in the parotid gland; however, the incidence of malignancy is higher in the submandibular and minor salivary glands. Histologically, the most common type of benign salivary gland neoplasms is a pleomorphic adenoma, and the most common malignant salivary gland tumors are mucoepidermoid and adenoid cystic carcinomas (3,8-10). Consistent with the literature, 85.2% of major salivary gland tumors were located in the parotid gland and 14.8% in the submandibular gland in our study. Based on final histopathology, 85.1% of 182 tumors were benign. The most common benign neoplasm is a pleomorphic adenoma and the malignant neoplasm is mucoepidermoid carcinoma.

FNAB is a safe, easy-to-apply, and inexpensive diagnostic procedure that is widely applied since the 1980s in the preoperative diagnosis of the salivary gland masses, while presenting low rates of complication and patient morbidity. However, the value of FNAB in preoperative diagnosis of salivary gland lesions is still being debated (11-13). In our study, 84% (n=153) of FNABs were benign, 4.9% (n=9) were malignant, and 10.9% (n=20) were non-diagnostic. Feinstein et al. (14) reported that when FNAB of the parotid gland was

Table 6. Measures of diagnostic accuracy among parotid gland, submandibular gland, solid tumors, cystic tumors, and overall

	Parotid gland	Submandibular gland	Solid tumors	Cystic tumors	Overall
Sensitivity %	43.7	33.3	45.4	37.5	42.1
Specificity %	99.1	100	99	100	99.3
PPV %	87.5	100	83.3	100	88.8
NPV %	93.1	90.4	94.2	89.5	92.8
Accuracy %	92.8	90.9	95.4	90	92.5

PPV: Positive predictive value, NPV: Negative predictive value

non-diagnostic, the final pathology was more likely benign than malignant and, when FNAB of the submandibular gland was non-diagnostic the final pathology was equally likely benign versus malignant. Similarly, when the FNAB of the parotid gland is non-diagnostic, the final histopathology was found to be most likely benign than malignant. In contrast with their findings, when the FNAB of the submandibular gland was non-diagnostic, the final histopathology was found to be most likely malignant than benign. Boursiquot et al. (7) stated that FNAB is frequently non-diagnostic in assessing cysts, as the aspirate only captures cystic fluid. In our study, similar non-diagnostic FNAB rates were found in solid (11.2%) and cystic (10.5%) tumors. This was related to our exclusion criteria of non-diagnostic cytology cases that depend on insufficient cellularity. In addition, they reported that, in line with our findings, no difference was found in the risk of malignancy between the cystic and solid tumors (7).

In the management of salivary gland tumors, distinguishing malignant tumors from benign ones is very important to determine the therapeutic approach. Olsen et al. (15) stated in their study that FNAB was found with a high false-negative rate, up to 20%, that limits its usefulness and, therefore they reported that they strictly rely on frozen section results for surgical decision making. False-negative rate was relatively small in our study, (6.7%, n=11) accounting for 20 (10.9%) non-diagnostic cases, wherein 35% (n=7) were malignant based on final histopathology.

The diagnostic value of FNAB in salivary gland tumors was assessed in several studies, and a wide range of results on accuracy for detecting malignancy was reported, with sensitivities ranging from 33% to 100% and specificities ranging from 67% to 100% (16). In the study by Kechagias et al. (4) they reported that the sensitivity and specificity of FNAB for salivary gland masses were 90% and 98%, respectively. In the study of Stow et al. (17) they reported that the sensitivity and specificity in their series were 86.9% and 96.3%, respectively. In our study, despite high specificity values, the sensitivity values for submandibular gland tumors, parotid gland tumors, cystic masses, solid masses, and overall were 33.3%, 43.7%, 37.5%, 45.4%, and 42.1%, respectively. Therefore, despite the relatively high sensitivity values of some other studies in the literature, approximately 58% of malignant tumors of the major salivary glands were undiagnosed by FNAB in our study (4,7,12,14,17). Based on our findings, benign FNAB cytology should not be trusted and FNAB should not overcome the clinical experience and intraoperative findings. An otorhinolaryngologist should be aware of the other signs of a malignant tumor, such as anamnesis of persistent pain in the area and a rapidly growing tumor of a salivary gland.

The major limitation of this study is its retrospective nature, which included only those patients who proceeded to surgery. FNAB results could not be evaluated in terms of determining the usefulness of FNAB for patients who did not elect surgery or who pursued surgery elsewhere. In addition, the impact of different pathologists on FNAB cytology was not assessed.

CONCLUSION

Study results revealed that when the FNAB of the submandibular gland was non-diagnostic, unlike the parotid gland, the final histopathology was most likely malignant than benign. In the context of a non-diagnostic FNAB, a high suspicion for salivary gland tumors especially for the submandibular gland tumors may be warranted for otorhinolaryngologists. Similar non-diagnostic FNAB rates in solid and cystic tumors were found. Our findings demonstrated that FNAB of the parotid gland, submandibular gland, cystic, and solid masses has limitations as an only diagnostic tool. FNAB has not had a reliable sensitivity in terms of screening malignancy. For a surgeon to complement FNAB diagnosis with a patient's medical history, physical examination, own clinical experience, and radiological imaging are more appropriate.

ETHICS

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital of Local Ethics Committee (protocol number: 2020/77, date:17.02.2020).

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: F.G., M.A.A., Z.M.Y., İ.S., Concept: F.G., M.A.A., E.A., Design: F.G., E.A., Z.M.Y., Data Collection or Processing: F.G., M.A.A., E.A., Analysis or Interpretation: F.G., M.A.A., E.A., Z.M.Y., İ.B., Literature Search: F.G., E.A., İ.S., Writing: F.G., Z.M.Y., İ.S.

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Research

Cognitive Dysfunction in Older Patients Undergoing Orthopedic Surgery: Analysis of Demographic, Clinical, and Intraoperative Risk Factors

Ortopedik Cerrahi Geçiren Yaşlı Hastalarda Bilişsel İşlev Bozukluğu: Demografik, Klinik ve İntraoperatif Risk Faktörlerinin Analizi

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ABSTRACT

Objective: We evaluated cognitive dysfunction in older patients undergoing orthopedic surgery regarding demographic, clinical, and intraoperative risk factors.

Methods: A total of 56 older patients (aged >65 years, mean ± SD age: 75.1±6.8 years, 75% were females) hospitalized for elective orthopedic surgery were included. Data on patient demographics (age, gender), comorbid diseases, planned surgery indications, American Society of Anesthesiologists (ASA) Classification category, intraoperative time (min), intraoperative blood loss (mL), and length of hospital stay (LOS; days) were recorded for each patient. Vital signs were recorded thrice, preoperatively, intraoperatively (every 15 min), and postoperatively (first hour), whereas pain was assessed based on visual analog scale (VAS) scores in the postoperative period (1st h, 12th h, 36th h). The Mini-Mental State Examination (MMSE) was administered preoperatively and in the postoperative 72nd h to assess cognitive status.

Results: Overall, a significant decrease was noted in MMSE scores from the preoperative to postoperative (72nd h) period [median (minimum-maximum) 26 (12-30) vs. 23.5 (12-30), p<0.001]. The significant decline in MMSE scores after the operation was evident in each subgroup, regardless of the gender, ASA category, presence of comorbidity, or acute renal failure (average SD change from baseline ranged from -1.5 to -1.0; p-values ranged from 0.024 to <0.001). Preoperative and postoperative MMSE scores were negatively correlated with age (r=0.415, p=0.002 and r=-0.406, p=0.003, respectively) and intraoperative time (r=-0.511, p<0.001 and r=-0.428, p=0.002, respectively).

Conclusion: Our findings revealed the likelihood of postoperative cognitive dysfunction in older patients undergoing orthopedic surgery, regardless of gender, comorbidities, or ASA category. However, there was an increased risk of postoperative cognitive decline with increased patient age and longer intraoperative time.

Keywords: Postoperative cognitive dysfunction, orthopedics, older patients, age, comorbidity, intraoperative characteristics

ÖZ

Amaç: Ortopedik cerrahi geçiren yaşlı hastalarda kognitif disfonksiyonu demografik, klinik ve intraoperatif risk faktörleri açısından değerlendirmeyi amaçladık.

Gereç ve Yöntem: Elektif ortopedik cerrahi için hastaneye yatırılan toplam 56 yaşlı hasta (>65 yaş, ortalama ± SS yaş: 75,1±6.8 yıl, %75 kadın) dahil edildi. Hastaların demografik özellikleri (yaş, cinsiyet), komorbid hastalıkları, planlanan cerrahi endikasyonları, Amerikan Anesteziyologlar Derneği (ASA) Sınıflandırma kategorisi, intraoperatif süre (dakika), intraoperatif kan kaybı (mL), hastanede kalış süresi her hastada kaydedildi. Hayati bulgular ardışık şekilde üç kez kaydedildi; preoperatif, intraoperatif (15. dakika) ve postoperatif (1. saat), ağrı postoperatif dönemde (1. saat, 12. saat, 36. saat) görsel analog skala (VAS) skorlarına göre değerlendirildi. Bilişsel durumu değerlendirmek için ameliyat öncesi ve ameliyat sonrası 72. saatte mini mental durum testi (MMSE) yapıldı.

Bulgular: Genel olarak, MMSE skorlarında preoperatif dönemden postoperatif (72. saat) döneme [medyan (minimum-maksimum) 26 (12-30) ve 23,5 (12-30), p<0,001] arasında anlamlı bir düşüş kaydedildi. Cinsiyet, ASA kategorisi, komorbidite varlığı veya akut böbrek yetmezliği varlığından bağımsız olarak her alt grupta operasyondan sonra MMSE puanlarında önemli düşüş belirgindi (başlangıca göre ortalama SS değişikliği -1,5 ile

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-1,0 arasında değişmekteydi; p değerleri 0,024 ve <0,001). Preoperatif ve postoperatif MMSE skorları yaş (sırasıyla; r=-0,415, p=0,002 ve r=-0,406, p=0,003) ve intraoperatif süre (sırasıyla; r=-0,511, p<0,001 ve r=-0,428, p=0,002).

Sonuç: Bulgularımız ortopedik cerrahi geçiren yaşlı hastalarda cinsiyet, komorbidite veya ASA kategorisine bakılmaksızın postoperatif kognitif disfonksiyon olasılığının, hasta yaşının artması ve intraoperatif sürenin uzaması ile postoperatif bilişsel gerileme riskinin arttığını ortaya koymuştur.

Anahtar Kelimeler: Postoperatif kognitif disfonksiyon, ortopedi, yaşlılık, yaş, komorbidite, intraoperative bulgular

INTRODUCTION

Postoperative cognitive dysfunction (POCD) refers to cognitive impairment affecting orientation, perception, attention, consciousness, and judgment that develop after anesthesia and surgery (1,2). It is a commonly recognized clinical phenomenon, particularly among older people with 40% and 10% rates in those over 60 years of age at discharge and the third-month post-discharge, respectively (2-4).

Alongside the risk of permanent cognitive dysfunction, the development of POCD in older patients has also been associated with prolonged hospital recovery, increased morbidity, and delays in functional recovery (1,5,6).

Given the aging of the population, an increasingly higher number of older patients are undergoing complex surgical procedures with advances in medicine, surgical techniques, and anesthetic care. Therefore, addressing the etiology, pathogenesis, and risk factors of POCD in older surgical patients has become clinically significant in developing appropriate preventive measures (2,4,6).

Orthopedic surgery is considered to have a particular role concerning the risk of postoperative cognitive impairment given the high prevalence of cerebral microemboli during procedures such as total knee replacements (1,7,8). This study was designed to evaluate cognitive dysfunction in older patients undergoing orthopedic surgery regarding demographic, clinical, and intraoperative risk factors.

METHODS

Study Population

A total of 56 older patients (aged >65 years, mean ± SD age: 75.1±6.8 years, 75% were females) hospitalized for elective orthopedic surgery were included in this prospective cross-sectional study conducted between April 2018 and October 2018. Patients with known cognitive dysfunction and foreign patients who did not speak Turkish were excluded from the study. Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee.

Assessments

Data on patient demographics (age, gender), comorbid diseases, planned surgery indications, American Society of Anesthesiologists (ASA) Classification category, intraoperative time (min), intraoperative blood loss (mL), and length of hospital stay (LOS; days) were recorded for each patient. Heart rate (bpm), systolic, diastolic and mean arterial blood pressure (mmHg) were recorded thrice; preoperatively, intraoperatively (every 15 min), and postoperatively (first hour), while hemoglobin (g/dL) and hematocrit (%) levels were also measured preoperatively and postoperatively. Pain was assessed based on visual analog scale (VAS) scores in the postoperative period (1st h, 12th h, 36th h). The mini-mental state examination (MMSE) was administered preoperatively and in the postoperative 72nd h to assess cognitive status.

Mini-Mental State Examination

MMSE, one of the most widely used screening instruments for estimating the severity of cognitive impairment, is an 11-item measure comprising five domains (orientation, registration, attention and calculation, recall, and language) of cognitive function. The MMSE total score ranges from 0 to 30, with a lower score indicating a higher degree of cognitive impairment (9-11).

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY) and PAST (Paleontological Statistics) Software Package (12). The Mann-Whitney U test and Kruskal-Wallis test with the Monte Carlo simulation technique analyzed numerical variables. The change over time was evaluated by the Wilcoxon test for two repeated measurements and by Friedman's Two-Way test with the Monte Carlo simulation and post hoc Dunn's test for more than two repeated measurements. A correlation analysis was performed via partial correlation analysis. Data were expressed as "mean ± standard deviation (SD)," median (minimum-maximum), and percent (%) where appropriate. A $p<0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics, Intraoperative Data, and Hospital Stay

Overall, the median age was 75 years (range, 65-92 years) and females comprised 75% of the study population. Comorbidities were evident in 50% of patients (hypertension in 25.0%), whereas acute renal failure was noted in 12.5% of patients. The ASA category was II in 42.9% of patients, and total hip replacement (39.3%) was the most common indication for surgery (Table 1).

The median intraoperative time, intraoperative blood loss, and LOS were 100 min (range, 60-180 min), 150 mL (range, 50-600 mL), and eight days (range, 4-27 days), respectively (Table 1).

Pre- vs. Postoperative Assessment for Hemogram Findings, Vital Signs, and Pain Scores

When compared with median (min-max) preoperative values, a significant decrease was noted in both hemoglobin [from 12.05 (8.9-15.9) g/dL to 9.5 (8.1-15.2) g/dL, p<0.001] and hematocrit [from 37.6% (28.4%-46.7%) to 30.05% (24.2%-43.9%), p<0.001) levels in the postoperative period. The median (min-max) systolic blood pressure [135 (65-175) vs. 140 (94-178] mmHg, p<0.001] and diastolic blood pressure [78.5 (55-148) vs. 71 (60-98) mmHg, p<0.05] levels were significantly lower in the intraoperative 15th min compared with preoperative values, with no significant change between pre- and postoperative records in vital signs.

VAS pain scores were significantly higher in the postoperative 36th hour compared with postoperative first and 12th hour values [median (min-max) 5 (3-8) vs. 3 (2-4) and 4.5 (2-7); p<0.001 and p<0.05, respectively]. VAS scores in the postoperative 12th hour were also significantly higher than in the postoperative first hour (p<0.001) (Table 2).

MMSE Scores According to Baseline Characteristics

Overall, a significant decrease was noted in MMSE scores from preoperative to postoperative (72nd h) period [median (min-max) 26 (12-30) vs. 23.5 (12-30), p<0.001]. The significant decline in MMSE scores after the operation was evident in each subgroup, regardless of gender, ASA category, presence of comorbidity, or acute renal failure (average SD change from baseline ranged from -1.5 to -1.0; p-values ranged from 0.024 to <0.001) (Table 3).

No significant difference was noted in preoperative MMSE scores, postoperative MMSE scores, and change from baseline according to gender, ASA category, and presence of comorbidity or acute renal failure (Table 3).

Correlation of MMSE Scores with Study Variables

Correlation analysis as adjusted for gender, ASA category, comorbidity and acute renal failure revealed that preoperative and postoperative MMSE scores were negatively correlated with age (r=-0.415, p=0.002 and r=-

Table 1. Baseline characteristics, intraoperative data, and hospital stay

Age (year)	Mean ± SD	75.1±6.8
	Median (min-max)	75 (65-92)
Gender, n (%)	Female	42 (75.0)
	Male	14 (25.0)
ASA category, n (%)	II	24 (42.9)
	III	21 (37.5)
	IV	11 (19.6)
Comorbidity, n (%)	None	28 (50.0)
	>3	2 (3.6)
	HT	14 (25.0)
	HT+DM	5 (8.9)
	CHF	3 (5.4)
	Stroke	2 (3.6)
	DM	1 (1.8)
	Hypothyroidism	1 (1.8)
Indication for surgery, n (%)	THR	22 (39.3)
	PHA	1 (1.8)
	TKP	19 (33.9)
	ORIF	7 (12.5)
	Upper	1 (1.8)
	MMR	3 (5.4)
	Other	3 (5.4)
Postoperative infection, n (%)		2 (3.6)
LFT, n (%)		1 (1.8)
Acute renal failure, n (%)		7 (12.5)
Intraoperative time (min)	Mean ± SD	108.0±34.8
	Median (min-max)	100 (60-180)
Intraoperative blood loss (mL)	Mean ± SD	183.9±108.3
	Median (min-max)	150 (50-600)
Length of hospital stay (day)	Mean ± SD	9.3±4.3
	Median (min-max)	8 (4-27)

ASA: American society of anesthesiologists, DM: Diabetes mellitus, HT: Hypertension, CHF: Congestive heart failure, THR: Total hip replacement, PHA: Partial hip replacement, TKP: Total knee prosthesis, ORIF: Open reduction internal fixation, Upper: Upper extremity surgeon, MMR: Medial meniscus repair, LFT: Liver function tests, SD: Standard deviation, Min: Minimum, Max: Maximum

0.406, p=0.003, respectively) and intraoperative time (r=-0.511, p<0.001 and r=-0.428, p=0.002, respectively) (Table 4). No significant correlation of MMSE scores was noted with intraoperative blood loss or LOS (Table 4).

DISCUSSION

Our findings in a cohort of older patients undergoing orthopedic surgery revealed a significant decrease in MMSE scores 72 h after surgery, regardless of gender, ASA category, or presence of comorbidity, or ARF.

Table 2. Pre- vs. postoperative assessment for hemogram findings, vital sings and pain scores

	Preoperative	Postoperative			p
Hemoglobin (g/dL)	12.05 (8.9-15.9)	9.5 (8.1-15.2)			<0.001[1]
Hematocrit (%)	37.6 (28.4-46.7)	30.05 (24.2-43.9)			<0.001[1]
	Preoperative	Intraoperative (15th min)		Postoperative (1st h)	
Heart rate (bpm)	74 (62-126)	80.5 (60-100)		80 (64-95)	0.099[2]
Systolic BPP	140 (94-178)	135 (65-175)**		138 (100-168)	0.004[2]
Diastolic BP	78.5 (55-148)	71 (60-98)*		72 (45-106)	0.026[2]
	Postoperative				
VAS Pain scores	**12th h**	**24th h**	**36th h**		
	3 (2-4)^{qq,w}	4.5 (2-7)^{q}	5 (3-8)		<0.001[2]

Data are shown as median (min-max). VAS: Visual analog scale; BP: Blood pressure
[1]Wilcoxon-Signed Ranks test (Monte Carlo), [2] Friedman test (Monte Carlo), Post Hoc test: Dunn's test
*p<0.05 and **p<0.01; compared to preoperative values
^{q}p<0.05 and ^{qq}p<0.001; compared to 36th hour values
^{w}p<0.001; compared to 12th hour values

Table 3. MMSE scores according to baseline characteristics

Preoperative median (min-max)		MMSE scores			
		Postoperative (72nd h)	Change from baseline	p pre vs. postop	
		median (min-max)	median (min-max)		
Total		26 (12-30)	23.5 (12-30)	-1 (-10-6)	**<0.001**[3]
Gender	Female	26 (12-30)	26 (12-30)	-1 (-10-6)	**<0.001**[3]
	Male	23 (13-30)	21.5 (12-29)	-1.5 (-6-1)	**0.001**[3]
	p	0.136[1]	0.136[1]	0.237[1]	
ASA category	II	25.5 (13-30)	23.5 (12-30)	-1 (-6-1)	**<0.001**[3]
	III	28 (12-30)	26 (16-30)	-1 (-6-6)	**0.004**[3]
	IV	25 (14-30)	20 (12-30)	-1 (-10-0)	**0.004**[3]
	p	0.295[2]	0.106[2]	0.459[2]	
Comorbidity	Absent	25.5 (12-30)	23 (12-30)	-1 (-6-6)	**<0.001**[3]
	Present	26 (14-30)	24.5 (12-30)	-1 (-10-1)	**<0.001**[3]
	p	0.307[1]	0.669[1]	0.582[1]	
Acute renal failure	Absent	26 (12-30)	23 (12-30)	-1 (-10-6)	**<0.001**[3]
	Present	28 (21-30)	27 (16-30)	-1 (-6-0)	**0.024**[3]
	p	0.314[1]	0.294[1]	0.945[1]	

ASA: American society of anesthesiologists, MMSE: Mini-Mental state examination
[1]Mann-Whitney U test (Monte Carlo), [2]Kruskal-Wallis test (Monte Carlo), [3]Wilcoxon Signed Ranks test (Monte Carlo)

Table 4. Correlation of MMSE scores with study variables

	MMSE scores					
	Preoperative		Postoperative		Change from baseline	
	r	p	r	p	r	p
Age	-0.415	**0.002**	-0.406	**0.003**	-0.019	0.895
Intraoperative time (min)	-0.511	**<0.001**	-0.428	**0.002**	0.133	0.346
Intraoperative blood loss (mL)	-0.182	0.195	-0.097	0.494	0.169	0.230
Length of hospital stay (day)	-0.193	0.169	-0.179	0.204	0.013	0.925

Adjusted for gender, ASA category, comorbidity, and acute renal failure. Partial Correlation test, r: correlation coefficient, MMSE: Mini-Mental state examination

When adjusted for gender, ASA category, or presence of comorbidity and ARF, MMSE scores were negatively correlated with age and intraoperative time. In contrast, no correlation of MMSE scores was noted with intraoperative blood loss or LOS. MMSE scores did not differ concerning gender, ASA category, or presence of comorbidity in the pre or postoperative period.

A postoperative decline of ≥1 SD from baseline MMSE scores in our patients indicated the likelihood of cognitive impairment in older patients undergoing orthopedic surgery. However, an increase in age and longer intraoperative time were determined as the two risk factors for the postoperative decline in MMSE scores. Hence, our findings emphasize that advanced age and complexity of the surgical procedure, rather than gender and comorbidities, were associated with a higher risk of new cognitive impairment arising after a surgical procedure in older orthopedic patients. This seems consistent with consideration of advanced age and extensive surgery with a higher risk of postoperative POCD in apparently previously cognitively well patients undergoing surgery with the most marked increase of risk among those over 65 years of age (2,13). In a systematic review of studies in surgery patients, increasing age was the most common risk factor for POCD. In contrast, the duration and type of surgery (cardiac, orthopedic, and vascular) were also reported among risk factors (13).

Indeed, a systematic review of 19 studies in 6477 patients concluded that the prevalence of POCD was 11.7% for non-cardiac surgery at three months of follow-up (14). However, older patients undergoing higher risk surgery are considered to have a higher risk. The POCD prevalence in patients undergoing elective hip surgery is estimated to be 22% (15).

Notably, in the older population undergoing hip fracture surgery, POCD, along with postoperative delirium, have been reported to be the two frequent complications leading to increased risk of morbidity, mortality, and prolonged hospital stays (16-18).

While potentially anesthetic-dependent modifiable factors (i.e., hypoxia, hypotension, and altered cerebral perfusion) have been postulated as contributing to POCD, there is no conclusive evidence (13,19,20). Indeed, hypotension as a common cause of cerebral hypoperfusion has been investigated regarding its relation to POCD, but the potential impact of prolonged hypotension was not confirmed in these studies (19,21-23). Accordingly, while there was a significant but transient decline in systolic and diastolic blood pressure intraoperatively, blood pressure was maintained at preoperative levels postoperatively in our patients. In addition, while a significant decline was noted in hemoglobin and hematocrit levels after the operation, there was no significant impact of intraoperative blood loss on MMSE findings.

Given the association of vascular risk factors (i.e., hypertension, obesity, diabetes mellitus) with cognitive decline in the general population, their optimization may have a role in lowering the risk of POCD (13,19,24). Our findings revealed comorbidities (i.e., diabetes, hypertension, congestive heart failure, stroke) in 50% of patients, whereas no significant impact of the presence of comorbidity on the postoperative decline in MMSE scores and on preoperative MMSE scores. These seem notable given that diabetes mellitus, hypertension, obesity, smoking, depression, cognitive inactivity, and physical inactivity are considered critical modifiable risk factors for cognitive dysfunction in Alzheimer's disease with the potential impact of related lifestyle interventions on cognitive improvement (25-27). Our findings revealed no such impact of comorbidities on the likelihood or severity of POCD among older orthopedic surgery patients.

Our findings based on assessing cognitive status 72 h after surgery seem consistent with considering POCD to be most prevalent in the immediate postoperative period and highly prevalent up to three months postoperatively, and mostly

resolving within days to weeks (6,13,28,29). Notably, while POCD may also persist 1-2 years after surgery, it has been considered to be reversible and rarely persists in the longer (10 years) term (6,13,28,29).

Notably, while the variance in the 7-day, 3-month, or 12-month cognitive assessment in the same patient has been considered likely, the longitudinal assessment has revealed that a single diagnosis of cognitive decline is associated with a higher incidence even with this fluctuating pattern of long-term dementia (25,30,31). Besides, while POCD is generally transient, it lasts longer and affects everyday life more severely in patients over age 60 (32). Also, the presence of POCD on discharge has been associated with diminished quality of life and increased risk of mortality within one year after surgery (2,3).

This also seems notable given the increasingly recognized role of perioperative consultation with older specialists among older patients undergoing major surgery to prevent POCD and improve quality of life (13). In orthopedic surgery, they are associated with good outcomes in hip fracture patients (33).

Although the MMSE was essentially developed as a cognitive screening tool, it performs well as a screening tool for dementia. Therefore, it was suggested that the MMSE has a limited role in assessing cognitive decline after anesthesia and surgery (13,25). It has been widely used as a tool to detect POCD, given the ease of administration (6-8 minutes) (25,34) and the consideration of the purpose of postoperative neuropsychological testing to detect the presence of general, rather than specific, changes in cognitive functioning (35).

Use of single-point testing for cognitive status before orthopedic surgery has been considered to give an incorrect opinion about patients' cognitive trajectory, given the likelihood of preoperative pain (i.e., for a patient about to undergo total hip replacement) to have a considerable impact on cognition and thus the impact of the postoperative reduction in pain and improved mobility on cognitive measures (13,24). This seems notable given the significant increase in VAS pain scores from the postoperative first h to 36th h in our patients.

Nonetheless, the heterogeneity of study populations, definition of cognitive impairment, instruments used to measure cognitive performance, and follow-up periods have been the major challenges limiting the likelihood of generalizing from the study findings available on POCD (2,13).

CONCLUSIONS

In conclusion, our findings revealed the likelihood of POCD in older patients undergoing orthopedic surgery, regardless of gender, comorbidities, or ASA category. However, there was an increased risk of postoperative cognitive decline with increased patient age and longer intraoperative time. This emphasizes the critical role of improved perioperative geriatric care in providing guidance and early recognition of risk factors among older patients prone to POCD. Larger-scale longitudinal clinical studies employing standardized test instruments are necessary to determine the risk for further cognitive deterioration in the longer term and develop strategies to minimize the cognitive impact for a better quality of life in older surgical patients.

ETHICS

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk training and Research Hospital of Local Ethics Committee (protocol number: 2018/126-17.05.2018).

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: G.S., Y.P., Concept: Y.T.Ş., Design: Y.T.Ş., Data Collection or Processing: D.Ö.B., G.S., Y.P., Analysis or Interpretation: Y.T.Ş., Literature Search: Y.T.Ş., Writing: Y.T.Ş.,

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Research

Spinal Anesthesia in Vaginal Natural Orifice Transluminal Endoscopic Surgery: Experience of 12 Patients

Vajinal Destekli Doğal Orifis Transluminal Endoskopik Cerrahide Spinal Anestezi: 12 Hasta Deneyimi

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ABSTRACT

Objective: Vaginal natural orifice transluminal endoscopic surgery (vNOTES) is an alternative to conventional laparoscopy with less postoperative pain and shorter hospital stay. Limited data on the vNOTES under spinal anesthesia (SA) was reported. Thus, this study aimed to evaluate the feasibility, effectiveness, and side effects of SA in patients with gynecologic problems who underwent vNOTES.

Methods: SA was administered to the L1-L2 interspinous space in 12 patients with gynecologic problems who underwent vNOTES. Intrathecal 10 µg of fentanyl (0.2 mL) and 0.5% of isobaric bupivacaine were administered. Patient demographic data, peroperative hemodynamic data, postoperative pain scores, and complications were retrospectively recorded.

Results: Between January 2019 and December 2019, gynecological surgery was successfully performed on 12 patients under SA via vNOTES. Right shoulder pain was observed in 3 patients (25%) and anxiety in 1 patient (8%), perioperatively. In the first 24 hours postoperatively, 1 patient (8%) had a headache, whereas postoperative nausea and vomiting were not observed. None had pain requiring analgesia in the first 6 hours postoperatively.

Conclusion: SA is applicable for vNOTES.

Keywords: Analgesia, anesthesia, spinal, natural orifice endoscopic surgery, pain, postoperative, postoperative nausea and vomiting

ÖZ

Amaç: Vajinal destekli doğal açıklık transluminal endoskopik cerrahi (vNOTES) tekniği, daha az postoperatif ağrı ve daha kısa hastanede kalış süresi ile geleneksel laparoskopik tekniğe bir alternatiftir. vNOTES tekniğinin spinal anestezi (SA) altında kullanımına ilişkin sınırlı veri vardır. Amacımız vNOTES tekniği ile ameliyat edilen jinekolojik hastalarda SA uygulanabilirliği etkinliğini ve yan etkilerini değerlendirmektir.

Gereç ve Yöntem: vNOTES tekniği ile ameliyat edilen 12 jinekolojik hastaya L1-L2 interspinöz aralıktan SA uygulandı. İntratekal 10 µg fentanil (0,2 mL) ve %0,5 izobarik bupivakain uygulandı. Hastaların demografik verileri, peroperatif hemodinamik verileri, postoperatif ağrı skorları ve komplikasyonlar geriye dönük olarak kaydedildi.

Bulgular: Ocak 2019-Aralık 2019 tarihleri arasında 12 hastaya SA altında vNOTES tekniği ile jinekolojik cerrahi başarıyla uygulandı. Ameliyat sırasında 3 hastada (%25) sağ omuz ağrısı ve 1 hastada (%8) anksiyete görüldü. Postoperatif ilk 24 saatte 1 hastada (%8) baş ağrısı olurken postoperatif bulantı ve kusma izlenmedi. Postoperatif ilk 6 saatte hiçbir hastada analjezi gerektiren ağrı olmadı.

Sonuç: SA, vNOTES tekniği için uygulanabilineceğini düşünmekteyiz.

Anahtar Kelimeler: Analjezi, anestezi, spinal, doğal ağızlı endoskopik cerrahi, ağrı, postoperatif, postoperatif bulantı ve kusma

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INTRODUCTION

The development of surgical techniques and technology allowed surgeons to perform an increased number of minimally invasive surgeries. In addition, it affected and improved anesthesia management. Conventional laparoscopy (CL) was reported to reduce postoperative morbidity, pain, and complications shorten the hospital stay and reduce the total cost compared to laparotomy (1,2).

However, hemodynamic and physiological effects of CL due to pneumoperitoneum with carbon dioxide insufflation pose difficulties for anesthetists in terms of hypercarbia and venous gas embolism (3,4).

In recent years, vaginal natural orifice transluminal endoscopic surgery (vNOTES) is a new developing alternative to CL. vNOTES is a minimally invasive surgery that allows transgastric or transvaginal access using endoscopes without skin incision (5,6). To date, minimal surgical trauma, early mobilization, less pain, and cosmetic benefits were reported in cholecystectomy, appendectomy, hysterectomy, and bilateral salpingoophorectomy cases performed via vNOTES (6,7).

Today, general anesthesia (GA) is the preferred route of anesthesia induction in minimally invasive surgeries (8,9) to reduce the risk of aspiration and abdominal discomfort, provide better bowel relaxation, and prevent hypercapnia due to carbon dioxide (CO2) during pneumoperitoneum (5,10). However, spinal anesthesia (SA) is an alternative, considering GA-related complications, in patients with respiratory distress and comorbidities. SA offers the advantages of less postoperative pain, nausea, and vomiting and early ambulation compared to GA (11,12). Previous studies reported that SA is utilized safely with less postoperative pain, fewer respiratory complications, and affordable cost in CL cases (13,14).

CO2 insufflation and Trendelenburg position are used to adequately display the abdominal cavity, as in the CL, in vNOTES. However, vNOTES was reported to provide better respiratory functions in the perioperative period due to lower intraabdominal pressure, shorter operative time, less perioperative opioid requirement, and less Trendelenburg position compared to CL (5,7).

Currently, limited literature is reported regarding the feasibility of vNOTES in gynecologic practice conducted with SA. Thus, this study aimed to evaluate the feasibility, effectiveness, and restrictions of SA for women who underwent the vNOTES technique.

METHODS

The ethical approval was obtained from University of Health Sciences Turkey, Bakirköy Dr. Sadi Konuk Training and Research Hospital Local Ethics Committee (protocal no: 2020/91), and the study was conducted with 12 patients having the American Anesthesiology Association (ASA) scores of 1-2, who were scheduled for benign gynecological pathology between January 2019 and December 2019. Investigate whether it complies with the 1964 Helsinki Declaration and the ethical standards of the national research committee.

The exclusion criteria were as follows: refusal to SA, any SA contraindications, coagulation disorder, cardiovascular diseases, history of local anesthetic allergies, and spinal deformities.

Information and written informed consent were obtained from patients regarding the SA and vNOTES procedures and conversion risk of GA in the presence of perioperative pain, severe anxiety, and abdominal discomfort.

The primary outcome was to evaluate the successful and safe applicability of SA in vNOTES cases. The secondary outcome was the visual analog scale (VAS) scores (VAS; 0, no pain; 10, severe pain), mean heart rate, and mean arterial pressures during the surgery, presence of postoperative pain, and nausea or vomiting, and postoperative headache.

Anesthesia Procedure

All patients were monitored for vital parameters with electrocardiography, pulse oximetry, and non-invasive pressure monitoring in a supine position. An 18-gage intravenous (IV) vascular access was established, and crystalloid fluid was given at a rate of 8-10 mL/kg/h and sufficient hydration was provided for volume loading before SA (approximately 20-25 min). A 0.03 mg/kg IV midazolam was administered before the procedure to eliminate anxiety.

SA was administered in the sitting position, paying attention to adequate antiseptic rules. A midline approach through the L1-L2 spinous space was preferred. A 25-gage Quincke spinal needle was advanced to the dura mater, and a 0.5% isobaric bupivacaine + 10 µg fentanyl was injected at 0.1 mL/s after confirming a free and clear cerebrospinal fluid flow. The amount of bupivacaine was determined by the anesthetist, considering the patient's height, weight, age, and the intended target sensory anesthetic level (approximately 12.5-15 mg of bupivacaine). Additional midazolam was given IV when a patient complains of increased anxiety during the operation.

All patients were placed in a supine position after the procedure. Anesthesia levels were evaluated with upper

and lower sensory pinprick test and motor block with the modified bromage scale (0: full flexion of the foot and knee; 1: knees and feet movements only; 2: no knee motion, just foot movement; and 3: full paralysis). When the sensory block reached the T4-5 level, the surgeon was allowed to start. All patients were noninvasively monitored for end tidal CO measurement (The ISA OR + sidestream multigaz analyzer Masimo Monitor Radikal 7; Masimo Corp., Irvine, CA, USA).

Adverse events during SA were treated as follows: 250 mL of isotonic saline infused over a 5-min period to treat hypotension [mean arterial pressure (MAP) <60 mmHg], 5 milligrams of IV ephedrine if inadequate with a systolic blood pressure of ≤90 mmHg, 0.5 mg of atropine IV for bradycardia (50 beats/min), 1-2 µg/kg of fentanyl for shoulder pain or abdominal pain/discomfort, and 0.03 mg/kg of midazolam for anxiety. The intraabdominal CO_2 pressure was set at ≤10 mmHg. Peak heart rate, non-invasive blood pressure, and peripheral oxygen saturation (SpO_2) values were recorded during the operation. Patient demographic data, perioperative anesthesia time, operative time, perioperative intraabdominal CO_2 pressure, and complications were obtained from the anesthesia files. Postoperative pain was evaluated at 1, 6, 12, and 24 hours postoperatively as VAS1, VAS6, VAS12, and VAS24, respectively. Additional analgesic drug requirements, length of hospital stay, and presence of postoperative headache, nausea, and vomiting were obtained from the patients' medical records.

NOTES Procedure

A posterior colpotomy was performed in the dorso-lithotomy position under SA. After revealing the cervix, the posterior lip was moved upwards using tenaculum forceps. A 2-3 cm posterior incision was performed. The vaginal mucosa was pushed with blunt and sharp dissections along with the cervical fascia. A self-constructed glove port with an X-Small Alexis Wound Retractor (Applied Medical, Rancho Santa Margarita, CA, USA) was inserted through the posterior colpotomy opening.

After achieving pneumoperitoneum, a 10 mm, 30-degree telescope was inserted (Karl Storz visualization system; Karl Storz Tuttlingen, Germany). Disposable conventional laparoscopic grasping forceps, scissors, bipolar forceps, and tissue sealing devices were used. A suction-irrigation device was used as necessary.

Statistical Analysis

The analyses were performed using the Statistical Package for Social Sciences (SPSS) 20 for Windows (IBM SPSS Inc., Chicago, IL). The normal distribution of data was evaluated with the Kolmogorov-Smirnov test. Normally distributed numerical variables were shown as mean ± standard deviation and minimum and maximum. Categorical variables were expressed as numbers and percentages.

RESULTS

The study group consisted of 12 patients who had ASA I (25%) and ASA II (75%). The mean age of the study population was 40.6±11.6 years (r=29-74) and the mean body mass index (BMI) was 25.9±5.5 kg/m^2 (r=21-39). Of these 12 patients, 50% (n=6) had bilateral salpingoophorectomy, 25% (n=3) had bilateral tubal ligation, 16% (n=2) had salpingectomy due to ectopic pregnancy, and 8.3% (n=1) had unilateral salpingoophorectomy due to unilateral adnexial mass. No laparotomy or CL conversion was performed in any patients and no surgical complication was observed. The mean operative time was 37.2±12.7 min (r=24-60). IV 1 µg/kg of fentanyl was administered to 3 patients (25%) due to perioperative shoulder pain complaints. None of the study participants developed respiratory depression and dyspnea. Hypotension (MAP ≤60mmHg) and bradycardia (peak heart rate ≤50/min) were not observed in any patient. No neurological deficits were observed on the neurological examinations performed postoperatively at 24 hours. The intraoperative data are presented in Table 1 and

Table 1. Perioperative findings

	Mean ± SD or n (%)	Min-max
HR 1st minute	83.8±9.3	69-98
HR 10th minute	77.1±5.3	65-83
HR 20th minute	81.6±12.5	65-101
MAP 1st minute	90.1±15.4	66-110
MAP 10th minute	81.7±14.1	60-105
MAP 20th minute	77.9±9.7	68-100
SPO_2 1st minute	100±0	100-100
SPO_2 10th minute	100±0	100-100
SPO_2 20th minute	100±0	100-100
Intraabdominal pressure	9.2±0.4	9-10
Operative time (min)	37.3±12.7	24-60
Additional fentanyl requirement	3 (25%)	-
Hypotension	-	-
Bradycardia	-	-
Nausea/Vomiting	-	-
Respiratory depression	-	-

HR: Heart rate, MAP: Mean arterial pressure, SPO_2: Saturation of peripheral oxygen, Min-max: minimum-maximum, SD: Standart deviation

Table 2. Postoperative findings

	Mean ± SD	Min-max
VAS1	0	0
VAS6	3.5±0.8	2-5
VAS12	4.4±0.9	3-6
VAS24	3.0±0.9	2-4
Analgesia requirement	1 (8.0%)	
Hospital stay (day)	1	1
Nausea/vomiting	-	-
Headache	1 (8.0%)	-

VAS: Visual analog scale, min-max: minimum-maximum, SD: Standart deviation

postoperative findings are demonstrated in Table 2.

DISCUSSION

Our study revealed that SA is a feasible and effective alternative to GA in benign gynecologic cases operated via vNOTES. SA has become a preferred technique for patient comfort due to its advantages, such as ease of application, absence of hypertensive attacks due to sympathetic stimulation, less postoperative respiratory complications, low cost, early ambulation, and less postoperative pain compared to GA (11,14,15). However, shoulder pain due to diaphragm irritation after CO_2 insufflation and anxiety limit the applicability of SA in CL (13,16). The incidence of shoulder pain for postoperative spinal and epidural anesthesia was reported to be 5.3%-16.6% (17,18). A CL cholecystectomy study under thoracic SA revealed that 25% of patients had shoulder pain and were treated with fentanyl, as in our study (15).

Both SA and pneumoperitoneum have hemodynamic effects. SA causes hypotension due to sympathetic efferent blockade resulting in peripheral vasodilation (19). Using low pneumoperitoneum pressure in patients with adequate hydration decreases splanchnic blood volume and increases venous return, cardiac output, and arterial pressure (14,20). In our study, patients were hydrated before SA was performed with 8-10 mL/kg crystalloid fluid infusion, and low (8-10 mmHg) pneumoperitoneum pressure was used. Therefore, decreased first measured MAP values of patients were observed; however, it was prevented from decreasing to values that require intervention (MAP <60 mmHg). In addition, the SA level was controlled with isobaric bupivacaine, which prevents hypotension that occurs due to a sudden SA level rise.

Hyperbaric SA agent is not recommended for CL cases since the Trendelenburg position causes cephalad spread of the regional anesthesia, causing a greater sympathetic block, bradycardia, and hypotension (4). In our study, isobaric bupivacaine was used and the operation was performed in around 15-degree Trendelenburg position. Thus, no drug-related respiratory distress, respiratory depression, and cardiac problems were observed in our patients.

Nausea and vomiting appear after GA due to the effect of opioids, nitrogen protoxide, tramadol, and other used anesthetics, and postoperative antiemetic requirement increase, which delays the discharge. One of the advantages of SA is the low incidence of postoperative nausea and vomiting (21). A study conducted by Cooper et al. (22) on patients who underwent cesarean with SA reported a statistically significant decreased intraoperative nausea by adding intrathecal fentanyl administration to the local anesthetic agent. None of our patients had nausea and vomiting during the perioperative period.

Under SA, the respiratory mechanism is preserved without significant change in the patient's ventilation parameters and CO_2 levels. The study by Ciofolo et al. (23) investigated the effects of regional anesthesia on ventilation in patients who underwent CL. Arterial blood gases were examined during epidural anesthesia performed at different levels, Trendelenburg position, and CO_2 insufflation. They reported no significant changes in ventilation parameters in the Trendelenburg position and respiratory rates. The ventilation in a minute increased during CO_2 insufflation, without an increased PCO_2 level during operation. According to their study, epidural anesthesia did not affect the ventilation values in CL cases and was a safe alternative to GA (23). Our study reported no problems with respiratory parameters and no increase in the end Tidal CO_2 levels.

Postoperative pain management plays a key role in terms of hospital stay and risk of morbidity. Kalaivani et al. (24) prospectively compared SA and GA techniques in patients with cholecystectomy, which stated no pain in the first postoperative hours and within 8 hours in SA. However, 8% of patients had a VAS score of 4 and rescue analgesia was needed (24). The study by Symeonidis et al. (25) reported significantly lower pain scores at 4, 8, 12, and 24 hours postoperatively and postoperative opioid use was found to be statistically lower in the SA group compared to the GA group in patients who had a cholecystectomy.

To compare the results of low and normal pneumoperitoneum pressure, Gurusamy et al. (26) reported that postoperative pain score, shoulder pain, and opioid use were lower in the group with low pressure (<12 mmHg) compared to normal pneumoperitoneum pressure. Our study reported a low

mean VAS score of patients and postoperative opioids were not used. We postulate that these low VAS scores are achieved due to SA and perioperative low abdominal pneumoperitoneum pressure as in the study by Gurusamy et al. (26).

Study Limitations

Our study had several limitations. Our study was conducted on patients with ASA1-2 and normal BMI to reveal the feasibility of the technique. SA is more appropriate than GA in patients with ASA3-4 and with obesity since these patients have more comorbidity to reduce the risk of postoperative respiratory distress. To generalize our findings, prospective studies with an increased number of patients are needed. In addition, the optimum sensory block level was not determined for vNOTES. The T4-T5 levels, which were determined for CL cases based on previous studies, are appropriate (15,27). One of the most important side effects of SA is urinary retention. Moreover, the postoperative urinary retention data of patients were not analyzed. SA and GA should be compared in the same patient group to reach a more objective conclusion.

CONCLUSION

SA is used without any major complications as a feasible anesthesia technique in vNOTES gynecologic cases with tolerable postoperative pain and a more comfortable postoperative period.

ETHICS

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Bakirköy Dr. Sadi Konuk Training and Research Hospital of Local Ethics Committee (protocal no: 2020/91).

Informed Consent: All patients were informed and written informed consent was obtained.

Authorship Contributions

Surgical and Medical Practices: G.Ö.Y., Concept: G.Ö.Y., C.K., Design: G.Ö.Y., C.K., Data Collection or Processing: G.S., Analysis or Interpretation: C.K., Literature Search: G.Ö.Y., A.F.T., G.S., Writing: G.Ö.Y., G.O.H., Z.Ç.

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Research

Management of Phyllodes Tumors of the Breast: Our Clinical Experience

Memenin Filloid Tümörlerinin Yönetimi ve Klinik Deneyimlerimiz

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ABSTRACT

Objective: This study aimed to examine the demographic features, clinicopathological findings, follow-up results, and treatment methods of patients treated for phyllodes tumors of the breast in light of the literature.

Methods: All patients with benign, borderline, or malignant phyllodes tumors admitted to our hospital between 2013 and 2020 were retrospectively analyzed.

Results: The study included a total of 14 patients with phyllodes tumors of the breast on histopathological examinations, wherein 6 were benign (42.9%), 3 were borderline (21.4%), and 5 were malignant (35.7%). The mean age was similar in the groups (p=0.654). Tumors were more frequently located in the right breast; however, no difference was found between the groups in terms of laterality (p=0.514). The tumor diameter was significantly smaller in benign phyllodes (benign: 32 mm, borderline: 72 mm, and malignant: 80 mm, p=0.036). Benign phyllodes were more frequently breast imaging-reporting and data system (BI-RADS) 3 and malignant phyllodes were BI-RADS 5; however, the difference was not significant. A core needle biopsy was performed in eight patients as the most common biopsy method (57.1%). The most common surgical procedure was segmental mastectomy (71.4%). Re-excision was performed in three patients due to the surgical margin positivity or closeness. One patient received chemotherapy and radiotherapy (RT) and 4 patients only received RT. The mean follow-up time was 53.8±25.4 months (13-96 months). Local recurrences occurred in two patients during the follow-up. Distant metastases or deaths were not observed.

Conclusion: Phyllodes tumors are rare, mixed-type breast tumors. Histopathological features and classification guide the management plan. Extensive analysis of phyllodes tumors is still lacking, and more studies are necessary to understand the behavior of this rare breast tumor.

Keywords: Breast, phyllodes tumor, fibroadenoma, surgery

ÖZ

Amaç: Filloid meme tümörü tedavisi gören hastalarımızın demografik özelliklerini, klinikopatolojik bulgularını, takip sonuçlarını ve tedavi yöntemlerini literatür ışığında incelemeyi amaçladık.

Gereç ve Yöntem: Kurumumuza 2013-2020 yılları arasında başvuran benign, borderline veya malign filloid tümörlü tüm hastalar retrospektif olarak incelendi.

Bulgular: Histopatolojik olarak 6 benign (%42,9), 3 borderline (%21,4) ve 5 malign filloid tümör (%35,7) olmak üzere toplam 14 hasta dahil edilebildi. Ortalama yaş gruplarda benzerdi (p=0,654). Sağ meme daha sık lokalize olmasına rağmen lokalizasyon açısından gruplar arasında farklılık yoktu (p=0,514). Benign grupta tümör çapı düşüktü (benign: 32 mm, borderline: 72 mm, malign: 80 mm, p=0,036). Benign grupta BIRADS 3 ve malign grupta BIRADS 5 ağırlık olmasına rağmen, istatistiksel olarak fark yoktu. En yaygın biyopsi yöntemi olarak 8 (%57,1) hastaya tru-cut biyopsi yapıldı. En yaygın cerrahi prosedür segmental mastektomiydi (%71,4). Üç hastaya cerrahi sınırın pozitifliği veya yakınlığı nedeniyle yeniden eksizyon yapıldı. Bir hasta kemoterapi ve radyoterapi aldı ve 4 hasta radyoterapi aldı. Ortalama takip süresi 53,8±25,4 aydı (13-96). Takip sırasında 2 hastada lokal nüks meydana geldi. Uzak metastaz veya ölüm yoktu.

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Sonuç: Phyllodes tümörler nadir görülen, mikst tip meme tümörleridir. Yönetim planına histopatolojik özellikler ve sınıflandırma rehberlik eder. Filloid tümörlerin kapsamlı analizi hala eksiktir ve bu nadir meme tümörünün davranışını anlamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Meme, filloid tümör, fibroadenom, cerrahi

INTRODUCTION

Phyllodes tumors are rare fibroepithelial lesions consisting of 2%-3% of all fibroepithelial tumors and 0.3%-0.5% of all breast tumors in women, which are clinically confused with fibroadenomas. A large, rapidly growing mass and detection of stromal hyperplasia and atypia on histopathological examination should suggest a phyllodes tumor. Due to their rarity, literature data are limited. The World Health Organization (WHO) classifies phyllodes tumors into three histological subtypes based on the number of histopathological parameters, i.e., benign, borderline, and malignant (1,2).

Surgery forms the basis for phyllodes tumor treatment. Preoperative diagnosis is very important for correct surgical planning. Difficulties in diagnosis during the preoperative period cause mistakes in treatment planning. Traditionally, considering the risk of local recurrences, wide local excision with a tumor-free border of 1 cm or more is recommended regardless of the subtype. Lumpectomy or partial mastectomy is the preferred surgical treatment. Total mastectomy is only necessary when negative margins are not achieved with breast-conserving surgery. Considering that a phyllodes tumor rarely metastasizes to the axillary lymph nodes (10%-15%), surgical axillary staging is not required unless lymph nodes are palpated upon clinical examination (3,4).

The borderline and malignant phyllodes tumor metastasis rate is approximately 25%-31%, whereas the overall rate of all phyllodes tumor metastases is 4%. In addition, WHO reported a local recurrence rate of 21% (17%, 25%, and 27% for benign, borderline, and malignant phyllodes tumors, respectively) (5,6). Surgical margin positivity was reported in the literature to be associated with tumor size, surgical treatment technique, and tumor-related histopathological features (7,8).

Evidence from randomized controlled trials and available data in the literature is insufficient to determine the treatment approach for phyllodes tumors. Therefore, this study aimed to examine the demographic characteristics, clinicopathological findings, follow-up results, and treatment methods of patients treated for phyllodes tumors of the breast in light of the literature.

METHODS

After obtaining approval from the Ethical Committee of Adana City Training and Research Hospital on January 27, 2021 (approval number: 75/1269), data about patients with phyllodes tumors treated in our hospital between 2013 and 2020 were retrospectively analyzed. Data were gathered from the results of pathological examinations and soft and hard copies of the patient files. Missing information about the patients was obtained by phoning them. Patients were divided into three groups based on the histopathological examination results, benign, borderline, and malignant. Age, complaints on admission, tumor location, surgery, breast imaging-reporting and data system (BIRADS) score, pathological features, tumor size, postoperative recurrence, and metastasis were compared in these groups.

Ultrasonography (USG) and mammography (MMG) were used as primary imaging methods. Histopathological diagnosis was made through core needle, excisional, and incisional biopsy methods. Pathologically, phyllodes tumors were classified into benign, borderline, and malignant based on the criteria reported by the WHO (1).

In the present study, the presence of 0-4 mitoses in each field examined under 10 X magnification, minimal stromal cellularity, and minimal and moderate stromal development was considered benign phyllodes tumor. The presence of 5-9 mitoses in each field examined under 10 X magnification, moderate stromal cellularity, and atypia was regarded as a borderline phyllodes tumor. The presence of >10 mitoses in each field examined under 10 X magnification, moderate or severe stromal cellularity, atypia, and overgrowth and infiltrates to the surrounding tissues indicated a malignant phyllodes tumor.

A wide local excision, breast-conserving surgery, mastectomy, or mastectomy, and axillary lymph node dissection [modified radical mastectomy (MRM)], which are the treatment methods to be performed, were selected to leave a clean surgical margin of at least 1 cm according to the location, size, and histopathological diagnosis of the tumor. The close surgical margin was considered a clean surgical margin of <1 cm in all types.

Statistical Analysis

Statistical analysis was made using the Statistical Package Program for Social Sciences 24.0 (IBM Corporation,

Armonk, NY, USA). Descriptive data were expressed in mean and standard deviation for quantitative variables and in frequency and percentage for qualitative variables. Data with a normal distribution were evaluated using the Student's t-test, whereas data without a normal distribution were evaluated using the Fisher's Exact or Pearson's chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 14 patients were included in the study, wherein 6, 3, and 5 were assigned into the benign, borderline, and malignant groups, respectively. The mean age was similar in the groups (p=0.654). Tumors were more frequently located in the right breast accounting for 78.6% of the cases; however, no significant difference was found in terms of laterality (p=0.514). All patients presented with a palpable mass. The tumor diameter was significantly smaller in the benign group (benign group: 32 mm, borderline group: 72 mm, and malignant group: 80 mm, p=0.036). A higher rate of tumors in the benign and malignant groups was BI-RADS 3 and BI-RADS 5; however, the difference was not statistically significant (p=0.141).

Regarding the biopsy method, a core-needle biopsy was performed in eight patients (57.1%), excisional biopsy in five patients (35.7%), and incisional biopsy in one patient (7.1%). No significant difference was found in the biopsy methods between groups (p=0.178)

Breast-conserving surgery was performed with a wide local excision in all six patients with benign phyllodes tumors. Two patients had close resection margins. These patients did not undergo re-excision surgery. No local recurrence was observed in the benign phyllodes group during the follow-up.

Three patients had borderline phyllodes tumors (5%). One of these patients immediately underwent a simple mastectomy after the core needle biopsy due to advanced tumor size. Another patient had a positive resection margin after breast-conserving surgery and underwent a mastectomy. She also had a submuscular prosthesis for cosmetic reasons. The last patient underwent re-excision due to the proximity of the surgical margin after wide local excision.

Five patients had malignant phyllodes tumors (35.7%). Three of them underwent breast-conserving treatment, one underwent a mastectomy, and one underwent MRM. One of the patients who underwent breast-conserving surgery was found with malignant phyllodes tumor and low nuclear grade intraductal carcinoma foci within the tumor on the permanent pathology sections after wide local excision. No tumor was left in the surgical resection margins. Sentinel lymph node biopsy was not performed. The tumor diameter was 12 cm in the patient who underwent a mastectomy, which was performed immediately after incisional biopsy. The patient who underwent MRM had a malignant phyllodes tumor accompanied by invasive ductal carcinoma detected on segmental resection. Positivity was found in some parts of the surgical margins and axillary lymph node involvement on USG. Therefore, MRM was subsequently performed.

None of the patients with benign and borderline phyllodes tumors received chemotherapy or radiotherapy (RT). All patients with malignant phyllodes tumors received RT, and the patient with invasive cancer and malignant phyllodes tumors received chemotherapy and hormone therapy.

The mean follow-up period was 53.8±25.4 months (13-96 months). Local recurrences occurred in two patients during the follow-up. One of them had a malignant phyllodes tumor and underwent re-excision surgery due to tumor-positive resection margins. However, she had a malignant relapse in the tenth month, thus a mastectomy was performed. The other patient developed a borderline phyllodes tumor recurrence locally 14 months after the primary breast-conserving surgery despite the excision surgery for borderline phyllodes tumor. This patient was also treated with mastectomy. No distant metastases or deaths were observed Table 1.

DISCUSSION

The uncertain and heterogeneous biological behavior of phyllodes tumors makes the diagnosis and management more difficult. Therefore, clinicians relied heavily on reports from retrospective studies of this tumor's behavior to improve early diagnosis and use correct treatment strategies. In the present study, data of 14 female patients diagnosed with phyllodes tumor in our tertiary hospital over an 8-year-period were reviewed, and obtained results will contribute to the reported data.

Phyllodes tumors are more common in women aged 35-55 years and the frequency of malignant phyllodes tumors increases in the older age group (9,10). The mean age in our series was 34.5 years; however, no relationship was found between the histological subtype and age. In a large case series in the literature, benign, borderline, and malignant tumors were found in 72.7%, 18.4%, and 8.9% of 605 patients, respectively. In another study, benign, borderline, and malignant phyllodes tumors were reported in 60%, 20%, and 20% of patients, respectively (11,12). In our series, benign, borderline, and malignant phyllodes tumors were

Table 1. The distribution of clinicopathological features by histopathological subtypes

	Total	Benign phyllodes	Borderline phyllodes	Malignant phyllodes	p
Number of patients	14	6 (42.9%)	3 (21.4%)	5 (35.7%)	-
Age (years) ± SD (min-max)	39.6±15 (22-70)	35.1±10.6 (25-55)	41.6±25.1 (22-70)	43.8±14.9 (26-63)	0.654
Laterality					
Right	11 (78.6%)	4 (28.6%)	3 (21.4%)	44 (28.6%)	0.514
Left	3 (21.4%)	2 (14.3%)	0	11 (7.1%)	
Size (mm) ± SD (min-max)	54.9±31 (22-120)	31.5±7 (22-40)	67.3±34 (32-100)	75±33.1 (40-120)	0.036
BI-RADS					
III	-	4 (28.6%)	1 (7.1%)	0	0.141
IV	-	2 (14.3%)	1 (7.1%)	2 (14.3%)	
V	-	0	1 (7.1%)	3 (21.4%)	
Biopsy method					
Core needle	8 (57.1%)	2 (14.3%)	2 (14.3%)	4 (28.6%)	0.178
Excisional	5 (35.7%)	4 (28.6%)	1 (7.1%)	0	
Incisional	1 (7.1%)	0	0	1 (7.1%)	
Surgical method					
Breast conserving	10 (71.5%)	6 (42.9%)	1 (7.1%)	3 (21.4%)	0.129
Mastectomy	3 (21.4%)	0	2 (14.3%)	1 (7.1%)	
MRM	1 (7.1%)	0	0	1 (7.1%)	
Re-excision for positive or close margin	3 (21.4%)	0	1 (7.1%)	2 (14.3%)	0.233
Margin status					
Negative	12 (85.7%)	4 (28.6%)	3 (21.4%)	5 (35.7%)	0.211
Close	2 (14.3%)	2 (14.3%)	0	0	
Positive	0	0	0	0	
Local recurrence	2 (14.3%)	0	1 (7.1%)	1 (7.1%)	0.364

BI-RAD: Breast imaging reporting and data system, MRM: Modified radical mastectomy, SD: Standard deviation, min-max: minimum-maximum

detected in 42.9%, 21.4%, and 35.9% of cases, respectively. The rate of malignant phyllodes tumors in this series was higher than that reported in the literature.

Phyllodes tumors widely vary in size. This variation in tumor size is due to the late diagnosis of the tumor caused by diagnostic difficulties and the benign or malignant nature of the tumor. In general, tumor size varies with benign and borderline or malignant phyllodes tumors (9). In a study by Wang et al. (9), the mean primary tumor diameter of the histological subtypes benign, borderline, and malignant phyllodes tumors was found to be 3.7 cm, 4.8 cm, and 7.5 cm ($p<0.000$), respectively. Based on multivariate analysis, larger masses were reported to be more likely malignant ($p=0.052$, odds ratio: 1.127) (9). In our series, the mean tumor diameter was 5.4 cm, and borderline and malignant tumors had a larger tumor diameter.

Phyllodes tumor diagnosis is difficult in daily clinical practice. Failure to preoperatively diagnose malignant phyllodes tumors causes short-term recurrences and even distant metastases due to insufficient excision. Contrarily, radiological and histopathological examinations of phyllodes tumors often yield similar findings to the examinations of fibroadenomas, thus clinical suspicion is essential for diagnosis (13). MMG and USG used in the diagnosis of breast masses are not very reliable methods in making the differential diagnosis of phyllodes tumors from fibroadenomas (14). Fine needle aspiration biopsies are generally inadequate due to their high false-negative rates. Core needle biopsies play an important role in the diagnosis of phyllodes tumors; however, false-negative results at the rates of 25%-30% were reported (15,16). In our series, the BIRADS 4-5 was more common in patients with the malignant subtype.

The treatment of phyllodes tumors is surgical excision with sufficient margins. Recurrences and the need for reoperation increase after inadequate surgery (17). In a multivariate analysis performed with 172 patients, insufficient margins were found to play a role in the emergence of local recurrences and metastases (18). Different opinions were presented on the surgical method to be chosen in the literature. Sotheran et al. (19) recommended a wide local excision in phyllodes tumors, whereas Sotheran et al. (19) recommended mastectomy (20). Kapiris et al. (21) could not find a difference between wide local excision and mastectomy in the presence of negative margins. In the current study, segmental mastectomy was performed in patients for whom clean surgical margins are achieved, and mastectomy or subcutaneous mastectomy was performed in the patients in whom clean surgical margins were not achieved or poor cosmetic results were likely to appear after excision.

The benefit of adjuvant chemotherapy for phyllodes tumors is controversial. No prospective or randomized studies were reported about the effect of adjuvant chemotherapy on this type of tumor. Using adjuvant RT is also controversial in the literature. The National Comprehensive Cancer Network and other studies recommend the use of RT in cases of recurrent malignant phyllodes. Other studies recommend adjuvant RT to reduce the likelihood of local recurrences in patients with borderline and malignant phyllodes tumors treated with breast-conserving surgery. Contrarily, RT did not increase the overall survival (OS) and disease-free survival rates (22,23). In our series, five patients received RT who had malignant phyllodes tumors. The patient receiving chemotherapy are those with invasive carcinoma.

In the series of the MD Anderson cancer center, 5-year OS rates in patients with benign and malignant phyllodes tumors were 91% and 82%, respectively (24). According to data from the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute, the 5-year OS rate was 91% for malignant cases (25). In another study, the 3-year survival rate for benign/borderline tumors was 100%, whereas 53.4% in patients with malignant phyllodes tumors (26). Acar et al. (14) reported that the 5-year OS rate was 89% for borderline and malignant cases. Kündeş et al. (27) found that the 5-year survival was 93.8% and 70% in borderline and malignant tumors, respectively. In our series, none of the patients died during the 53-month follow-up.

Local recurrence rates in the follow-up of patients with phyllodes tumors were reported to range from 10% to 40% in different studies. Local recurrences are often detected in the breast tissue, but rarely, local-regional recurrences including the chest wall can be detected. Especially in borderline and malignant phyllodes tumors, the probability of local recurrence was reported to be 21% even if the surgical margins are negative (28,29). Metastasis rates for phyllodes tumors vary between 13% and 40%. The most common site of metastasis is in the lungs. The current approach to the treatment of metastatic lesions is surgical excision (28). In our series, during the 53-month follow-up period, local recurrences developed in two patients with borderline and malignant phyllodes tumors. Three patients underwent re-excision due to positive surgical margins and one of them had a local recurrence. In the present study, the total local recurrence rate was 14% and none of the patients developed distant organ metastasis.

CONCLUSIONS

Phyllodes tumors are rare breast tumors with variable biological behavior and heterogeneous radiological and clinical manifestations. Histopathological features and classification guide the management plan. Extensive analysis of phyllodes tumors is still lacking, and more studies are necessary to understand the behavior of this rare breast tumor.

ETHICS

Ethics Committee Approval: The study were approved by the Adana City Training and Research Hospital of Local Ethics Committee (protocol number: 27.01.2021/75/1269).

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: O.İ., O.E., Concept: O.E., A.P., Design: O.E., O.İ., Data Collection or Processing: Z.A.T., U.T., Analysis or Interpretation: U.T., O.E., Literature Search: U.T., O.E., O.İ., Writing: O.E., U.T.

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

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Research

The Relationship Between Warfarin Resistance and CYP2C9*2 and CYP2C9*3 Variatıons

Varfarin Direnci CYP2C9*2 ve CYP2C9*3 Varyasyonlarıyla İlişkili Olabilir

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ABSTRACT

Objective: Warfarin is one of the most widely used anticoagulants worldwide. Some patients need >15 mg/day of warfarin to get their therapeutic international normalized ratio (INR). This condition is known as warfarin resistance (WR). WR is related to enzyme deficiencies, which play a role in warfarin metabolism. One of the most important enzyme-related drug metabolism is the cytochrome P450, family 2, subfamily C, member 9 (*CYP2C9*). Therefore, this study aimed to investigate the relationship between *CYP2C9* variations and WR.

Methods: To find patients with WR, 650 patients who used warfarin for at least 6 months were screened. Then, patients were grouped into two according to the INR values, wherein 30 patients with INR levels not reaching the therapeutic range (<2) despite using 15 mg of warfarin per day were included in the non-responder group and 30 randomly selected patients who received low-dose warfarin, whose INR levels were within the therapeutic range (2-3), were included in the responder group. After the genomic deoxyribonucleic acid isolation from the peripheral blood, *CYP2C9*2* and *CYP2C9*3* variations were investigated using the real-time polymerase chain reaction. Results were statistically evaluated.

Results: Heterozygous genotype of *CYP2C9*3* was statistically high in responders (33.3%), whereas the wild-type genotype was statistically high in nonresponders (90%) ($p<0.05$). In addition, the T allele of *CYP2C9*2* (18.3%) and the C allele of *CYP2C9*3* (16.7%) were statistically high in responders ($p<0.05$).

Conclusion: Patients with gene variations that reduced the *CYP2C9* activity are termed poor metabolizers. These individuals metabolize warfarin more slowly and require smaller doses of the drug to reach the therapeutic INR values. Therefore, adjusting the warfarin dose is possible depending on the genotype of patients.

Keywords: Warfarin resistance, *CYP2C9*, RT-PCR

ÖZ

Amaç: Varfarin, dünyada en yaygın kullanılan antikoagülanlardan biridir. Bazı hastalar, uluslararası normalize oranlarını (INR) terapötik aralığa getirmek için günde 15 mg'dan fazla varfarine ihtiyaç duyar. Bu durum, varfarin direnci (VD) olarak bilinir. VD, varfarin metabolizmasında rol oynayan enzim eksiklikleri ile ilişkili olabilir. İlaç metabolizması ile ilgili en önemli enzimlerden biri sitokrom P450, aile 2, alt aile C, üye 9'dur (*CYP2C9*). Bu nedenle bu çalışmada, VD ile *CYP2C9* varyasyonları arasındaki ilişkinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: VD olan hastaları bulabilmek için en az 6 ay varfarin kullanan 650 hasta tarandı. Daha sonra hastalar içerisinden INR değerlerine göre 2 grup oluşturmak için hasta seçimi yapıldı. Günde 15 mg varfarin kullanmasına rağmen INR düzeyi terapötik aralığa gelmeyen (<2) 30 kişi ilaca yanıt vermeyen gruba, düşük doz varfarin kullanarak INR düzeyleri terapötik aralığa (2-3) gelen ve tüm hastalar arasından rastgele seçilen 30 hasta ise tedaviye yanıt veren gruba dahil edildi. Periferik kandan genomik DNA izole edildikten sonra, *CYP2C9*2* ve *CYP2C9*3* varyasyonları gerçek zamanlı polimeraz zincir reaksiyonu (GZ-PZR) kullanılarak araştırıldı. Sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: Tedaviye yanıt veren grupta *CYP2C9*3* heterozigot genotipinin (%33.3), tedaviye yanıt vermeyen grupta ise yabani tip genotipin istatistiksel olarak yüksek olduğu (%90) belirlendi ($p<0,05$). Ek olarak, tedaviye yanıt veren grupta *CYP2C9*2* T alleli (%18.3) ve *CYP2C9*3* C alleli (%16,7) istatistiksel olarak yüksek bulundu ($p<0,05$).

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Sonuç: Hastalarda *CYP2C9* aktivitesinde azalmaya neden olan gen varyasyonları varsa, bunlar zayıf metabolize ediciler olarak adlandırılır. Bu kişiler, varfarini daha yavaş metabolize ederler ve terapötik INR değerlerine ulaşmak için daha küçük dozlarda ilaca ihtiyaç duyarlar. Bu nedenle hastaların genotipine bağlı olarak varfarin dozunun ayarlanması mümkündür.

Anahtar Kelimeler: Varfarin direnci, *CYP2C9*, GZ-PZR

INTRODUCTION

Atrial fibrillation (AF) is one of the most known abnormal heart rhythm disorders that affects >30 million people worldwide. Patients with a high risk of AF usually take anticoagulants, such as warfarin (1). The risk of embolism, especially the risk of ischemic stroke, increases in AF associated with thrombus formation in the left atrium (2). Pulmonary embolism (PE) is a life-threatening condition and is one of the most common causes of cardiovascular death, which is a known complication of deep venous thromboembolism (DVT) (3,4). Mechanical heart valves are implanted in patients with long life expectancy, which are more durable than bioprostheses. However, the disadvantage is the lifelong requirement of anticoagulants, such as warfarin, due to their higher thrombogenicity (5).

Warfarin is one of the most used anticoagulants worldwide, which reduces vitamin K formation by inhibiting vitamin K epoxide reductase, a multiprotein enzyme complex that activates vitamin K. Prothrombin and clotting factors VII, IX, and X have reduced coagulation ability without adequate active vitamin K. Anticoagulant protein S and C are also inhibited in this process. Thus, blood clotting decreases (6). Age, gender, body mass index, smoking, drug therapy, concurrent hepatic or renal disease, and dietary vitamin K intake also play a role in warfarin metabolism (7-9).

The international normalized ratio (INR), developed by the World Health Organization in the early 1980s, is widely used to monitor oral anticoagulation and evaluate patients with coagulation disorders, which was designed to eliminate problems in oral anticoagulants (10). It is the test of choice for patients taking vitamin K antagonists (VKA). INR is also used to assess patients' risk of coagulation or bleeding. Patients receiving oral anticoagulants need to monitor the INR values to adjust their VKA dose, as this value differs between patients (11). INR value is around 1 in healthy individuals. INR ratio differs according to each disease. INR value should be between 2-3 in patients with cerebro-cardiovascular occlusion or with DVT/PE and 2.5-3.5 in patients with heart rhythm disorders, such as heart valve disease or AF (12). Patients usually receive 5 mg of warfarin daily; however, some patients require >15 mg of warfarin daily to get the INR value into the therapeutic range. This condition is defined as warfarin resistance (WR) (13). WR is associated with enzyme deficiencies involved in warfarin metabolism (14). cytochrome P450, family 2, subfamily C, member (*CYP2C9*) is one of the cytochrome p450 enzymes involved in the metabolism of many drugs, which is also involved in warfarin metabolism (15). *CYP2C9*2* and *CYP2C9*3* variations are frequently encountered in the *CYP2C9* gene encoding the *CYP2C9* enzyme. *CYP2C9*2* (rs1799853) variation causes Arg144Cys amino acid change due to a C430T nucleotide change in exon 3. *CYP2C9*3* (rs1057910) variation causes Ile359Leu amino acid change due to the A1075C nucleotide change in exon 7. Both variations decrease enzymatic activities (16). The variations in the *CYP2C9* gene are associated with WR, thus this study aimed to investigate the relationship between the *CYP2C9* gene variations and WR.

METHODS

Study Population

WR is a rare condition. Therefore, 650 patients who applied to the cardiology clinic of Haydarpaşa Numune Training and Research Hospital and used warfarin for at least 6 months and had AF, mechanical aortic prosthetic valve, and DVT or PE were screened to find those with WR. Then, patients were grouped into two according to the INR values, wherein 30 patients, whose INR level did not reach the therapeutic range (<2) despite using 15 mg of warfarin per day, were included in the non-responder group and 30 randomly selected patients who received low-dose warfarin, with INR levels within the therapeutic range (2-3) were included in the responder group. Patients who have liver disease, heart failure, or hematologic disorders were excluded from the study. Patients who used aspirin, non-steroidal anti-inflammatory drugs, antifungals (fluconazole), antibiotics, statin group drugs, tricyclic antidepressants, and thyroid drugs within the last week were excluded from the study, together with those alcoholic and pregnant. The study, which is compatible with the Helsinki Declaration, was approved by the Clinical Research Ethics Committee of Haydarpaşa Numune Training and Research Hospital (approval date: december 30, 2019; approval number: 2019/166-1096). Each individual was informed about the study and written informed consent was obtained from each participant.

Blood Sampling and Genotyping

Whole blood samples were obtained from all patients. Deoxyribonucleic acid (DNA) was extracted from 200 µL of peripheral blood using commercially available kits (Qiagen, Foster City, USA) according to the manufacturer's instructions. DNA purity and concentrations were determined by NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA). Real-time polymerase chain reaction (RT-PCR) reactions for *CYP2C9*2* and *CYP2C9*3* were carried out on 7500 fast RT-PCR System (Applied Biosystems). The reaction was performed according to the manufacturer's instructions.

Statistical Analysis

Statistical Package for the Social Science 25.0 was performed for statistical analysis (IBM Corp. released 2017, IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.). Continuous variables are expressed as mean ± standard deviation and discrete variables are expressed as counts or percentages. Normal distribution assumption was checked with the Kolmogorov-Smirnov test. Two independent samples t-test was used to compare continuous variable means between two groups, which were normally distributed. The Kruskal-Wallis tests were performed to investigate the difference between genotypes and risk factors (which are not normally distributed). The Mann-Whitney U test was performed for pairwise comparison in statistically significant differences, and Bonferroni correction was applied to the p-values. The p-values of <0.05 ($p<0.05$) were considered statistically significant.

RESULTS

Study Population

The demographic characteristics of patients are shown in Table 1. Demographic characteristics comparison between groups revealed a statistically significant relationship between age and INR values.

CYP2C9 Genotyping

Table 2 shows the nucleotide distributions among the groups. The genotype distribution comparison between the groups revealed a statistically high heterozygous genotype of *CYP2C9*3* (33.3%) in responders, whereas a statistically high wild-type genotype was found in nonresponders (90%) ($p<0.05$).

Allele Frequencies of CYP2C9 Variations

Table 3 shows the allele frequencies of groups. The group comparison in terms of allele frequencies revealed a statistically high T allele of *CYP2C9*2* (18.3%) and C allele of *CYP2C9*3* (16.7%) in responders ($p<0.05$).

Table 1. Demographic characteristics of patients

Demographic characteristics		Groups: Non-responders (n=30)	Groups: Responders (n=30)	p
Age (year)		53.17±13.48	66.4±12.46	<0.001**
Average INR value		1.96±0.39	2.59±0.24	<0.001**
Warfarin indication	Atrial fibrillation	11	18	0.118
	Deep vein thrombosis or pulmonary embolism	6	6	
	Mechanical aortic prosthetic valve	13	6	
Gender	Female	21	17	0.284
	Male	9	13	

**p<0.001, INR: International normalized ratio

Table 2. Distribution of nucleotide variations between groups

Genes, genotypes, nucleotide variations	Groups: Non-responders (n=30)	Groups: Responders (n=30)	p
CYP2C9*2			
CC	27(90%)	22 (73.3%)	0.135
CT	3 (10%)	5 (16.7%)	
TT	0 (2.8%)	3 (10%)	
CYP2C9*3			
AA	*27 (90%)	20 (66.7%)	0.029*
AC	3 (10%)	*10 (33.3)	
CC	0 (0%)	0 (0)	
*p<0.05			

Table 3. Allele frequencies of groups

Alleles	Groups: Non-responders (n=30)	Groups: Responders (n=30)	p
CYP2C9*2			
C	57 (95%)	49 (81.7%)	**0.012***
T	3 (5%)	*11 (18.3%)	
CYP2C9*3			
A	57 (95%)	50 (83.3%)	**0.037***
C	3 (5%)	*10 (16.7%)	
*p<0.05			

DISCUSSION

Warfarin is frequently used for venous thromboembolism treatment after mechanical heart valve implantation and thromboembolic complication prevention in AF (15). It is metabolized by *CYP2C9*, one of the cytochrome P450 enzymes, to the 7-hydroxylated form. Many variants were identified in the *CYP2C9* gene which encodes the CYP2C9 enzyme. *CYP2C9*2* and *CYP2C9*3* are best-characterized variations of *CYP2C9* (13). Individuals with *CYP2C9*2* and *CYP2C9*3* genotypes have 12% and 5% lower enzyme activity than the wild type, respectively, and are prone to bleeding after warfarin treatment (17). *CYP2C9*2* reduced warfarin metabolism by 30% and *CYP2C9*3* by 80% compared with *CYP2C9*1*, which is a wild-type variant of *CYP2C9* (18). Individuals carrying the *CYP2C9*2* and *CYP2C9*3* variants reach the targeted INR level with lower amounts of warfarin (13). Ozer et al. (19) found that individuals with the *CYP2C9*1*1* genotype had a higher daily warfarin dose than those with the *1*3/*2*3 genotype. The same study revealed a similar result of undetected *1*2 genotype. Another study revealed no significance between the WR and *CYP2C9* variations (13). Dilge Taşkın et al. (18) showed that *CYP2C9* variants were detected at the rates of *1/*1 (54.6%), *1/*2 (16.4%), *1/*3 (24.2%), *2/*3 (2.9%), and *3/*3 (1.9%). The same study concluded that patients with variations needed lower warfarin doses (18). Another study found that *CYP2C9*2* and *CYP2C9*3* variations of *CYP2C9* decrease the rates of warfarin clearance. Another study found a significant association between *CYP2C9*3* with warfarin therapy (20). The allele frequency comparison between the groups in our study revealed a statistically high T allele in *CYP2C9*2* (18.3%) and C allele in *CYP2C9*3* (16.7%) in responders ($p<0.05$). In addition, the genotype distribution comparison between the groups revealed a statistically high CYP2C9*3 (33.3%) variation in responders ($p<0.05$). The wild-type genotype of *CYP2C9*3* was significantly high in nonresponders ($p<0.05$).

The limitation of our study is the small number of patients (n=60). However, considering that WR is an extremely rare condition, it is acceptable to screen approximately 650 patients and include 30 patients with WR in the study.

CONCLUSION

In conclusion, individuals with *CYP2C9* homozygous wild-type genotype required higher daily warfarin dose compared to the *2 and *3 variants. Particularly, patients with gene variations reduced *CYP2C9* activity, which is termed as poor metabolizers. These individuals metabolize warfarin more slowly and require smaller doses of the drug to reach the therapeutic INR values. Therefore, adjusting the warfarin dose is possible depending on the genotype of patients.

ETHICS

Ethics Committee Approval: The study, which is compatible with the Helsinki Declaration, was approved by the Clinical Research Ethics Committee of Haydarpaşa Numune Training and Research Hospital (approval date: december 30, 2019; approval number: 2019/166-1096).

Informed Consent: All patients and/or legal guardians included in the study provided their written informed consent.

Authorship Contributions

Surgical and Medical Practices: A.E.Y., Concept: D.K., A.E.Y., Design: D.K., A.E.Y., E.Ç.A., Data Collection or Processing: D.K., A.E.Y., T.D., E.Ç.A., Analysis or Interpretation: D.K., A.E.Y., T.D., E.Ç.A., Literature Search: D.K., T.D., Writing: D.K., T.D., E.Ç.A.

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

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Research

Does High-Density Lipoprotein Have a Role in Dysregulated Host Response in Sepsis?

Sepsisteki Disregüle Konak Yanıtında HDL'nin Rolü Var Mı?

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ABSTRACT

Objective: Serum lipoprotein levels [high-density lipoprotein (HDL), low-density lipoprotein (LDL)], total cholesterol, and triglyceride) have protective functions against sepsis. Reduced levels are associated with organ dysfunction and mortality. This retrospective cohort study aimed to investigate the relationship between the serum lipoprotein levels and sepsis in a large population of patients admitted in the intensive care unit.

Methods: Serum lipoprotein levels of 151 patients diagnosed with sepsis and 450 without sepsis were analyzed retrospectively. The level of lipoprotein decreased in the sepsis and non-sepsis groups, and appropriate statistical methods were used.

Results: Lipoprotein levels were significantly lower in the sepsis group ($p<0.05$). No significant difference was found between the survivor group and non-survivor group in terms of lipoprotein levels ($p>0.05$). Among patients with intra-abdominal sepsis and pneumosepsis, a significant difference was found in the levels of HDL, LDL, and total cholesterol between patients admitted in the medical and surgical intensive care units ($p<0.05$). HDL levels were lower in patients with gram-negative bacterial infection than those with gram-positive bacterial infection.

Conclusion: This study provides evidence of the role of serum lipoproteins, particularly HDL, in the pathogenesis of sepsis, suggesting that lipoproteins may offer new avenue in the treatment of sepsis.

Keywords: Sepsis, lipoprotein, albumin effect, HDL

ÖZ

Amaç: Serum lipoprotein düzeylerinin [yüksek yoğunluklu lipoprotein (HDL) ve düşük yoğunluklu lipoprotein (LDL)] total kolesterol ve trigliserid sepsise karşı koruyucu fonksiyonu vardır. Azalmış düzeyler, organ disfonksiyonu ve mortalite ile ilişkilidir. Bu retrospektif kohort çalışmasının amacı, geniş bir yoğun bakım hasta popülasyonunda sepsis ve serum lipoprotein düzeyleri arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntem: Sepsis tanısı konan 151 hasta ve 450 sepsis olmayan hastanın serum lipoprotein düzeyleri retrospektif olarak analiz edildi. Sepsis ve sepsis olmayan hastalarda lipoprotein düzeyleri karşılaştırıldı.

Bulgular: Lipoprotein düzeylerinin sepsis hastalarında daha düşük olduğu belirlendi ($p<0,05$). Hayatta kalan ve mortalite gelişen hastalar arasında lipoprotein düzeyleri açısından anlamlı fark yoktu ($p>0,05$). İntraabdominal sepsis ve pnömosepsis nedeniyle takip edilen medikal ve cerrahi yoğun bakım hastalarında HDL, LDL ve total kolesterol düzeyleri anlamlı farklılık gösterdi ($p<0,05$). Gram-negatif bakteriyel enfeksiyonu olan hastalarda, gram-pozitif enfeksiyonu olanlara göre HDL düzeyleri daha düşük bulundu.

Sonuç: Bu çalışmanın bulguları serum lipoproteinlerinin ve özellikle HDL'nin sepsis patogenezindeki rolü hakkında yeni kanıtlar sunmakta, sepsis tedavisinde lipoproteinlerin yeni bir yol açabileceğini göstermektedir.

Anahtar Kelimeler: Sepsis, lipoprotein, albümin efekt, HDL

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INTRODUCTION

Sepsis is a life-threatening organ dysfunction-associated syndrome that results from a dysregulated host response to infection. Sepsis is a global problem with high morbidity and mortality rates. The mortality rate of sepsis range from 17% to 26%, and this may increase in middle- and low-income countries (1).

Lipopolysaccharide (LPS, gram-negative) and lipoteichoic acid (gram-positive) are known endotoxins, lipophilic, and bacterial cell wall components responsible for bacterial virulence. LPS initiates the release of cytokines. It results in intravascular coagulopathy, hypotension, multiple organ failure, and ultimately death if these progress to uncontrolled inflammatory response. These are the clinical signs and symptoms of sepsis and septic shock (2).

Numerous studies have shown that lipoproteins can bind and neutralize gram-negative and gram-positive bacterial toxins, which serves as the so-called pathogen lipid sink (3).

High-density lipoprotein cholesterol (HDL-C) neutralizes LPS and gram-positive lipoteichoic acid and mediates toxin clearance (4-6). Changes in HDL-C function occur during sepsis. This dysfunctional and pro-inflammatory form of HDL was shown to predict organ failure (7). Decreased levels of HDL-C were associated with mortality in sepsis (8,9). Low-density lipoprotein cholesterol (LDL-C) can also bind LPS and lipoteichoic acid and facilitate bacterial toxin clearance (4,5). Decreased levels of LDL-C can predict organ failure and mortality in patients with sepsis (10).

In a long-term observational study investigating the association of lipid and lipoprotein levels with clinical benefit, low cholesterol levels were found to be associated with increased mortality, while the Kaiser Permanente study of 15,000 healthy individuals revealed that it was associated with the development of infectious diseases (11,12). Hypolipidemia was associated with an increased risk of infection and poor clinical outcomes in patients with critical conditions (13,14).

This study aimed to investigate the relationship between serum lipoprotein levels and sepsis in a large population of patients admitted in the intensive care unit (ICU).

METHODS

Patients (Obtaining Patient Data)

This study was approved by the Clinical Research Ethics Committee of the public Bakırköy Dr. Sadi Konuk Training and Research Hospital where the study was conducted (dated: 14.10.2019 and no: 2019/459). Data of 8.738 patients in the registry of "ImdSoft-Metavision/QlinICU Clinical Decision Support Software (Canada)," who were hospitalized between January 01, 2013, and August 19, 2019, in the general ICU of the anesthesia and reanimation clinic of this hospital were obtained using Structured Query Language queries. The levels of HDL-C, LDL-C, total cholesterol, and triglyceride were available in 1.372 of these patients on the first admission to the ICU. A total of 151 patients with sepsis and calculated serum lipoprotein levels, as well as procalcitonin, white blood cell, C-reactive protein (CRP), lactate, blood gas, and biochemical parameters (sepsis group) and 450 patients without sepsis (non-sepsis group) who met the same conditions, were included in the study. Blood gas samples were checked with the ABL800 (Radiometer, Denmark, Copenhagen) device. Biochemical tests were performed with the AU5800 biochemistry autoanalyzer (Beckman Coulter, Inc., Brea, CA, USA). The mean systolic, diastolic, and mean arterial pressures measured noninvasively at 5-min intervals during the ICU stay as well as the total balance, which is the total input-total output difference during the ICU stay, were calculated using appropriate software.

Urine, sputum, endotracheal aspirate, wound site, and intracatheter and peripheral blood culture data collected from patients with infection foci were recorded. Blood cultures were maintained in the BACTEC 9000 MB automation system (Becton Dickinson, USA) for 7 days if there was no bacterial reproduction. Necessary pretreatments were performed in the reproductive cultures, and identification and antibiotic susceptibility of microorganisms were determined in the VITEC 2® system (bioMerieux, France).

Patients with liver cirrhosis, renal failure receiving dialysis treatment, and end-stage chronic obstructive pulmonary disease and who did not stay in the ICU for more than 24 h were not included in the study. Moreover, patients whose initial and final age, Acute Physiology and Chronic Health Evaluation (APACHE II-IV) score, Simplified Acute Physiology score 3 (SAPS3) score, and Sequential Organ Failure Assessment (SOFA) score were not calculated in the software were not included in the study.

Diagnosis of Sepsis

Patients who previously had sepsis-2 and sepsis-3 septic shock events defined according to the sepsis 2012 (15) and sepsis 2016 guidelines (16) were included in the analysis (supplementary data) based on the following criteria:

1) Patients with bacterial growth in one of the two blood cultures because of suspected infection or bacterial growth

in two of the four blood cultures in the presence of a central catheter (two vials) according to the medical histories, clinical examinations, and laboratory and radiological findings.

2) Patients with bacterial growth more than 100,000 cfu/ml'in one of the urine, tracheal, body fluid, wound swab samples, and catheter cultures.

3) Patients without bacterial growth in the blood culture, who met the sepsis and septic shock diagnosis criteria according to the sepsis 2016 guideline (2):

a) Fever >38.0 °C, b) CRP >0.02 mcg/L, c) 10,000< leukocytosis <4,000 10e3/UL, d) neutrophil >%80, e) ICU admission SOFA >2 or patients with high SOFA score calculated after the admission value indicated sepsis (supplementary data).

Septic Shock Diagnosis

Despite adequate fluid rescucitation, MAP< 65 mmHg and lactate > 2.5 mmol, requiring at least one intravenous vasopressor medication to maintain MAP> 65 mmHg.

Statistical Analysis

The GraphPad Prism (v 5.01) program was used for the statistical analysis of data obtained in the study. Frequency distribution and percentages of qualitative variables such as gender were calculated, and the chi-square test was used to determine significant differences. After determining the homogeneity of the variables by using the Shapiro-Wilk normality test, Student's t-test was used to compare independent and homogeneous binary variables, and the Mann-Whitney U test was used to compare non-homogeneous binary variables. The Spearman correlation test was used to analyze the correlation of data. Chi-square test was used to compare variables according to gender. The log-rank (Mantel-Cox) test and Gehan-Breslow-Wilcoxon test were used to determine significant differences in the intergroup survival analyses in terms of survival percentages, median survival, median ratio, and 95% confidence interval (CI). The median interquartile range (IQR) values were taken as the reference. Values of $p<0.05$ were considered significant. For the graphical representation, p-values are summarized as follows: *$p<0.05$, **$p<0.01$, ***$p<0.001$, $p\geq0.05$= not significant (ns).

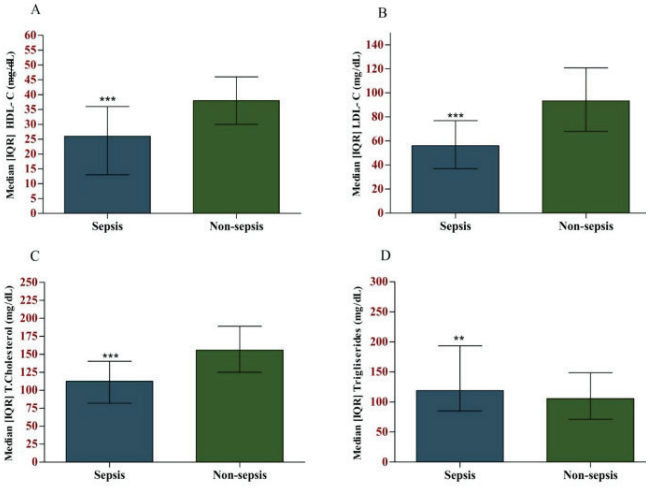

Figure 1. In the Mann-Whitney U test analyses of different blood serum lipoproteins of patients with and without sepsis. (A) High-density lipoprotein cholesterol. (B) Low-density lipoprotein cholesterol. (C) Total cholesterol. (D) Triglycerides
**$p<0.01$, ***$p<0.001$

RESULTS

In this study, serum lipoprotein levels (HDL-C, LDL-C, total cholesterol, and triglyceride) on ICU admission of patients with sepsis (n=151) and without sepsis (n=450) were compared retrospectively (Figure 1).

When the ICU admission HDL-C, LDL-C, total cholesterol, and triglyceride values of the sepsis group (n=151) and non-sepsis group (n=451) were compared using the Mann-Whitney U test, the median and IQR values were 26 (13-36) and 38 (30-46) mg/dL, respectively, for the HDL-C. The median and IQR values were 56 (36-76) and 93 (68-120) mg/dL, respectively, for the LDL-C, and those for total cholesterol were 112 (82-140) and 156 (125-189) mg/dL, respectively. Differences among HDL-C, LDL-C, and total cholesterol values were significant (Figure 1A,B,C, $p<0.0001$). The median and IQR values for triglycerides were 119 (85-193) and 105 (71-148) mg/dL, and the difference was significant (Figure 1D, $p=0.002$).

The age values of the sepsis and non-sepsis groups were compared using Student's t-test. The initial and final values of the height, body weight, body mass index, length of ICU stay, invasive mechanical ventilation time, APACHE II score, APACHE IV score, SOFA score, and biochemical parameters were compared with the Mann-Whitney U test, and the median, IQR values, and p values are shown in Table 1. Categorical variables such as gender were compared with the chi-square test, and the frequency, percentage, and p values are shown in Table 1. Patients with sepsis were divided into the surgical and medical subgroups of intra-abdominal sepsis and pneumosepsis. However, the gram-positive and gram-negative subgroups were established as having culture-positive and culture-negative results, and the parameters were compared in binary using the Mann-Whitney U test. The sepsis and non-sepsis groups were analyzed in terms of survival according to their duration of ICU stay (day) (Table 1).

In the comparison of patients with intra-abdominal sepsis (33%, n=49) and pneumosepsis (46%, n=69), the median and

Table 1. Characteristics of patients with and without sepsis

Patient characteristics	Sepsis (n=151)	Non-sepsis (n=450)	p
Gender, female, no (%)	54 (35%)	215 (48%)	0.018*
Age (year)	59.6±19.6	60.8±19.5	0.4976
Height, median (IOR 25-75) (m)	1.65 (1.6-1.735)	1.67 (1.6-1.75)	0.2909
Tracheostomy time, median (IQR) (hour)	290.2 (69.11-604.8)	265.4 (137.7- 411)	0.1657
Body weight, median (IQR) (kg)	75 (60-85)	75 (65-85)	0.628
Body mass index, median (IQR) (kg/m^2)	25.39 (22.49-27.85)	26.12 (24.22-29.14)	0.0537
Length of stay in the ICU, median (IQR) (day)	5 (3-15)	2 (1-4)	<0.0001***
Invasive MV time, median (IQR) (hour)	138.2 (79.73- 396.6)	94.33 (38.74- 211.7)	0.0019**
SOFA score first, median (IQR)	8 (4-11)	4 (2-6)	<0.0001***
SOFA score last, median (IQR)	8 (4-12.25)	3 (1-5)	<0.0001***
APACHE II score first, median (IQR)	20.5 (14-24)	14 (9-20)	<0.0001***
APACHE II score last, median (IQR)	18 (12-23)	13 (9-19)	<0.0001***
APACHE IV score first, median (IQR)	84 (64-107)	59 (45-81)	<0.0001***
APACHE IV score last, median (IQR)	74 (54-106)	55 (41-81)	<0.0001***
SAPS 3 score first, median (IQR)	48.50 (40-57.75)	40 (31-49)	<0.0001***
SAPS 3 score last. median (IQR)	45 (37-56)	38 (29-47)	<0.0001***
Vital parameters			
HR mean, median (IQR) (1/min)	96 (84-109)	83 (72-96)	<0.0001***
NIBP systole mean, median (IQR) (mmHg)	113 (103-126)	118 (106-132)	0.0059**
NIBP diastole mean, median (IQR) (mmHg)	62 (55-72)	65 (58- 72)	0.1043
NIBP mean, median (IQR) (mmHg)	79 (70-87)	82 (74- 92)	0.0023**
Urine amount, median (IQR) (mL/day)	500 (200-1115)	692 (350-1264)	0.0047**
Body temperature max, median (IQR) (C)	37 (36.2-37.6)	36.6 (36.2-37.1)	0.0006***
Laboratory parameters			
Procalcitonin, median (IQR) (ng/mL)	3.75 (0.96-13.82)	0.32 (0.16-0.51)	<0.0001***
WBC, median (IQR) (10e^3/Ul)	11.95 (7.16-18.03)	11.8 (8.3-15.60)	0.9399
CRP, median (IQR) (mg/L)	0.019 (0.008-0.032)	0.003 (0.0007-0.010)	<0.0001***
CKMB, median (IQR) (IU/L)	33.5 (21.75-55.25)	29 (20-39)	0.0592
PT, median (IQR) (second)	33.5 (21.75-55.25)	29 (20-39)	<0.0001***
APTT, median (IQR) (second)	35.5 (30.15-46.7)	26.75 (24.1-32.03)	<0.0001***
Urea, median (IQR) (mg/dL)	60 (36-103)	37(25 - 57)	<0.0001***
Creatinine, median (IQR) (mg/dL)	1.12 (0.68-2.17)	0.79 (0.59-1.04)	<0.0001***
Bilirubin, median (IQR) (mg/dL)	0.66 (0.42-1.33)	0.58 (0.39-0.89)	0.0146*
AST, median (IQR) (IU/L)	39 (22-77)	26 (18-44)	<0.0001***
ALT, median (IQR) (IU/L)	21 (12-44)	19 (13-33)	0.159
INR, median (IQR)	1.26 (1.11-1.52)	1.08 (1.01- 1.21)	<0.0001***
Albumin, median (IQR) (g/dL)	2.6 (2.1-3.03)	3.4 (2.9-3.8)	<0.0001***
Total protein, median (IQR) (g/dL)	5.6 (4.8-6.2)	6.2 (5.6-6.79)	<0.0001***

Student's t-test, Mann-Whitney U, chi-square tests were used. Summary of p values: *p<0.05, **p<0.01, ***p<0.001, not significant (ns); p≥0.05

SOFA score: Sequential organ failure assessment score, APACHE II-IV score: Acute physiology and chronic health evaluation, SAPS: Simplified acute physiology score, ICU: Intensive care unit, MV: Mechanical ventilation, WBC: White blood cell, CRP: C-reactive protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, INR: International normalized ratio, NIBP: Non-invasive blood pressure, HR: Heart rate

IQR values for HDL-C were 20 (10-31) and 29 (15-40) mg/dL, respectively (p>0.01). The median and IQR values for LDL-C were 48 (31-65) and 62 (39-89) mg/dL, respectively (p>0.014), while those for total cholesterol were 103 (77-123) and 121 (95-159) mg/dL, respectively (p>0.002), and their difference was significant. The median and IQR values for triglyceride were 100 (71-200) and 140 (88-190) mg/dL, respectively, and the difference in values was not significant (p=0.4).

In the comparison of surgical (33%, n=50) and medical (67%, n=101) subgroups of patients with sepsis, the median and IQR values for HDL-C were 18 (8-30) and 29 (14-39) mg/dL, respectively (p>0.003), those for LDL-C were 44 (31-61) and 62 (41-84) mg/dL, respectively (p>0.0008), and those for total cholesterol were 98 (73-130) and 120 (96-150) mg/dL, respectively (p>0.0007), and their differences were significant. The median and IQR values for triglyceride were 110 (71-200) and 130 (86-190) mg/dL, respectively, but the difference was not significant (p=0.3).

In the comparison of gram-negative (25%, n=39) and gram-positive (23%, n=35), subgroups, the median and IQR values for HDL-C were 23 (14-36) and 35 (28-44) mg/dL, respectively, and the difference was significant (p=0.025). The median and IQR values were 65 (36-87) and 69 (42-91) mg/dL (p=0.5) for LDL-C, 120 (66-150) and 130 (100-160) mg/dL (p=0.8) for total cholesterol, and 90 (63-169) and 127 (86-185) mg/dL (p=0.19), for triglyceride, respectively, and their differences were not significant.

In the similar analysis for culture-positive (48%, n=73) and culture-negative (44%, n=67) subgroups, the median and IQR values for HDL-C were 30 (17-40) and 19 (9-32) mg/dL, respectively (p=0.006), and the difference was significant. The median and IQR values were 65 (41-90) and 53 (33-72) mg/dL (p=0.037) for LDL-C, 120 (87-150) and 110 (82-140) mg/dL (p=0.14) for total cholesterol, and 100 (68-185) and 138 (88-203) mg/dL (p=0.051) for triglyceride, respectively, and the differences were not significant.

When the ICU admission procalcitonin values of sepsis subgroups were analyzed with the Mann-Whitney U test, the median and IQR values of the intra-abdominal sepsis and pneumosepsis subgroups were 6.1 (1.2-33.8) and 2.4 (0.4-8.8) mg/dL, respectively (p=0.045). The median and IQR values of the surgical and medical subgroups were 7.5 (1.4-27.4) and 2.3 (0.5-8.2) mg/dL, respectively (p=0.02). The median and IQR values of the gram-negative and gram-positive subgroups were 3.4 (0.6-13) and 1.5 (0.4-6.5) mg/dL, respectively (p=0.18); the values for culture-positive and culture-negative subgroups were 2.2 (0.5-7.9) and 3.5 (1.0-14.3) mg/dL, respectively, and a significant difference was determined only in the comparison of the surgical and medical subgroups (p=0.18).

In the survival non-survival analysis made according to the duration of ICU stay of patients with sepsis (n=151) and without sepsis (n=451), the median survival values were 9 and 2 days, and the medial ratio was 4.5 with 95% CI of 3.697-5.303. The hazard ratio was 0.9284, with 95% CI of 0.6108-1.411, and the values were considered significant (p<0.0001)

In the sepsis group, when the survivor (n=61) and non-survivor (n=51) subgroups were compared, the median and IQR values for HDL-C were 28 (15-35) and 26 (9-38) mg/dL, respectively, and the difference was not significant (p=0.9). The values obtained from the survivor-non-survivor analyses for LDL-C, total cholesterol, and triglyceride were not significant.

DISCUSSION

Adequate levels of HDL-C and LDL-C are thought to be protective against sepsis. LDL-C facilitates bacterial toxin clearance via hepatic LDL receptors or HDL-C. Bacterial toxin binding, inhibition of inflammatory cytokine release, inhibition of vascular and intercellular adhesion molecule expression, and stimulation of endogenous corticosteroid secretion have been reported as prevention mechanisms of HDL-C-associated sepsis (17).

HDL particles have been shown to protect endothelial cells by reducing leukocyte extravasation in inflammation (18,19). HDL is a protective factor in sepsis because of its antioxidant effects. HDL-C can increase nitric oxide production of endothelial cells, and HDLs can bind to enzymes such as paraoxonase-1 or platelet-activating factor acetylhydrosis, which exhibits antioxidant and endothelial protective properties. The positive effects of HDLs on cleansing liposaccharides of microorganisms from tissues and organs were shown in a previous study (20).

In vivo studies have shown that LPS interacts very early with HDL within 3 min of intravenous administration and remains associated with HDL until clearance. However, a new study on human lipoprotein fractions showed the absence of LPS detoxification activity in the serum. In addition, some HDL apoproteins may function as coenzymes of LPS-inactivating enzymes different from arylesterase. A coenzyme function of apoprotein A-I and C-II was described in lipid metabolism (21). Further, only binding of LPS to HDL can determine the biological effect and *in vivo* distribution of LPS and facilitate or delay the internalization into phagocytic system cells. More work is ongoing on the biological significance of HDL-LPS interaction.

In this study, the initial lipoprotein values (HDL-C, LDL-C, and total cholesterol) of patients who were taken to the ICU

in the last 6 years because of sepsis and serum lipoprotein values of patients without sepsis were compared. The lipoprotein levels of patients with sepsis were significantly lower than those of patients without sepsis (Figure 1A, B, C). In a previous study, HDL-C levels were found to decrease rapidly in the early stage of sepsis development (17). Tanaka et al. (22) compared patients with sepsis and those with trauma and found that HDL-C levels were lower in those with sepsis. Another study of patients who underwent cardiac surgery reported lower preoperative cholesterol levels in the sepsis group (23,24).

In the subgroup analyses of patients with sepsis, the HDL-C values were significantly lower and the procalcitonin values were higher in the intra-abdominal, surgical, and gram-negative subgroups. This was thought to be due to the source control of patients with intra-abdominal sepsis required surgery, which was brought under control lately. Interestingly, although no significant difference was found between the procalcitonin values of the culture-positive and culture-negative groups, their HDL-C levels were significantly low. The suspicion of anaerobic infection should also be taken into consideration because 45% of patients with negative cultures were those with intra-abdominal sepsis and a routine anaerobic culture test was not performed in the center where the study was conducted (Table 2). Barlage et al. (25) suggested that the beneficial effects of HDLs on gram-positive infections may result from other anti-inflammatory properties, including modulation of neutrophil activation.

Several studies have reported a relationship between HDL-C and morbidity/mortality. The total cholesterol, HDL-C, and LDL-C values were significantly lower in the non-survivor group than in the survivor group (8). However, in the present study, no significant difference was found in the serum HDL-C, LDL-C, total cholesterol, and triglyceride levels of patients with sepsis who were discharged from the ICU and deceased patients. These findings suggest that serum lipoprotein levels may affect the development of infection-induced sepsis. Similarly, although the findings of the present study support the pathological mechanism that serum lipoproteins may play a role in endotoxin neutralization, it does not support the "abnormal host response" to infection highlighted in the definition of sepsis, or, in other words, the role of serum HDL-C levels in host immune dysregulation emphasized in the "current sepsis guideline," which is the current consensus (2).

Chien et al. (8) observed significant increases in the sepsis-associated 30-day mortality, overall mortality, risk of prolonged ICU stay, and hospital-acquired infection rate in patients with sepsis with HDL-C<20 mg/dL.

In the light of these publications showing the protective effect of cholesterol on sepsis, studies are ongoing to determine the effect of lipid emulsions on the clinical outcome by stabilizing cholesterol levels early in sepsis. Although sepsis is not included in the treatment guideline, many researchers recommend that HDL be given to patients with severe sepsis and septic shock based on the results of studies supporting the suggestion that the restoration of the physiological immunomodulatory activity of HDL may provide a new therapeutic opportunity for sepsis (23).

In a Mendelian randomization study, the authors found significant reductions in HDL-C concentrations during sepsis in individuals with cholesteryl ester transfer protein-rs1800777 missense variant and associated the reductions with increased mortality and morbidity (26).

CONCLUSION

This study highlights the important role of serum lipoproteins in the neutralization of endotoxins at the onset of sepsis. The subgroup analysis revealed a significant decrease in the HDL-C level in patients with sepsis, which suggests that serum HDL-C level has immunomodulatory properties as well as endotoxin-neutralizing function.

Table 2. Characteristics and numerical distribution of microorganisms in the culture-positive and culture-negative subgroups among patients with sepsis

	Abdominal sepsis n=49		Pneumosepsis n=69		Urosepsis n=10		Other sepsis (fasit, meningitis, etc.) n=20	
Microorganisms cultured, n=148	Medical	Surgical	Medical	Surgical	Medical	Surgical	Medical	Surgical
Gram-negative, n=36	3	8	18	0	2	0	5	0
Gram-positive, n=35	2	2	22	1	2	1	4	1
Fungus, n=10	0	4	2	1	1	1	1	0
Culture-negative, n=67	7	23	25	0	2	1	7	2

ETHICS

Ethics Committee Approval: The present study was approved by the Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinical Research Ethics Committee of the public hospital where the study was conducted, with the decision dated 14.10.2019 and no: 2019/459.

Informed Consent: Retrospective study.

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Authorship Contributions

Surgical and Medical Practices: S.A., M.S.S., E.K.T., Ö.A., Z.Ç., Concept: S.A., M.S.S., E.K.T., Ö.A., Z.Ç., Design: S.A., M.S.S., E.K.T., Ö.A., Z.Ç., Data Collection or Processing: S.A., M.S.S., E.K.T., Ö.A., Z.Ç., Analysis or Interpretation: S.A., M.S.S., E.K.T., Ö.A., Z.Ç., Literature Search: S.A., M.S.S., E.K.T., Ö.A., Z.Ç., Writing: S.A., M.S.S., E.K.T., Ö.A., Z.Ç.

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Research

The Roles of Thwaites' and Marais' Diagnostic Scoring Indexes and a Clinical Prediction Model in the Diagnosis of Tuberculous Meningitis

Tüberküloz Menenjit Tanısında Thwaites ve Marais'in Tanı Skor İndeksleri ve Klinik Bir Tahmin Modelinin Rolü

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ABSTRACT

Objective: Tuberculous meningitis (TBM) is the most severe form of extra pulmonary tuberculosis. Bacteriological confirmative tests based on mycobacterial cultures or polymerase chain reaction tests are time-consuming process may not help diagnose every TBM patient. Therefore, practical and easily applicable scoring systems may be helpful in the early diagnosis of TBM and should be applied in the clinical follow-up process.

Methods: The features of 20 patients with TBM were retrospectively evaluated according to the Thwaites' diagnostic scoring indexes (TDSI) and Marais' diagnostic scoring indexes (MDSI) beside a clinical prediction model (CPM) in this study. MDSI, CPM, viral, brucellar, and fungal etiologies were excluded by microscopic, serological, and molecular examinations of cerebrospinal fluid (CSF) and blood in all patients.

Results: All patients were assessed for TBM according to TDSI (100%). Of these, 5 (25%) were considered probable TBM, and 15 (75%) were possible TBM, according to MDSI. The scores were greater than or equal to 6 in all the cases with TBM by CPM, 13 of which were scored as 9 points (65%).

Conclusion: According to the outcomes of our study, the TDSI, MDSI, and CPM assessment methods are easily applicable and helpful techniques for rapid and accurate TBM diagnosis.

Keywords: Tuberculosis, tuberculous meningitis, Thwaites, Marais, clinical prediction model

ÖZ

Amaç: Tüberküloz menenjit (TBM), ekstrapulmoner tüberkülozun en ciddi formudur. Mikobakteriyel kültür ve polimeraz zincir reaksiyonu testlerine dayanan bakteriyolojik doğrulama testleri hem zaman alıcı testlerdir hem de TBM'li her hastanın tanısında yardımcı olmayabilir. Bu yüzden, pratik ve uygulaması kolay diagnostik skorlama indeksleri TBM'nin erken tanısında faydalı olabilir ve klinik takipte kullanılmalıdır.

Gereç ve Yöntem: Bu çalışmada 20 TBM hastasının özellikleri, klinik tahmin modelinin yanı sıra Thwaites ve Marais'in diagnostik skorlama indekslerine (TDSI ve MDSI) göre retrospektif olarak değerlendirildi. Tüm hastalarda BOS ve kanın mikroskobik, serolojik ve moleküler incelemeleri ile viral, brusellar ve fungal etiyolojiler dışlandı.

Tartışma: Tüm hastalar TDSI'ya göre (%100) TBM olarak değerlendirilirken, sırasıyla 5'i (%25) ve kalan 15'i (%75) MDSI'ye göre olası TBM ve olası TBM olarak değerlendirildi. CPM ile TBM olan tüm olgularda puanlar 6'dan büyük veya eşit olup, 13'ü 9 puan (%65) olarak puanlanmıştır.

Sonuç: Çalışmamızın sonuçlarına göre TDSI, MDSI ve CPM değerlendirme yöntemleri TBM'nin hızlı ve doğru teşhisi için uygulanması kolay ve kullanışlı tekniklerdir.

Anahtar Kelimeler: Tüberküloz, tüberküloz menenjit, Thwaites, Marais, klinik tahmin modeli

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INTRODUCTION

Tuberculous meningitis (TBM) is the most severe form of tuberculosis (TB), as its exact incidence and prevalence rates are unknown. However, its incidence is expected to be relatively high, especially in children, human immunodeficiency viruses-infected individuals, and people living in countries with a high burden of pulmonary TB (1). According to the 2019 World Health Organization (WHO) Global TB report, approximately 10 million people were diagnosed with TB in 2018. They lived mainly in Southeast Asia (44%) and Africa (24%). Extra pulmonary TB (EPTB) constitutes 15% of all cases. Nevertheless, the patients from high-incidence countries may manifest a higher prevalence rate. Turkey is among the countries with a prevalence rate of EPTB higher than 30% (2). In Turkey, the total case rate is 15.6 per hundred thousand in 2016, according to the 2018 report of TB (3). Globally, about 1.5 million TB-related deaths occurred worldwide. Without the prompt diagnosis and early treatment of TBM, mortality and morbidity rates are exceptionally high. Also, delayed diagnosis and subsequent therapy significantly contribute to the current high mortality rate (1,4-6).

The gold standard of TBM diagnosis is the identification of Mycobacterium tuberculosis bacilli in the cerebrospinal fluid (CSF). Novel culture methods based on liquid media, such as mycobacteria growth indicator tube, have higher positivity rates than the classical Löwenstein-Jensen method. However, the sensitivity and positivity rates of the current laboratory confirmation methods based on mycobacterial culture or polymerase chain reaction (PCR) tests do not exceed 60% (7). These methods require a long-lasting process and a well-trained authorized staff capable of using specific equipment for a positive result (8). Therefore, scoring indexes or clinical prediction models based on clinical, laboratory, radiological, and CSF findings have been developed by researchers (9-11). This study aims to evaluate 20 patients with TBM according to the Thwaites' scoring indexes (TDSI) and Marais' scoring indexes (MDSI) and a clinical prediction model (CPM) and to assess these practical diagnostic approaches to diagnose TBM.

METHODS

This retrospective study included patients with TBM over 18 years of age followed in our hospital between 2015 and 2019. TBM was diagnosed based on clinical, CSF, radiological, and laboratory findings in the cases that presented the clinical picture of meningitis (12) (Table 1). Viral, brucellar, and fungal etiologies were excluded by microscopic, serological, and molecular examinations of CSF and blood. Ethics Committee approval was obtained from University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital (2018/470; 2018-23-18). Since the study was retrospective, patient consent forms were not obtained.

Table 1. Diagnostic criteria for TBM and ABM (12)

TBM	ABM
Mycobacterium tuberculosis isolation from CSF or clinical signs of meningitis	Isolation of bacterial agent from CSF or clinical signs of meningitis
With negative Gram or Indian ink stain and negative culture for bacterial or fungal agents, in the presence of at least one of the following features:	In the presence of all of the following features:
Cranial imaging findings compatible with TB	Pleocytosis in CSF
The signs of accompanying pulmonary TB	CSF/plasma glucose ratio <50%
Positive family history for TB	Negative CSF and blood cultures
Close contact with an active TB case	-
Clinical response to antituberculous therapy	Clinical response to non-specific therapy

TBM: Tuberculous meningitis, ABM: Acute bacterial meningitis, CSF: Cerebrospinal fluid, TB: Tuberculosis

The TDSI, MDSI, and a clinical prediction method supported the diagnosis (Tables 2,3). TDSI included five clinical and laboratory variables. The cases with scores ≤4 were considered as having TBM, whereas patients with scores >4 were classified as bacterial meningitis, according to TDSI. MDSI included criteria for clinical and biochemical analysis of CSF, cerebral imaging, and evidence of extra-neural tuberculosis. Patients with scores of ≥12 were considered probable, whereas those with scores of 6-11 were evaluated as possible TBM, according to MDSI criteria. The cases with culture or PCR positivity were assessed as "definite TBM." The following four rules were accepted in the CPM for TBM (11): 1) Duration of symptoms before admission ≥5 days; 2) neurological stage II and III; 3) CSF/blood glucose ratio ≤0.5; 4) CSF protein level ≥100 mg/dL. This model accurately predicts 89.1% of cases with TBM, according to Hristea et al. (11). TBM staging was performed according to the British Medical Research Council criteria: Patients with mild and non-specific symptoms were considered as stage I, those with mild alteration of consciousness or with cranial nerve palsies were considered as stage II, and those with major neurological deficits or coma were considered as Stage III (13). All cases were treated with classical four-drug antituberculous therapy for 12 months and with additional

Table 2. Thwaites' diagnostic scoring index (9)

Parameters	DI
Age ≥36	2
Age <36	0
Blood WBC ≥15000	4
Blood WBC <15000	0
History of illness ≥6 days	(-5)
History of illness <6 days	0
CSF WBC ≥900 ise	3
CSF WBC <900 ise	0
CSF % neutrophils ≥75	4
CSF % neutrophils <75	0
Total score ≤4	**TBM**

CSF: Cerebrospinal fluid, DI: Diagnostic index, TBM: Tuberculous meningitis, WBC: White blood cell

dexamethasone therapy for 8 weeks in the presence of severe neurological deficits.

RESULTS

Patients over 18 years of age who presented the clinical picture of meningitis between 2015 and 2019 were assessed. TBM diagnosis was established based on clinical, CSF, radiological, and laboratory features. Viral, brucellar, and fungal etiologies were excluded by microscopic, serological, and molecular examinations of CSF and blood in all patients. Of the patients with TBM, 16 (80%) were male, whereas the ages of patients ranged between 18 and 72 years (range, 39.05±15.52 years). The most common symptoms were headache (90%), altered consciousness (75%), and neck rigidity (70%). Convulsion (25%), paresis/plegia (25%), and cranial nerve palsy (20%) were the other neurological findings detected in the TBM cases. Twelve (60%), five (25%), and three (15%) patients were assessed as stage II, stage III, and stage I, respectively. According to CSF analysis, 76% of patients had a CSF/blood glucose ratio below 0.3 and had a protein concentration above 100 mg/dL, whereas pleocytosis (>20/mm^3) was detected in 90% of patients. PCR and culture positivity rates were 45% separately, whereas five patients showed both culture and PCR positivity, four patients had only PCR, and four patients had only culture positivity. The rate of concomitant pulmonary TB was 25%. Four (20%) patients died despite antituberculous therapy. All patients were assessed as TBM according to TDSI (100%), whereas five (25%) and the remaining 15 (75%) were considered as probable TBM and possible TBM according to MDSI. The scores were greater

Table 3. Marais' diagnostic scoring index (10)

Parameters	
Clinical criteria	**Max score =6**
Symptom duration more than 5 days	4
Systemic symptoms suggestive of TB (one or more of the following):	2
Weight loss, night sweats, persistent cough >2 weeks	
History of recent close contact with an individual with pulmonary TB or positive TST or IGRA	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
Altered consciousness	1
CSF criteria	**Max score =4**
Clear appearance	1
Cells: 10-500 per µL	1
Lymphocytic predominance	1
CSF to plasma glucose ratio less than 50% or absolute CSF glucose less than 2.2 mmol/L	1
Cranial imaging criteria	**Max score =6**
Hydrocephalus	1
Basal meningeal enhancement	2
Tuberculoma	2
Infarction	1
Pre-contrast basal hyperdensity	2
Evidence of TB elsewhere	**Max score =4**
Chest radiograph suggestive of active TB: Signs of TB = 2; miliary TB = 4	2/4
CT/MRI/USG evidence for TB outside of CNS	2
AFB identified or *Mycobacterium tuberculosis* cultured from another source-	4
i.e., sputum, lymph node, gastric washing, urine, blood culture	
Positive commercial *M. tuberculosis* NAAT from extra-neural specimen	4
Patients with no cranial imaging	
Total score 6-9	Possible
Total score ≥ 10	Probable
Patients with cranial CT/MRI	
Total score 6-11	Possible
Total score ≥12	Probable

TB: Tuberculosis, CSF: Cerebrospinal fluid, CT/MRI/USG: Computed tomography/Magnetic resonance imaging/Ultrasonografi

than or equal to 6 in all the cases with TBM by CPM, 13 of which were scored as 9 points (65%).

Table 4 summarizes the cases with TBM and their outcomes according to TDSI, MDSI, and CPM criteria with additional findings supporting TBM.

DISCUSSION

Despite its decreased incidence in recent years, TB, particularly TBM, remains a leading cause of infection-related deaths worldwide. TBM is a severe and fatal disease that presents diagnostic and treatment difficulties. Antituberculous therapy prevents mortality and disability in less than half of the cases (14). A significant rate of morbidity and mortality related to TM results from the dysregulated immune response and delayed diagnosis and treatment (15,16). Therefore, early and accurate TBM diagnosis remains of critical importance regarding disease eradication. Definitive diagnosis of TBM is based on isolation of Mycobacterium tuberculosis from CSF by staining and/or culture or PCR techniques. However, the identification of the agent by culture or PCR techniques is not possible in every patient.

In this study, PCR or culture positivity was determined in only 45% of patients with TBM. Heemskerk et al. (17) have compared sensitivity levels of MDSI and other laboratory methods, including conventional Ziehl-Neelsen (ZN) staining, modified ZN staining, culture test, and Xpert PCR analysis for TBM diagnosis. Compared with MDSI, the sensitivity levels of the techniques mentioned above were 33.9%, 34.5%, 31.8%, and 25.1%, respectively. In another study conducted by Feng et al. (18), the sensitivity levels of the conventional and modified ZN procedures were compared with TB culture. They were determined to be 3.3%

Table 4. The features of TBM cases according to three diagnostic indexes

Patients	TDSI						MDSI					CPM				
	Age ≥ 36	WBC ≥ 15.000	DSBA ≥ 6 days	Pleocytosis ≥900	CSF PNL ≥ 75%	Score	Clinical criteria	CSF criteria	Imaging criteria	EMTB	Score	DSBA ≥5 days	Stage II/III	CSF Glucose < 0.5	CSF protein >100 mg/dL	Score
1	0	0	-5	0	0	**-5**	5	4	0	0	**9**	**3**	**0**	**3**	**1**	7
2	0	0	-5	0	0	**-5**	5	4	0	0	**9**	**3**	**2**	**3**	**0**	8
3	0	0	-5	3	0	**-2**	5	4	0	0	**9**	**3**	**2**	**3**	**1**	9
-4	0	0	-5	0	0	**-5**	4	4	1	0	**9**	**3**	**0**	**3**	**1**	7
5	0	0	-5	0	0	**-5**	5	3	0	0	**8**	**3**	**2**	**0**	**1**	6
6	0	4	-5	0	0	**-1**	1	3	0	2	**6**	**3**	**2**	**3**	**1**	9
7	0	0	-5	0	0	**-5**	5	4	2	2	**13**	**3**	**2**	**3**	**1**	9
8	2	0	-5	0	0	**-3**	5	4	1	0	**10**	**3**	**2**	**3**	**1**	9
9	2	0	-5	0	0	**-3**	6	3	0	4	**13**	**3**	**2**	**3**	**1**	9
10	2	0	-5	0	0	**-3**	6	4	2	0	**12**	**3**	**2**	**3**	**1**	9
11	2	0	-5	0	0	**-3**	5	4	3	2	**14**	**3**	**2**	**3**	**1**	9
12	2	0	-5	0	0	**-3**	5	3	1	0	**9**	**3**	**2**	**3**	**1**	9
13	0	0	-5	0	0	**-5**	4	4	1	2	**11**	**3**	**0**	**3**	**0**	6
14	0	4	0	0	0	**4**	1	4	1	1	**7**	**0**	**2**	**3**	**1**	6
15	0	0	-5	0	0	**-5**	6	3	2	0	**11**	**3**	**2**	**3**	**1**	9
16	2	0	-5	0	0	**-3**	4	2	1	0	**7**	**3**	**2**	**0**	**1**	6
17	2	4	-5	0	0	**1**	5	4	2	0	**11**	**3**	**2**	**3**	**1**	9
18	0		-5	0	0	**-5**	6	4	2	2	**14**	**3**	**2**	**3**	**1**	9
19	2	0	-5	0	0	**-3**	5	4	1	0	**10**	**3**	**2**	**3**	**1**	9
20	2	0	-5	0	0	**-3**	2	4	0	0	**6**	**3**	**2**	**3**	**1**	9

TDSI: Thwaites' diagnostic scoring indexes, TBM: Tuberculous meningitis, WBC: White blood cell

and 82.9% versus 15.4%, respectively. Hooker et al. (19) have determined that the rates of culture positivity assessed by the Löwenstein-Jensen and BACTEC methods were 20.7% and 35.7% in patients with TBM, respectively. Hence, ZN staining and culture methods are known as the cornerstone and gold standards for diagnosing TBM. However, their positivity rates do not exceed 60%, even in the best-trained hands or best-equipped laboratories (18). According to a review by WHO in 2014, the nucleic acid test sensitivity rate was higher than that of culture methods for diagnosing TBM (80.5% versus 62.8%, respectively) (20). As a consequence, highly sensitive diagnostic tests for TBM remain elusive and impractical. Therefore, we need to improve affordable diagnostic methods or develop novel practical diagnostic approaches to achieve better TBM outcomes (21).

Clinical-based diagnostic approaches, such as TDSI, MDSI, and CPM, have been developed to eliminate these disadvantages, proven efficacy (11,22-26). Sunbul et al. (23) have applied this method in 126 patients with TBM and reported it as practical, sensitive, and specific. In contrast, Sulaiman et al. (25) evaluated the TDSI method in 391 patients with TBM and detected it in 99% of cases. They reported that the TDSI method showed weak positivity in differentiating TBM from subacute meningitis and chronic meningitis. They also reported high specificity (99.1%) despite having low sensitivity (1.2%), whereas 50% of the 162 cases diagnosed with TBM were evaluated as either possible TBM or probable TBM, according to the MDSI method. The same study determined that MDSI was statistically significant in discriminating TBM from fungal and viral meningitis leading to chronic meningitis, except in undiagnosed subacute meningitis cases. A study conducted in our country found that all the TBM cases were scored according to TDSI, and 70% of patients had maximum scores (26). Likewise, in another study from our country, Erdem et al. (24) have analyzed both TDSI and MDSI methods and demonstrated that the TDSI method was effective in the cases with brucellar and tuberculous meningitis. The cases with TBM had statistically higher scores than those with brucellar meningitis assessed by MDSI. They found that the number of the cases classified as "probable" among the cases with TBM and "possible" among the cases with brucellar meningitis were higher when assessed by MDSI. Considering these results, they have noted that some cases with brucellar meningitis may be incorrectly diagnosed as TBM, according to both scoring indexes. Therefore, it is recommended to consider neurobrucellosis regarding etiology, particularly in chronic meningitis cases diagnosed with TBM in endemic regions, according to TDSI and MDSI. In addition, all the cases in our study had scores of ≤4 by TDSI, whereas 75% and 25% of the 20 cases were classified in the possible and probable TBM categories by MDSI, respectively. However, in all our study patients categorized as possible TBM by MDSI, neurobrucellosis was excluded by serological and cultural methods.

A CPM developed by another study conducted in Romania presented a rapid clinical diagnostic model with good sensitivity and specificity to differentiate TBM from viral meningitis (11). This CPM model was evaluated in another study. This is a rapid clinical scoring method with a sensitivity of 96.7% and specificity of 81.1% in differentiating cases with TBM from those with viral meningitis by scores of ≥6 (27). Similarly, the scores were greater than or equal to 6 in all TBM cases in our study by CPM, 13 of which scored 9 points (65%). According to the outcomes of our study, the TDSI, MDSI, and CPM assessment methods are easily applicable and useful techniques for rapid and accurate TBM diagnosis.

CONCLUSION

Considering that available microbiological diagnostic methods used for TBM are impractical, time-consuming, and have low sensitivity, clinicians should use rapid diagnostic methods, such as TDSI, MDSI, or CPM to avoid complications due to delayed TBM diagnosis. An evaluation of the role of these valuable methods in the rapid and accurate TBM diagnosis by comprehensive studies involving larger sample sizes is needed.

ETHICS

Ethics Committee Approval: Ethics Committee approval was obtained from University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital (2018/470; 2018-23-18).

Informed Consent: Patient consent forms were not obtained.

Authorship Contributions

Concept: K.K.Y., Design: Ş.N.K., Data Collection or Processing: Ş.N.K., G.Ü., S.Ş., E.C.Ü., R.K., Analysis or Interpretation: Ş.N.K., Literature Search: Ş.N.K., Writing: Ş.N.K., G.Ü., S.Ş.

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Research

Pulmonary to Systemic Flow Ratio in Precapillary Pulmonary Hypertension Without Left-to-Right Shunts: A Prognostic Implication of the Fick Method

Soldan Sağa Şantı Olmayan Prekapiller Pulmoner Hipertansiyonda Pulmoner/Sistemik Akım Oranı: Fick Yönteminin Prognostik Bir Sonucu

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ABSTRACT

Objective: We routinely calculate the pulmonary to systemic (Qp/Qs) ratio to avoid missing any left-to-right shunts in patients with precapillary pulmonary hypertension (PH). However, the pulmonary artery oxygen saturation ($SpaO_2$) was lower than the mixed venous oxygen saturation ($SmvO_2$) calculated using the formula; hence, the Qp/Qs ratio was calculated as less than "1" in some patients despite the absence of any detectable shunt. We hypothesized that this observation might have prognostic significance; however, to the best of our knowledge, it has not been investigated yet. Therefore, we aimed to examine the prognostic value of the Qp/Qs ratio in precapillary PH without left-to-right shunts.

Methods: In this retrospective cohort study of 173 consecutive patients, hospital files were scanned for clinical, echocardiographic, and hemodynamic data including the Qp/Qs ratio calculated using the Fick method.

Results: During a median follow-up of 25 months, 74 patients died. Nonsurvivors had lower Qp/Qs ratio than survivors (0.76±0.19 vs. 1.02±0.07 p<0.001). The multivariate logistic regression analysis showed that a decreased Qp/Qs ratio and poor functional capacity (World Health Organization class III-IV) were independent predictors of mortality. The receiver operating characteristic curve analysis revealed that the optimal cutoff value of the Qp/Qs ratio for predicting mortality was 0.90 with a sensitivity of 76% and specificity of 98%.

Conclusion: The Qp/Qs ratio calculated using the Fick method was an independent predictor of mortality. This prognostic implication was based on the difference between $SpaO_2$ and $SmvO_2$ calculated using the formula. Nevertheless, this result might be a reflection of a potential intrinsic methodological flaw of the Fick method.

Keywords: Pulmonary hypertension, prognosis, fick method, oxygen, mortality

ÖZ

Amaç: Prekapiller pulmoner hipertansiyonlu (PH) hastalarda soldan sağa şantları kaçırmamak için pulmoner/sistemik oranı (Qp/Qs) rutin olarak hesaplamaktayız. Şaşırtıcı bir şekilde pulmoner arter oksijen satürasyonunun ($SpaO_2$) formülle hesaplanan mikst venöz oksijen satürasyonundan ($SmvO_2$) daha düşük olduğunu gözlemledik. Bu nedenle, saptanabilir herhangi bir şant olmamasına rağmen bazı hastalarda Qp/Qs değeri "1"den küçük olarak hesaplandı. Bu gözlemin prognostik öneme sahip olabileceğini varsaydık ve bildiğimiz kadarıyla bu henüz çalışılmadı. Bu nedenle soldan sağa şantı olmayan prekapiller PH'de Qp/Qs'in prognostik değerini araştırmayı amaçladık.

Gereç ve Yöntem: Ardışık 173 hastayı içeren bu retrospektif kohort çalışmada, hastane dosyaları, Fick yöntemiyle hesaplanan Qp/Qs'i de içeren klinik, ekokardiyografik ve hemodinamik veriler için tarandı.

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Bulgular: Ortanca 25 ay takipte 74 hasta öldü. Ölenler, hayatta kalanlardan daha düşük Qp/Qs'e sahiplerdi (0,76±0,19'a karşılık 1,02±0,07; p<0,001). Çok değişkenli lojistik regresyon analizi, düşük Qp/Qs ve kötü fonksiyonel kapasitesinin (Dünya Sağlık Örgütü sınıf III-IV) mortalitenin bağımsız öngörücüleri olduğunu gösterdi. ROC eğrisi analizi, mortaliteyi tahmin etmek için Qp/Qs'in optimal kestirim değerinin %76 duyarlılık ve %98 özgüllük ile 0,90 olduğunu ortaya koydu.

Sonuç: Fick yöntemiyle hesaplanan Qp/Qs'in mortalitenin bağımsız bir öngördürücüsü olduğu gösterildi. Bu prognostik çıkarım, $SpaO_2$ ile formül aracılığıyla hesaplanan $SmvO_2$ arasındaki farka dayanmaktadır. Bununla birlikte, bu sonuç, Fick yönteminin potansiyel bir içsel metodolojik kusurunun bir yansıması olabilir.

Anahtar Kelimeler: Pulmoner hipertansiyon, prognoz, fick yöntemi, oksijen, mortalite

INTRODUCTION

Pulmonary hypertension (PH) is characterized by elevated mean pulmonary artery pressure (mPAP) of ≥25 mmHg at rest during right-sided cardiac catheterization (RSCC) (1). PH is a life-threatening condition that ultimately causes right ventricular failure and death. Since the prognostic evaluation is critical for PH, several risk assessment tools were developed to predict its prognosis and optimize treatment (2-7).

RSCC is accepted as the gold standard diagnostic tool for the definitive diagnosis of PH and used for prognostic purposes (1). The pulmonary to systemic flow (Qp/Qs) ratio is a calculation for estimating the direction and severity of shunting, especially in congenital heart diseases. This ratio is typically calculated using the Fick method (8), and it is essential in the differential diagnosis and management of PH in patients with left-to-right shunts (9). We routinely calculate the Qp/Qs ratio to avoid missing any left-to-right shunts in patients with precapillary PH. However, the pulmonary artery oxygen saturation ($SpaO_2$) was lower than the mixed venous oxygen saturation ($SmvO_2$) calculated using the formula; hence, the Qp/Qs ratio was calculated as less than "1" in some patients despite the absence of any detectable shunt. We hypothesized that this observation might have prognostic significance; however, to the best of our knowledge, it has not been investigated yet. Therefore, we aimed to investigate the prognostic value of the Qp/Qs ratio in patients with precapillary PH without any left-to-right shunt.

METHODS

Study Population

This retrospective cohort study was conducted in the Department of Cardiology of Dokuz Eylül University Hospital. This study was approved by the Local Ethics Committee. Informed consent was obtained for the RSCC procedure from all patients.

The medical records of 225 consecutive patients who underwent RSCC combined with oximetric study through the Fick method (8) for differential diagnosis of PH were screened from the hospital database. All patients were diagnosed with precapillary PH by an algorithm recommended in the guideline (1). After excluding 52 patients with confirmed left-to-right shunt not only through the oximetric analysis but also with other diagnostic modalities, the remaining 173 patients with precapillary PH were considered for the final analysis. Precapillary PH was defined as mPAP ≥25 mmHg along with pulmonary artery wedge pressure (PAWP) ≤15 mmHg (1).

Transthoracic Doppler Echocardiography

Thorough transthoracic echocardiography that specifically focused on investigating the function and pressure within the right side of the heart was performed by using a Philips HD11 XE Ultrasound system with 3.2 MHz transducer (Philips Healthcare, Best, Netherlands).

Data Collection and Patient Follow-up

All patients were routinely evaluated every 3 months in the PH outpatient clinic. The functional capacity (FC) of the patients was recorded according to the World Health Organization (WHO) functional classification (10). Results of the 6-min walking distance (6MWD) test, echocardiographic values, and laboratory parameters were obtained. All-cause mortality was noted during follow-up, and patients were compared as survivors versus nonsurvivors for analysis.

Right-Sided Cardiac Catheterization

All patients underwent RSCC at rest that was performed by experienced cardiologists. Neither sedation nor supplemental oxygen was administered during the study. Pressure records and the oximetric study were performed with multipurpose angled catheters (end hole only). The right atrial pressure and PAP, along with the PAWP, were recorded. Blood samples from the pulmonary artery, right atrium, right ventricle, superior vena cava (SVC), inferior vena cava (IVC), and systemic artery were obtained routinely, and the Qp/Qs ratio was calculated using the following formula: systemic artery oxygen saturation - $SmvO_2$/pulmonary artery wedge oxygen saturation - $SpaO_2$ (8). To calculate $SmvO_2$, this formula was used: [(3×SVC oxygen saturation ($SsvcO_2$) + IVC oxygen saturation ($SIvcO_2$))/4] (11). In cases with no record of the pulmonary artery wedge saturation,

it was assumed to be similar to the systemic artery oxygen saturation. The cardiac index was calculated by dividing Qs values by the measure of the total surface area of the body. Transpulmonary gradient determined by subtracting PAWP from mPAP was divided by Qp to calculate the pulmonary vascular resistance (PVR).

Statistical Analysis

Statistical analyses were performed using SPSS 25.0 (institutionally registered software). Normality was assessed with the Kolmogorov-Smirnov test. Data were reported as percentages for categorical variables and mean ± standard deviation or median (interquartile range) for continuous variables. Student's t-test or Mann-Whitney U test was used to compare continuous variables, and the appropriate chi-squared test was performed to compare categorical variables. To predict mortality, the optimal cutoff threshold for the Qp/Qs ratio was obtained by analyzing the receiver operating characteristics (ROC) curve. The Kaplan-Meier analysis with a cutoff value of 0.90 for the Qp/Qs ratio was used to designate the survival curves. Logistic regression analyses were performed to define predictors of mortality. Variables with p-value <0.1 in the univariate regression analysis were included in the multivariate logistic regression analysis. For all statistical analyses, p-value of ≤0.05 was accepted as significant.

RESULTS

This study included 45 men and 128 women, with a mean age of 60.2±15.5 years. The follow-up duration ranged from 25 months to 128 months. A total of 74 (42.7%) deaths were noted during the follow-up. Baseline demographics and clinical and echocardiographic comparisons of survivors versus nonsurvivors are presented in Table 1. According to functional class, 91.8% of the nonsurvivors and 54.5% of the survivors had WHO FC III-IV. Nonsurvivors had significantly lower 6MWD, tricuspid annular plane systolic excursion, and right ventricular outflow tract maximum systolic velocity values. Pericardial effusion more frequently occurred in nonsurvivors, and they had significantly higher brain natriuretic peptide and lactate dehydrogenase levels (Table 1).

Baseline hemodynamic data obtained from RSCC are shown in Table 2. Nonsurvivors had significantly lower Qp and Qp/Qs ratio (3.52±1.48 vs. 4.53±1.43, p<0.001; 0.76±0.19 vs. 1.02±0.07, p<0.001) than survivors, respectively. The mPAP and PVR levels were significantly higher in nonsurvivors.

The multivariate logistic regression analysis showed that the Qp/Qs ratio and poor FC (WHO FC III-IV) (p=0.002,

Table 1. Baseline clinical and echocardiographic characteristics of the study population

Variables	Survivors (n=99)	Non-survivors (n=74)	p
Female n (%)	72 (72.7)	56 (75.7)	0.662
Age (year)	58.97±15.3	61.0±16.3	0.384
WHO/FC III-IV n (%)	48 (54.5)	67 (91.8)	**<0.001**
6-min walking distance (m)	330 (221-380)	264 (120-320)	**0.043**
BNP (pg/mL)	206 (100-442)	614 (253-1186)	**<0.001**
Hemoglobin (mg/dL)	12.43±2.23	12.29±2.32	0.714
Uric acid (mg/dL)	6.89±2.44	7.02±2.38	0.751
Serum LDH (mg/dL)	223 (183-263)	265 (188-344)	**0.027**
sPAP (mmHg)	79.38±20.19	82.76±16.92	0.255
TAPSE (mm)	18.1±5.3	16.1±4.8	**0.020**
RVOT maximum systolic velocity (m/sec)	0.87±0.20	0.75±0.18	**0.002**
Pericardial effusion, n (%)	14 (15.7)	27 (43.5)	**<0.001**

WHO/FC: World Health Organization/functional capacity, BNP: Brain natriuretic peptide, LDH: Lactate dehydrogenase, sPAP: Systolic pulmonary arterial pressure, TAPSE: Tricuspid annular plane systolic excursion, RVOT: Right ventricular outflow tract

Table 2. Baseline hemodynamic data obtained from right-sided cardiac catheterization

Variables	Survivors (n=99)	Non-survivors (n=74)	p
Qp (L/min)	4.53±1.43	3.52±1.48	**<0.001**
Qs (L/min)	4.5±1.5	4.7±1.7	0.431
Qp/Qs ratio	1.02±0.07	0.76±0.19	**<0.001**
Systolic PAP at catheterization (mmHg)	73.4±23.3	77.7±18.0	0.207
Mean PAP at catheterization (mmHg)	43.0±12.5	47.1±12.1	**0.038**
Diastolic PAP at catheterization (mmHg)	25.57±10.10	28.23±9.42	0.093
Pulmonary vascular resistance (wood unit)	6.5 (4.1-9.5)	8.6 (5.0-12.8)	**0.006**
Right atrial pressure (mmHg)	10.7±5.3	10.2±6.0	0.598
Cardiac index (L/min/m^2)	2.5±0.8	2.7±0.9	0.147

Qp: Pulmonary blood flow, Qs: Systemic blood flow, Qp/Qs: pulmonary to systemic blood flow ratio, PAP: Pulmonary arterial pressure

p<0.001 respectively) were independent predictors of mortality (Table 3). The optimal Qp/Qs cutoff for predicting mortality was 0.90 with 76% sensitivity and 98% specificity (area under the curve=0.845, 95% CI: 0.778-0.913, p<0.001) based on the ROC curve analysis (Figure 1). The Kaplan-Meier analysis of Qp/Qs >0.90 and Qp/Qs ≤0.90 yielded diverging survival curves (p<0.001) (Figure 2). Qp/Qs ≤0.90 was noted in 34.7% of the patients, and Qp/Qs ratio ≤0.90 was significantly more common in nonsurvivors than in survivors (75.7% vs. 4%; p<0.001).

DISCUSSION

Hemodynamic parameters obtained from RSCC are valuable for predicting the prognosis of PH (1). The Fick method relies on measuring oxygen concentrations in blood samples obtained by a catheter positioned at several points and serves as a reference procedure for the evaluation of blood flow (Qp/Qs) (8) and shunts (9). Qp, Qs, and Qp/Qs ratio are routinely calculated in patients

Table 3. Multivariate logistic regression analysis to predict mortality

Variables	Univariate OR, 95% CI	p	Multivariate OR, 95% CI	p
WHO/FC III-IV	6.306 (3.654-8.696)	**<0.001**	7.297 (2.410-9.257)	**<0.001**
6MWD	0.997 (0.995-1.0)	**0.046**	0.993 (0.984-1.002)	0.149
BNP	1.001 (1.000-1.001)	**0.001**	0.999 (0.998-1.001)	0.561
Serum LDH	1.005 (1.001-1.008)	**0.010**	1.009 (0.996-1.022)	0.164
TAPSE	0.925 (0.865-0.989)	**0.023**	1.124 (0.851-1.486)	0.410
RVOT maximum systolic velocity	0.162 (0.023-1.165)	**0.071**	0.205 (0.027-0.826)	0.504
Pericardial effusion,	0.443 (0.267-0.737)	**<0.001**	0.729 (0.371-1.432)	0.246
Qp/Qs	0.599 (0.467-0.770)	**<0.001**	0.117 (0.030-0.450)	**0.002**
Mean PAP at catheterization	1.027 (1.027-1.054)	**0.040**	0.995 (0.893-1.108)	0.206

Variables entered into the univariate logistic regression analysis: sex, age, WHO/FC III-IV, 6MWD, BNP, hemogram, uric acid, serum LDH, systolic pulmonary arterial pressure, TAPSE, RVOT maximum systolic velocity, pericardial effusion, Qp/Qs ratio, systolic PAP at catheterization (mmHg), mean PAP at catheterization (mmHg), pulmonary vascular resistance, right atrial pressure, cardiac index. WHO/FC: World Health Organization/functional capacity, 6MWD: 6-min walking distance, BNP: Brain natriuretic peptide, LDH: Lactate dehydrogenase, TAPSE: Tricuspid annular plane systolic excursion, RVOT: Right ventricular outflow tract, Qp/Qs: pulmonary to systemic blood flow ratio, PAP: Pulmonary arterial pressure

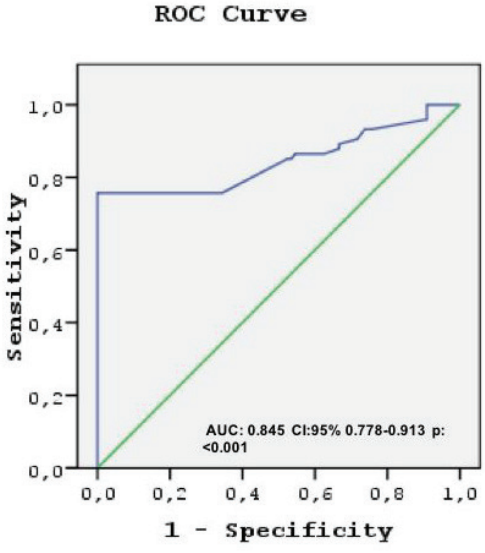

Figure 1. Receiver operating characteristic curves of the Qp/Qs ratio for predicting mortality
Qp/Qs: pulmonary to systemic blood flow ratio, ROC: Receiver operating characteristics, CI: Confidence interval, AUC: Area under curve

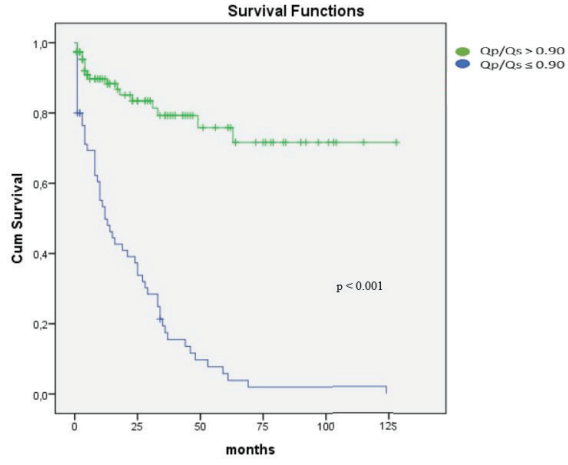

Figure 2. Kaplan-Meier survival estimates for mortality in all patients with precapillary pulmonary hypertension based on the Qp/Qs ratio of ≤0.90 vs. >0.90
Qp/Qs: pulmonary to systemic blood flow ratio

with PH, except in settings with technical obstacles in our clinic. In real-life practice, without any shunting, the Qp/Qs ratio should be equal to 1. However, during routine RSCC

procedures in patients with high-risk status and precapillary PH, the Qp/Qs ratio calculated using the Fick method was <1. $SpaO_2$ was lower than $SmvO_2$ calculated using the formula; therefore, the Qp/Qs ratio was calculated as <1. In the absence of shunting, the downward deviation of the Qp/Qs value from the expected normal value of 1 might initially appear to be strange in a closed-loop system and might be ignored as test result variability. However, the Qp/Qs ratio calculated using the Fick method is based on oxygen consumption, and the main determinant of this ratio is a difference between $SpaO_2$ and $SmvO_2$. Moreover, $SmvO_2$ is measured directly from the pulmonary artery (12) or calculated using a formula (11). However, various potential flow sources such as the coronary sinus (CS) can be neglected by calculating $SmvO_2$ from $SsvcO_2$ and $SivcO_2$. This may not matter in patients with normal cardiac function. However, this phenomenon becomes important in patients with heart failure (13). CS oxygen saturation ($ScsO_2$) was a strong predictor of the severity of heart failure due to deranged metabolic demands (14). Similarly, we thought that in the presence of right ventricular dysfunction caused by a chronic pressure overload in PH, the metabolic demand of the right ventricle increases, and it may result in reduced $ScsO_2$. This causes a difference between $SpaO_2$ and $SmvO_2$ calculated using the formula. A study that supported our hypothesis showed that the major reason for the calculation between average venous oxygen saturation and $SpaO_2$ was the deoxygenated blood of the CS in patients who underwent cardiac surgery (15). While the contribution of deoxygenated blood derived from the myocardium via the CS to venous SO_2 can be taken into account in the measurement of $SmvO_2$ from the pulmonary artery, the effect of the myocardium can be ignored in the measurement of SO_2 from the central veins. Therefore, direct measurement of $SmvO_2$ through pulmonary artery catheterization is more accurate in patients with critical illness (16), including patients undergoing cardiac surgery and/or patients with impaired cardiac function (17,18). The contribution of CS drainage to venous SO_2 could have significant effects in the late stages of PH that might also serve as a basis for the Qp/Qs deviation in the Fick method (19). In the present study, we think that the $SpaO_2$ value is lower than that of $SmvO_2$ because of right ventricular dysfunction in patients with precapillary PH. The low $SpaO_2$ indicates that the heart cannot meet the tissue oxygen demand. The Qp/Qs ratio was calculated as <1 because a low $SpaO_2$ and, not surprisingly, decreased Qp/Qs ratio was an independent predictor of mortality. Similarly, $SpaO_2$ was shown to be a more valuable prognostic factor than the cardiac index in patients with PH (20).

Study Limitations

This retrospective cohort analysis had several limitations. First, our study was conducted on a limited number of patients from a single center without any data about treatment-related issues, which can potentially influence outcomes. Second, blood samples from the CS were not obtained. Analyses of $ScsO_2$ that can reflect an intrinsic error in the calculation of $SmvO_2$ should be ideally incorporated in the final evaluation of patients with PH and right ventricular dysfunction. However, the potential value of this issue in calculating the Qp/Qs ratio through the Fick method remains to be established. Third, we could not compare $SmvO_2$ with $SpaO_2$ because of the absence of oxygen saturation values in our hospital records. Fourth, thermodilution, which is the preferred method for determining cardiac output in patients with PH (19), was not utilized in this cohort because it was not available during the study period. Therefore, we could not compare the cardiac output obtained by thermodilution with Qp and Qs calculated using the Fick method. Essentially, further prospective randomized controlled studies with larger sample sizes are strongly recommended for the validation of the results of this study.

CONCLUSION

In this study, decreased Qp/Qs ratio calculated using the Fick method was an independent predictor of mortality in patients with precapillary PH without left-to-right shunts. This prognostic implication was based on the difference between $SpaO_2$ and $SmvO_2$ calculated using the formula. Nevertheless, it remains to be elucidated whether this is a reflection of an intrinsic methodological flaw in the Fick method.

ETHICS

Ethics Committee Approval: This retrospective cohort study was conducted in the Department of Cardiology of Dokuz Eylül University Hospital. This study was approved by the Local Ethics Committee (no: 2018/07-32, date: 15.03.2018).

Informed Consent: Informed consent was obtained for the RSCC procedure from all patients.

Authorship Contributions

Surgical and Medical Practices: B.Ş., Concept: B.Ş., B.A., E.Ö., Design: B.Ş., B.A., M.B.Y., M.B., C.S., Data Collection or Processing: B.Ş., B.A., E.Ö., D.S., B.Ö.K., K.C.T., Analysis or Interpretation: B.Ş., B.A., M.B.Y., E.Ö., D.S., B.Ö.K., B.Ac., M.B., C.S., Literature Search: B.Ş., Writing: B.Ş., B.A., M.B.Y., E.Ö., D.S., B.Ö.K., B.Ac., K.C.T., M.B., C.S.

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Research

Identification of Factors Associated with Prolonged Stay in the Intensive Care Unit

Yoğun Bakım Ünitesinde Uzun Süre Kalış ile İlişkili Faktörlerin Belirlenmesi

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ABSTRACT

Objective: This study aimed to determine the factors associated with prolonged intensive care unit length of stay (ICU-LOS), which is increasingly common in ICU, and to evaluate its effect on patient outcomes.

Methods: This retrospective study was evaluated by obtaining data of 5,022 patients who were followed in the ICU of a tertiary education and research hospital between January 2014 and January 2021 and met the research criteria in electronic environment.

Results: Patients were divided into two groups as patients with ICU-LOS <14 days (n=4,083, 81.3%) and patients with ICU-LOS ≥14 days (n=939, 18.7%). Sepsis and pulmonary diseases were more common in the prolonged ICU-LOS group than in the non-prolonged ICU-LOS group (p<0.05). While 61.8% (2,525) in the non-prolonged ICU-LOS group needed mechanical ventilator support, this rate increased to 97.4% (915) in the prolonged ICU-LOS group (p<0.001). The duration of MV was higher in the prolonged ICU-LOS group [20.3 (14.9-29.2)] than in the non-prolonged ICU-LOS group [3.4 (1.7-6.2)] (p>0.001). Although 18.7% of the patients had prolonged ICU-LOS, they consumed 66.3% and 59.7% of all mechanical ventilator days and ICU hospitalization days, respectively. ICU mortality was higher in the prolonged ICU-LOS group (n=376; 40%) than in the non-prolonged ICU-LOS group (n=1219; 29.9%). The development of acute kidney injury (odds ratio (OR): 1,807; 95% confidence interval (CI) 1,434-2,277), development of pressure sores (OR: 3,572; 95% CI: 2,663-4,792), total parenteral nutrition use (OR: 2,014; 95% CI: 1,639-2,475), increase in body mass index (OR: 1,015; 95% CI: 1.001-1.031), and mechanical power increase (OR: 1.041; 95% CI: 1.002-1.082) were associated with prolonged ICU-LOS (OR: 1.015; 95% CI: 1.001-1.031).

Conclusion: Prolonged ICU-LOS is associated with increased costs, use of resources, and morbidity and mortality.

Keywords: Intensive care units, length of stay, mechanical ventilation, mortality

ÖZ

Amaç: Bu araştırma yoğun bakım ünitesinde (YBÜ) gittikçe daha sık rastlanan uzamış YBÜ kalış süresi (YBÜ-LOS) ile ilişkili faktörleri belirlemek ve hasta sonuçlarına etkisini değerlendirmek amacı ile planlandı.

Gereç ve Yöntem: Bu retrospektif araştırma Ocak 2014- Ocak 2021 döneminde üçüncü düzey bir eğitim ve araştırma hastanesinin YBÜ'de takip edilen ve araştırma kriterlerini karşılayan 5.022 hastanın verileri elektronik ortamda elde edilerek değerlendirildi.

Bulgular: Araştırmaya dahil edilen hastalar YBÜ-LOS <14 gün olan 4.083 (81,3) hasta ve YBÜ-LOS ≥14 gün olan 939 (18,7) hasta olacak şekilde iki gruba ayrıldı. Uzamış YBÜ-LOS grubunda sepsis ve pulmoner hastalıklar uzamamış YBÜ-LOS grubundan daha sık görüldü (p<0,05). Uzamamış YBÜ-LOS hastalarının %61,8'i (2.525) mekanik ventilatör desteğine ihtiyaç duyarken bu oran uzamış YBÜ-LOS hastalarında %97,4'e (915) yükseldi (p<0,001). Mekanik ventilasyon süresi uzamış YBÜ-LOS hastalarında [20,3(14,9-29,2)], uzamamış YBÜ-LOS hastalarına [3,4(1,7-6,2)] göre daha yüksekti (p>0,001). Uzamış YBÜ-LOS hastalarının oranının %18,7 olmasına rağmen tüm mekanik ventilatör günlerinin %66,3'ünü ve tüm YBÜ yatış günlerinin %59,7'sini işgal ettikleri saptandı. YBÜ mortalitesi uzamış YBÜ-LOS grubunda (376; %40), uzamamış YBÜ-LOS grubuna göre (1.219; %29,9) daha yüksek bulundu. YBÜ'de akut böbrek hasarı gelişmesinin [risk ornaı (OR): 1.807; güven aralığı (CI): %95 1.434-2.277],

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University of Health Sciences Turkey, bası yarası gelişmesinin (OR: 3.572; CI: %95 2.663-4.792), TPN kullanımının (OR: 2.014; CI: %95: 1.639-2.475) vucüt kitle indeksi artışın (OR: 1.015; CI: %95: 1.001-1.031) ve mechanical power artışının (OR: 1.041; CI: %95: 1.002-1.082) uzamış YBÜ-LOS ile ilişkili olduğu belirlendi (OR: 1.015; CI: %95: 1.001-1.031).

Sonuç: YBÜ uzun süre kalma, artan maliyet ve kaynak kullanımı ile ilişkilidir. Ek olarak hastaların morbidite ve mortalitesinin de artmasına neden olmaktadır.

Anahtar Kelimeler: Yoğun bakım üniteleri, kalış süresi, mekanik ventilasyon, mortalite

INTRODUCTION

The science of intensive care has developed significantly in the last decade, and emerging technological developments and new treatments have reduced the mortality rates in intensive care units (ICUs) (1). Increasing survival and aging population as a result of advances in the early management of critical illnesses have led to the formation of a patient profile that is increasingly encountered in the ICUs and requires prolonged ICU follow-up. The number of patients with this profile, who are dependent on a particular type of technology or medical care, is expected to increase in the coming years (2,3).

There is no consensus on the definition of long-term intensive care. Different criteria have been used for its definition, and it has been variously defined as staying in the ICU for >10 days or >21 days (4-7). In a more recent study, ICU stay of at least 14 days was defined as a prolonged ICU length of stay (ICU-LOS) (6). Depending on the definition used, 4%-11% of patients admitted to the ICU have been determined to have a prolonged ICU-LOS (8-10). Patients with ICU-LOS >14 days use approximately half of all ICU hospitalization days and mechanical ventilation (MV) use days, although they constitute a small proportion of patients admitted in the ICU (10,11). Prolonged ICU-LOS is associated with increased cost and resource use and may affect morbidity and mortality. Long-term ICU-LOS can adversely affect health by increasing the risk of infection, complications, and possibly mortality (9,12). Operationally, it affects ICU bed availability and results in the cancellation of elective surgeries, leading to long waiting times (9). Bed waiting time is prolonged before admission to the ICU, a factor known to affect patient outcomes (13). Identifying patients at risk of long-term stay can assist ICU management and prevent ICU bed shortage.

In patients followed up in the ICU, prolonged LOS causes increased costs and resource use, as well as prolonged ICU waiting time. Therefore, early identification of patients at risk of prolonged ICU-LOS is important. This study aimed to determine the factors associated with prolonged ICU-LOS and to evaluate its effect on patient outcomes.

METHODS

Data Center

This study was performed retrospectively in the ICU of a third-level training and research hospital in Istanbul, the most populous city in Turkey. Consisting of 27 tertiary hospital beds, the ICU accepts an average of 1340 patients per year. The nurse-patient ratio in these ICUs, which is controlled by the clinical decision support system (CDSS) and where extracorporeal treatments (ECMO, hemodialysis, and plasmapheresis) can be applied, is 1:2.

When a patient is admitted to the ICU, the patient's identity information, anamnesis, and examination findings are recorded in the patient file created in CDSS. Information that requires dynamic measurement, such as urine output and glasgow coma scale (GCS), is recorded in the CDSS patient file at regular intervals by the patient's physician. Intensive care scores are calculated and recorded with the algorithms defined in CDSS, taking into account the worst values in the last 24 h. In addition, all bedside monitor parameters, MV data, extracorporeal applications, laboratory test results, and dose information of all infusions administered for treatment are transmitted to the CDSS from the applied devices, thanks to the electronic ecosystem created during the patient's ICU follow-up. Thus, all patient data are collected in the patient file in CDSS.

Data Collection

Data of patients admitted to the ICU between January 2014 and January 2021 from the EMRall-QlinICUImd Soft Metavision CDSS database used in the ICU were obtained by using Structured Query Language queries. In addition to the patients' LOS in the ICU, demographic data, admission diagnoses, comorbid diseases, scores calculated in the ICU, blood gas and biochemical values, MV data, treatments, and interventions, developed complications, and mortality results were evaluated.

Sample

A total of 9,380 patients were admitted to the ICU during the research period, of which 4,358 did not meet the research and exclusion criteria. The remaining 5,022 patients constituted the study population (Figure 1).

In the present study, similar to the study by Zampieri et al. (6), a 14-day or longer stay in the ICU was considered

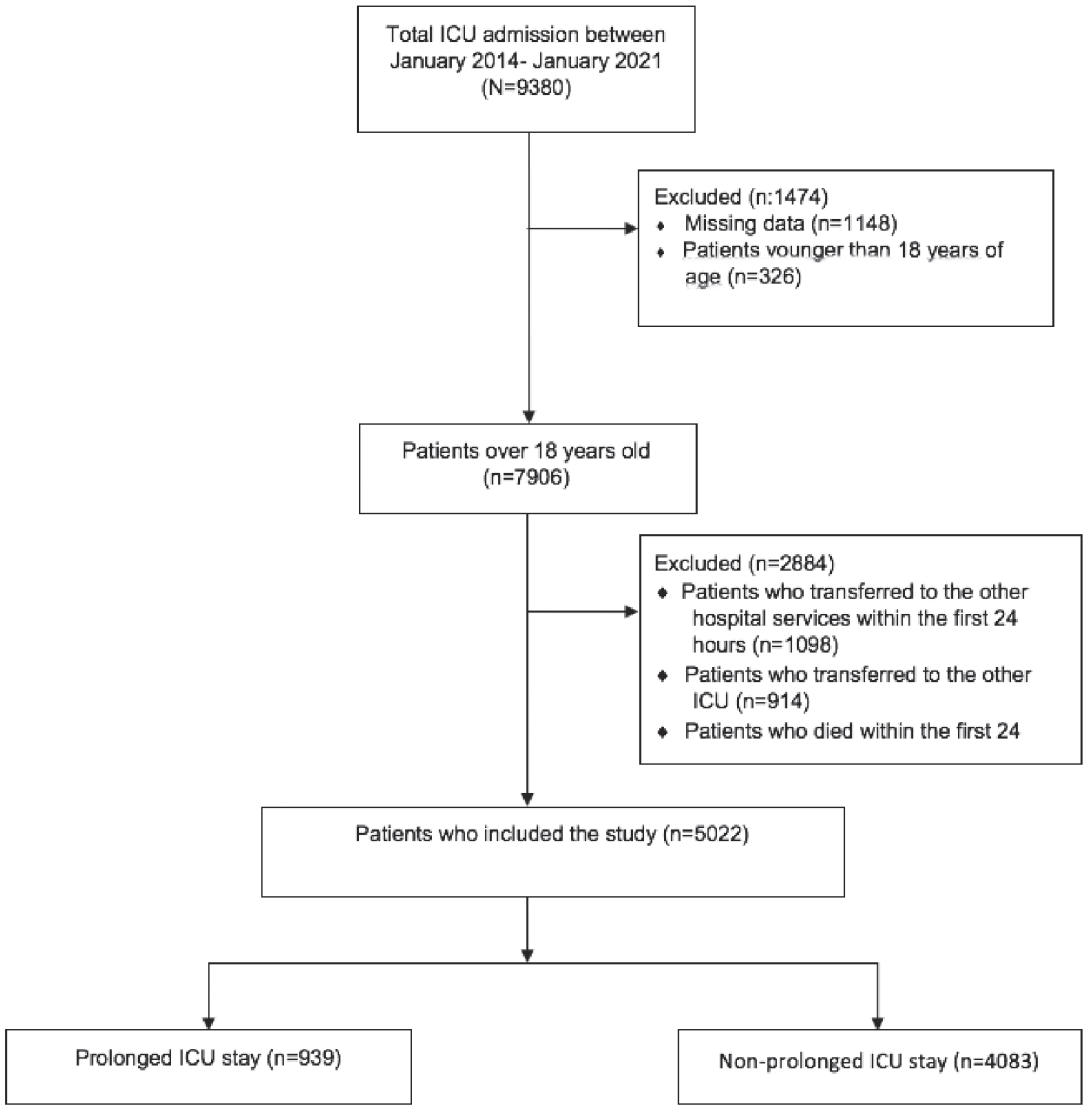

Figure 1. Flow diagram of patient selection
ICU: Intensive care unit

a prolonged ICU-LOS. Patients included in the study were divided into two groups: 4,083 (81.3) patients hospitalized for <14 days and 939 (18.7) patients with a hospitalization period of ≥14 days.

Admission Criteria

The study planned to include the entire patient population aged ≥18 years who stayed in the ICU for >24 h.

Exclusion Criteria

Patients aged <18 years (n=326), admitted to the service within 24 h of ICU admission (n=1,098), died within the first 24 h in the ICU (n=872), referred to another ICU (n=914), and had missing data (n=1,148) were excluded from the study.

Primary Outcome

The primary outcomes were factors associated with prolonged hospital stay in the ICU and their effects on patient outcomes.

Secondary Outcomes

The secondary outcomes were comorbid diseases, admission diagnoses, scores, treatment, and interventions.

Ethical Issues

Before the study, ethical committee approval and institutional permission were obtained from the Clinical Research Ethics Committee (protocol code: 2021/405; decision no. 2021-16) of the public hospital where the study was conducted.

Statistical Analysis

SPSS 22.00 program was used in the analysis of the research data. The Shapiro-Wilk test was used to determine the normal distribution of numerical data. Normally distributed numerical data were expressed as mean ± standard deviation, and non-normally distributed numerical data were expressed as median and interquartile ranges. The independent samples t-test was used to compare the numerical data between groups, and the Mann-Whitney U test was used when the conditions of this test were not met. Categorical variables were expressed as frequency and percentage. The chi-square test was used to compare categorical variables between groups, and Fisher's Exact test was used when the conditions of the chi-square test were not met. Logistic regression analysis was used to determine parameters associated with the risk of prolonged ICU hospitalization; $p<0.05$ was accepted for the significance level.

RESULTS

In this study, 56.9% of the 5022 patients constituting the study population were male, and the mean age was 60.49±19.24 years. The mean body mass index (BMI) was 27.11±5.58 kg/m^2. Moreover, 74.6% of the patients had at least one comorbid disease. The most common comorbid disease was hypertension (n=1,835; 36.5%). Sepsis (n=1,116; 22.2%) was the most common admission diagnosis in the ICU, and 68.5% (n=3,440) of the patients with a calculated acute physiology and chronic health evaluation (APA-CHE) II score of 20 (n=1327) needed MV support. Acute kidney injury (AKI) was detected in 3,309 (65.8) patients. The median ICU-LOS of the patients was 4.54 (1.91-10.45) days. The ICU mortality rate was 31.7% (n=1595).

Comparison of Groups in Terms of Clinical Characteristics

Patients were divided into two groups as non-prolonged ICU-LOS group with <14 days and prolonged ICU-LOS group with ≥14 days. While ICU-LOS was <14 days in 4,083 (81.3%) of 5,022 patients, ICU-LOS was ≥14 days in 939 patients (18.7). The general characteristics of the groups are given in Table 1. No significant difference was found in age and gender between the groups. The frequency of the male gender was higher in both groups. The frequency of comorbid disease was lower in the prolonged ICU-LOS group (671; 71.5) than in the non-prolonged ICU-LOS group (3,075; 75.3) ($p<0.05$). Hypertension was the most common comorbid disease in both groups. Considering other comorbidities, the frequency of chronic obstructive pulmonary disease (COPD), malignancies, and hepatic diseases was higher in the non-prolonged ICU-LOS group,

Table 1. Characteristics of patient groups and admission diagnosis

Parameters	ICU-LOS of ≥ 14 n=939 (18.7%)	ICU-LOS<14 n=4083 (81.3%)	p
Age (year)	59.78±19.55	60.66±19.16	0.210
18-65	502 (53.5)	2145 (52.6)	0.608
65-74	174 (18.5)	821 (20.1)	0.274
75-84	174 (18.5)	732 (17.9)	0.665
≥85	89 (9.5)	385 (9.4)	0.963
Gender			0.298
Female	390 (41.5)	1772 (43.4)	
Male	549 (58.5)	2311 (56.6)	
Body mass index (kg/m^2)	26.12 (24.22-29.38)	25.95 (23.87-29.29)	0.003
Comorbidity	671 (71.5)	3075 (75.3)	0.014
Hypertension	327 (34.8)	1508 (36.9)	0.226
Diabetes	189 (20.1)	925 (22.7)	0.093
Cerebrovascular disease	69 (7.3)	288 (7.1)	0.751
CAD	106 (11.3)	424 (10.4)	0.416
COPD	106 (11.3)	574 (14.1)	0.025
CRF	81 (8.6)	381 (9.3)	0.500
Malignancy	78 (8.3)	536 (13.1)	<0.001
Hepatic disease	9 (1.0)	79 (1.9)	0.040
Psychiatric disorder	20 (2.1)	84 (2.1)	0.888
Dementia	35 (3.7)	173 (4.2)	0.480
Obesity	214 (22.8)	790 (19.3)	0.017
Other	95 (10.1)	390 (9.6)	0.597
Admission diagnosis			
Cerebrovascular disease	141 (15.0)	587 (14.4)	0.616
Cardiac	56 (6)	227 (5.6)	0.628
Pulmonary	159 (16.9)	454 (11.1)	<0.001
Pneumonia	96 (10.2)	252 (6.2)	<0.001
COPD	48 (5.1)	134 (3.3)	0.007
Pulmonary, others	15 (1.6)	68 (1.7)	0.883
Renal-metabolic	44 (4.7)	234 (5.7)	0.207
Hepatic cirrhosis	1 (0.1)	54 (1.3)	<0.001*
Trauma	153 (16.3)	399 (9.8)	<0.001
Sepsis	245 (26.1)	871 (21.3)	0.002
Pneumosepsis	46 (4.9)	180 (4.4)	0.513
Intra-abdominal sepsis	116 (12.4)	405 (9.9)	0.027
Urosepsis	22 (2.3)	79 (1.9)	0.422
Sepsis, other	61 (6.5)	207 (5.1)	0.080
Malignancy	44 (4.7)	325 (8.0)	<0.001
Postoperative	31 (3.3)	471 (11.5)	<0.001
Intoxication	13 (1.4)	162 (4.0)	<0.001
GIB-hemorrhage	19 (2.0)	123 (3.0)	0.099
Others	33 (3.5)	176 (4.3)	0.271

CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, CRF: Chronic renal failure, GIB: Gastrointestinal bleeding, *Fisher's Exact test

and the prevalence of obesity was higher in the prolonged ICU-LOS group (p<0.05). The BMI values were 26.12 (24.22-29.38) in the prolonged ICU-LOS group and 25.95 (23.87-29.29) in the non-prolonged ICU-LOS group (p<0.05). The frequency of any other comorbid diseases was not significantly different between the groups. In both groups, the most common diagnosis on ICU admission was sepsis. Furthermore, a diagnosis of sepsis (n=245; 26.1%) was more common in the prolonged ICU-LOS group than in the non-prolonged ICU-LOS group (n=871; 21.3%) (p<0.05). Although the most common sepsis source was intra-abdominal sepsis in both groups, intra-abdominal sepsis was more common in the prolonged ICU-LOS group (p<0.05). While pulmonary diseases (n=96; 10.2%) and trauma (n=153; 16.3%) were more common in the prolonged ICU-LOS group (due to COPD, pneumonia, and other causes), malignancy (n=325; 8.0%), intoxication (n=162, 4.0%), hepatic diseases (n=54; 1.3%), and postoperative follow-up (n=471; 11.5%) were more common in the prolonged ICU-LOS group (p<0.05) (Table 1).

The scores of the patients calculated during ICU admission, interventions, and treatments are shown in Table 2. APACHE II, APACHE IV, simplified acute physiology (SAPS) III, sequential organ failure assessment, and therapeutic intervention scoring system 28 scores were higher in the prolonged ICU-LOS group, whereas GCS and richmond agitation and sedation scale scores were lower (p<0.001). Arterial and central venous catheterization was performed more frequently in the prolonged ICU-LOS group. MV and dialysis were more needed and the rate of tracheostomy was higher in the prolonged ICU-LOS group than in the non-prolonged ICU-LOS group (p<0.001). The rates of using antibiotics, total parenteral nutrition (TPN), and vasoactive drugs were higher in the prolonged ICU-LOS group (p<0.001). The average daily energy amounts given in the ICU of both groups were comparable (p>0.05). The frequency of pressure sores and AKI was higher in the prolonged ICU-LOS group than in the non-prolonged ICU-LOS group (p<0.001).

Table 2. Scores, interventions, and treatments during ICU-LOS

Parameters	ICU-LOS of ≥ 14 n=939 (18.7%)	ICU-LOS<14 n=4083 (81.3%)	p
APACHE II	24 (19-29)	19 (12-27)	<0.001
APACHE IV	85 (64-107)	71 (49-100)	<0.001
SAPS III	47 (39-53)	42 (33-53)	<0.001
SOFA	7 (5-9)	5 (2-9)	<0.001
TISS	22 (17-27)	19 (15-24)	<0.001
GCS	10 (8-13)	12 (7-15)	<0.001
RASS	-2 [-4- (-1)]	-1 (-3-0)	<0.001
Interventions			
Arterial catheter	783 (83.4)	2704 (66.2)	<0.001
Central catheter	763 (81.3)	1783 (43.7)	<0.001
MV	915 (97.4)	2525 (61.8)	<0.001
Tracheostomy	617 (65.7)	284 (7.0)	<0.001
Dialysis	323 (34.4)	624 (15.3)	<0.001
Treatments			
Nutrition (kcal/day)	1765 (1537-2089)	1752 (1523-2039)	0.135
TPN	582 (62.0)	1218 (29.8)	<0.001
Antibiotics	938 (99.9)	3355 (78.2)	<0.001
Vasoactive agents	730 (77.7)	2102 (51.5)	<0.001
Complications			
AKI	699 (74.4)	2610 (63.9)	<0.001
Pressure sores	221 (23.5)	171 (4.2)	<0.001
MV (day)	20.3 (14.9-29.2)	3.4 (1.7-6.3)	<0.001
LOS ICU (day)	23.6 (17.9-32.9)	3.6 (1.8-6.5)	<0.001
28-day mortality	248 (26.4)	1219 (29.9)	0.036
ICU mortality	376 (40.0)	1219 (29.9)	<0.001

APACHE: Acute physiology and chronic health evaluation, SAPS: Simplified acute physiology, SOFA: Sequential organ failure assessment, TISS: Therapeutic intervention scoring system, GCS: Glasgow coma score, RASS: Richmond agitation and sedation scale, MV: Mechanical ventilation, TPN: Total parenteral nutrition, AKI: Acute kidney injury, LOS: Length of stay, ICU: Intensive care unit

MV and Blood Gas Parameters

Patients with prolonged ICU-LOS needed mechanical ventilator support more frequently. While 61.8% (2525) of the patients in the non-prolonged ICU-LOS group needed mechanical ventilator support, this rate increased to 97.4% (915) in the prolonged ICU-LOS group (p<0.001). The duration of MV was longer in the prolonged ICU-LOS group [20.3 (14.9-29.2)] than in the non-prolonged ICU-LOS group [3.4 (1.7-6.2)] (p>0.001). Although the proportion of patients with prolonged ICU-LOS was 18.7%, they consumed 66.3% of all mechanical ventilator days. When the mechanical ventilator parameters were examined, the positive end-expiratory pressure [6.6 (5.6-7.4)], peak pressure [14.8 (13.2-16.6)], and minute respiratory rate [19 (17-21)] values in the prolonged ICU-LOS group were higher, and work of breathing ventilator, pulmonary compliance, and tidal volumes were comparable. The mechanical power value was higher in the prolonged ICU-LOS group [9.72 (8.54-11.54)] than in the non-prolonged ICU-LOS group [8.85 (7.42-10.88)]. Partial pressure of carbon dioxide and bicarbonate levels

were higher and lactate levels were lower in the prolonged ICU-LOS group when blood gas results were compared between the groups. Moreover, pH and PO_2 values were comparable. Considering other laboratory parameters, blood glucose and procalcitonin levels were higher in the prolonged ICU-LOS group. Hemoglobin, hematocrit, platelet, and white blood cell counts were comparable between the groups. International normalized ratio and activated partial thromboplastin time were increased in the prolonged ICU-LOS group (Table 3).

Table 3. Mechanical ventilator values and blood parameters of the patients during ICU follow-up

Parameters	ICU-LOS of ≥ 14 n=939 (18.7%)	ICU-LOS<14 n=4083 (81.3%)	p
Mechanical ventilation			
FiO_2 (%)	43 (40-46)	44 (40-49)	0.063
PEEP (cm H_2O)	6.6 (5.6-7.4)	6.0 (5.1-6.7)	<0.001
P peak (cm H_2O)	14.8 (13.2-16.6)	14.1 (12.3-16.2)	<0.001
Tidal volume	492 (439-556)	499 (444-559)	0.455
Tidal volume (mL/kg)	6.83 (6.17-7.60)	6.80 (6.08-7.60)	0.344
Respiratory rate (min)	19 (17-21)	17 (15-20)	<0.001
Compliance (mL/cm H_2O)	37.0 (30.1-46.0)	37.6 (30.9-46.8)	0.068
WOBv (j/L)	1.13 (0.99-1.26)	1.12 (0.98-1.30)	0.134
Mechanical power (J/min)	9.72 (8.54-11.54)	8.85 (7.42-10.88)	<0.001
Blood gas			
PH	7.40 (7.36-7.44)	7.40 (7.35-7.44)	<0.092
PO_2 (mm Hg)	89.5 (72.1-104.9)	85.5 (61.2-108.6)	0.400
PCO_2 (mm Hg)	44.6 (40.1-51.4)	40.6 (36.1-46.0)	<0.001
HCO_3 (mEq/L)	26.7 (24.2-30.0)	24.4 (21.2-27.1)	<0.001
Lactate (mmol/L)	1.61 (1.33-2.14)	1.87 (1.36-3.35)	<0.001
Laboratory			
Glucose (mg/dL)	146 (126-178)	141 (117-178)	<0.001
Hemoglobin (g/dL)	10.5 (8.9-12.5)	10.8 (9.2-12.8)	0.140
Hematocrit (%)	33.0 (27.6-39.0)	33.5 (28.4-38.7)	0.231
Platelet (x10^9/L)	216 (152-295)	218 (157-293)	0.971
White blood cell (x10^9/L)	12.7 (9.5-16.8)	13.0 (10.4-16.2)	0.284
Procalcitonin (µg/L)	1.03 (0.40-5.07)	0.82 (0.35-3.98)	0.026
INR	1.20 (1.09-1.38)	1.17 (1.06-1.41)	0.045
APTT (sec)	36.6 (28.9-46.0)	33.3 (27.1-43.3)	<0.001

FiO_2: Fraction of inspired oxygen, PEEP: Positive end-expiratory pressure, WOBv: Work of breathing ventilator, PCO_2: Partial pressure of carbon dioxide, PO_2: Partial pressure of oxygen, HCO_3: Bicarbonate, CRP: C-reactive protein, INR: International normalized ratio, APTT: Activated partial thromboplastin time, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, IQR: Interquartile range

LOS in the ICU and Mortality

The ICU-LOS in the prolonged ICU-LOS group was 23.6 (17.9-32.9) days and that in the non-prolonged ICU-LOS group was 3.6 (1.8-6.5) days. Although only 18.7% (n=939) of the patients had ICU-LOS ≥14 days, they consumed 59.7% of the total hospitalization days. The 28-day mortality rate was 26.4% (n=248) in the prolonged ICU-LOS group and 29.9% (n=1219) in the non-prolonged ICU-LOS group (p<0.05). Despite the lower 28-day mortality in the prolonged ICU-LOS group, ICU mortality was higher in the prolonged ICU-LOS group (n=376; 40%) than in the non-prolonged ICU-LOS group (n=1,219; 29.9%) (Table 2). In the subgroup analysis performed according to the admission diagnoses of the patients, the mortality rate in the prolonged ICU-LOS group was higher in patients followed up with the diagnoses of pulmonary diseases, sepsis, malignancy, postoperative follow-up, intoxication, and gastrointestinal bleeding (p<0.05). The mortality rate in other admission diagnoses was not different between the groups (Table 4). The logistic regression model created to determine the factors associated with prolonged ICU-LOS found that the development of AKI in the ICU increased the probability of prolonged ICU-LOS by 1.8 times (OR: 1,807; 95% CI: 1,434-2,277), development of pressure sores by 3.5 times (OR: 3,572; 95% CI: 2,663-4.792), and TPN use by 2 times (OR: 2,014; 95% CI: 1,639-2,475) (p<0.001). In addition, the increase in BMI was associated with prolonged ICU-LOS (OR: 1,015; 95% CI: 1,001-1,031). Additionally, a one-unit increase in mechanical power increased the probability of prolonged ICU-LOS by 4.1% (OR: 1,041; 95% CI: 1,002-1,082). Patient age, APACHE II score, pulmonary compliance values, and need for vasoactive agents were not associated with prolonged ICU-LOS (Table 5).

DISCUSSION

This study, which aimed to determine the factors associated with prolonged ICU hospitalization, determined that increased BMI, TPN use, AKI, and pressure ulcer development were associated with prolonged ICU. In addition, the increase in mechanical power in patients who were intubated increased the possibility of prolonged ICU, and pulmonary compliance was not associated with prolonged ICU. Moreover, patient age, APACHE II score,

Table 4. Mortality rates of the groups according to the diagnosis upon ICU admission

Admission diagnosis	ICU-LOS of ≥ 14 n=939 (18.7%)	ICU-LOS<14 n=4083 (81.3%)	p
Cerebrovascular disease	41 (29.1)	177 (30.2)	0.802
Cardiac	22 (39.3)	98 (43.2)	0.598
Pulmonary	65 (40.9)	136 (30.0)	0.012
Pneumonia	41 (42.7)	89 (35.3)	0.203
COPD	16 (33.3)	29 (21.6)	0.107
Pulmonary, Other	8 (53.3)	18 (26.5)	0.042
Renal-metabolic	21 (47.7)	84 (35.9)	0.138
Hepatic cirrhosis	0 (0)	29 (53.7)	0.286*
Trauma	31 (20.3)	91 (22.8)	0.519
Sepsis	127 (51.8)	363 (41.7)	0.005
Pneumosepsis	21 (45.7)	74 (41.1)	0.578
Intra-abdominal sepsis	56 (48.3)	165 (40.7)	0.148
Urosepsis	11 (50)	17 (21.5)	0.008
Sepsis, other	39 (63.9)	107 (51.7)	0.091
Malignancy	24 (54.5)	85 (26.2)	<0.001
Postoperative	16 (51.7)	54 (11.5)	<0.001
Intoxication	6 (46.2)	17 (10.5)	<0.001
GIB-hemorrhage	15 (78.9)	53 (43.1)	0.004
Other	8 (24.2)	32 (18.2)	0.417
28-day mortality	248 (26.4)	1219 (29.9)	0.036
ICU mortality	376 (40.0)	1219 (29.9)	<0.001

COPD: Chronic obstructive pulmonary disease, GIB: Gastrointestinal bleeding, *Fisher's Exact test

Table 5. Parameters associated with prolonged ICU-LOS

Parameters	OR	95% CI	P-value
Age	0.995	0.989-1.000	0.055
Body mass index	1.015	1.001-1.031	0.036
APACHE II	0.990	0.978-1.004	0.156
AKI	1.807	1.434-2.277	<0.001
Pressure sores	3.572	2.663-4.792	<0.001
TPN	2.014	1.639-2.475	<0.001
Vasoactive agents	0.977	0.749-1.274	0.861
Pulmoner compliance	0.998	0.990-1.005	0.525
Mechanical power	1.041	1.002-1.082	0.040

APACHE: Acute physiology and chronic health evaluation, AKI: Acute kidney injury, TPN: Total parenteral nutrition

and use of vasoactive agents were not associated with prolonged ICU-LOS. The rate of prolonged ICU-LOS was higher in patients diagnosed with sepsis and pulmonary diseases.

The increase in MV application, hemodynamic support, and organ support systems together with the developing technology in the ICU has enabled more patients to recover from acute critical illnesses (7). Decreased mortality in the acute period has led to the formation of a patient population that remains dependent on MV and other supportive treatments applied in the ICU for a long time. Despite the different definitions of long-term ICU-LOS in the literature, regardless of the definition employed, the proportion of this patient population was small. The small number of patients who stayed in the ICU for long periods tended to overuse resources disproportionately for their number. As a result of this study, although only 18.7% (n=939) of the patients has prolonged ICU-LOS, they consumed 66.3% of MV days and 59.7% of total hospitalization days. This finding is consistent with those of previous studies. In a similar study conducted in Canada, the average ICU-LOS was 4.74 days and 7.3% of the applications remained >14 days (14). In another study, 11% of the patients had prolonged ICU-LOS, but they consumed 45% of intensive care days and 56% of ventilation days (9). The modest number of patients with prolonged ICU-LOS consuming more than half of the MV days can be explained by the increased mechanical power in this patient group. Considering the potential causes of prolonged ICU-LOS, the suppression of the immune system is considered common after trauma, sepsis, and other serious diseases, and this immune dysfunction plays a role in patients who stayed in the ICU for a long time (15). Another study suggested endocrinopathy acquired in the ICU (16). In another study, early nutritional needs of patients in the ICU were given sufficient attention, and the resulting iatrogenic malnutrition may lead to long-term hospitalization (17).

In line with the results of this study, which determined that prolonged ICU-LOS is more common in patients hospitalized in the ICU with the diagnosis of pulmonary diseases and sepsis, other studies have reported that long-term hospitalization is associated with sepsis and acute respiratory failure requiring MV (9,18,19). Pulmonary diseases and sepsis increased proinflammatory activity that can lead to organ failures, neuromuscular weakness, and dysfunction, leading to prolonged hospitalization (20). This finding indicates that early treatment of pulmonary diseases that cause sepsis and acute respiratory failure is an important goal for the prevention of long-term hospitalization. Treatments and practices such as early

resuscitation, conservative fluid management after the shock period, early mobilization, low tidal volume, and avoidance of excessive sedation, which can prevent early organ damage in the course of critical illness, may help the clinician to prevent long-term hospitalization (20-25). The present study also found that the usage rate of vasoactive drug was higher in the prolonged ICU-LOS group. However, vasoactive drug use was not associated with prolonged ICU-LOS. A similar study reported that vasoactive drug use and prolonged ICU-LOS were not associated (6). This is probably because patients who need vasopressors are in the more severe patient group and are more likely to die early after ICU admission.

In the present study, APACHE II, SAPS III, and other disease severity scores were higher in the prolonged ICU-LOS group, but they were not associated with prolonged ICU-LOS. Contrary to our results, a multicenter study, which was conducted with APACHE II, examined ICU-LOS for 10 months in 5,881 patients and found that patients with short ICU-LOS had high APACHE II scores (26). Another study found that patients with very high disease severity (APACHE II ≥30) had a short ICU-LOS because they died early in the ICU (18). Patients with low disease severity have relatively short ICU-LOS and are then discharged from the ICU, probably because they only need intensive care for a short time. By contrast, patients with very high disease severity have a shorter ICU-LOS because mortality develops in the early period. Considering the studies on SAPS III, which is another disease severity score, a study evaluated the SAPS III scores of the patients during admission to the ICU and found that patients with high SAPS III scores had a longer ICU-LOS (6). In another study, patients with a higher SAPS III scores stayed in the postoperative ICU for 3 more days (27). This situation can be explained by the high chronic disease burden of most of the patients with high SAPS III scores (6).

The present study also found that the increase in BMI and development of pressure sores were associated with prolonged ICU-LOS. Similar studies have reported that obesity increases ICU time, which is consistent with our results (28,29). In a meta-analysis including 23 studies, a trend was found to increase ICU-LOS in patients who were obese (29). In another meta-analysis study including 14 studies, obesity was associated with increased ICU-LOS and MV time (28). Nursing care of patients who are obese is more difficult than those with normal weight because of the large body weight and large body surface area. Difficult position changes in these patients may cause pressure sores and skin lacerations. Disruption of the protective skin barrier may prolong ICU-LOS in patients who are obese by increasing complications such as infection or bleeding (30,31).

Another finding of this study was that AKI development was associated with prolonged ICU-LOS of patients with critical illness. Our results are compatible with the literature. Previous studies have shown that AKI development is associated with prolonged ICU-LOS and increased morbidity, hospital costs, and worse outcomes in patients with critical illness (32,33). Impairment of pulmonary dynamics due to hypervolemia, which is common with decreased pulmonary compliance in patients with AKI, may increase respiratory workload and prolong MV time (34). In the present study, another parameter related to prolonged ICU-LOS was TPN. Similarly, a previous study showed that TPN use prolonged ICU-LOS (35). TPN was associated with infectious complications due to hyperalimentation and hyperglycemia, which may explain its association with prolonged ICU-LOS (36,37).

Study Limitations

In addition to its strengths, such as minimized data loss since the study data were obtained by the CDSS with electronic queries and all ICU processes were followed in our clinic, this study has some limitations. First, its single-center design prevents generalization of the results despite the diversity in the patient population. Second, given its retrospective nature, confounding factors may affect the results and increase bias risk. Third, data on long-term survival and functional outcomes of the patients could not be obtained. Fourth, since data on IV fluids and diuretic treatments given before the ICU follow-up could not be obtained, the weights measured in the ICU and urine volume of patients may have affected the BMI and AKI data. Fifth, comorbidities may have been underestimated because of deficiencies in patient's statement during anamnesis. Finally, the lack of a common consensus for the need for prolonged intensive care and various definitions in previous studies that accepted it as 10, 14, and 21 days may lead to differences in results, where we determined the need for prolonged intensive care as ≥14 days.

CONCLUSION

Patients who had prolonged ICU-LOS occupied 66.3% of MV days and 59.7% of total hospitalization days. The rate of prolonged ICU-LOS was higher in patients diagnosed with sepsis and pulmonary diseases. An increase in BMI, TPN use, AKI, and pressure ulcer development was associated with prolonged ICU. The increase in mechanical power increased the probability of prolonged ICU in patients who needed MV. Patient age, APACHE II score, and use

of vasoactive agents were not associated with prolonged ICU-LOS. A full understanding of the factors associated with prolonged ICU-LOS will be achieved through prospective studies that will generalize well-designed results and randomize patients with prolonged ICU-LOS at the center of the study.

ETHICS

Ethics Committee Approval: The study were approved by the Bakırköy Dr. Sadi Konuk Training and Research Hospital of Local Ethics Committee (protocol code: 2021/405-decision number: 2021-16).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: M.S.S., S.A., Concept: M.S.S., S.A., Design: M.S.S., S.A., Data Collection or Processing: M.S.S., S.A., Analysis or Interpretation: M.S.S., Literature Search: M.S.S., S.A., Writing: M.S.S., S.A.

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Research

Do Histopathological Findings of Kidney Biopsies Performed in Patients with Acute Kidney Injury Differ with Age?

Akut Böbrek Hasarı Nedeniyle Yapılan Böbrek Biyopsilerinin Histopatolojik Bulguları Yaşla Değişir Mi?

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ABSTRACT

Objective: Acute kidney injury (AKI) occurs in various conditions with different clinical presentations. Kidney biopsy (KB) is recommended when pre-renal and post-renal causes have been excluded, and the cause of AKI is unclear. We aimed to determine the histopathological features in patients with AKI and compare the frequency of the underlying causes in patients with different age groups.

Methods: This retrospective study was performed on all patients who underwent KB for AKI at our institution between January 2006 and April 2021. Demographic, clinical, and laboratory data and histopathological results were retrieved from the patients' files. Patients aged 60 years and older were regarded as elderly. The distribution of renal diseases was compared between the age groups.

Results: Overall, 767 kidney biopsies were performed at our institution for 15 years. Of these, 171 (22.2%) had unexplained AKI, of whom 35% were elderly. Males were predominated in both groups. The most common diagnosis was glomerular diseases (71.9%), followed by tubulointerstitial diseases (21.7%) and vascular lesions (5.3%). Immunoglobulin A nephropathy was the most frequent diagnosis in adults, whereas pauci-immune glomerulonephritis was the most frequent cause of AKI in the elderly.

Conclusion: Our data documents the different patterns of biopsy-confirmed causes of AKI according to the age groups and points the value of biopsy since most of the pathologic diagnoses are entities that are both difficult to diagnose without biopsy and are treatable.

Keywords: Acute kidney injury, histopathology, kidney biopsy

ÖZ

Amaç: Akut böbrek hasarı (ABH), farklı klinik bulgular ve farklı nedenler ile ortaya çıkabilir. ABH'ye yol açabilecek prerenal ve postrenal nedenler dışlandığında ve ABH'nin nedeni saptanamadığında böbrek biyopsisi (BB) önerilir. Bu çalışmada, ABH ile başvuran hastalarda histopatolojik özelliklerin belirlenmesi ve farklı yaş gruplarındaki hastalarda altta yatan nedenlerin sıklığının karşılaştırılması amaçlandı.

Gereç ve Yöntem: Ocak 2006 ve Nisan 2021 tarihleri arasında ABH nedeniyle BB yaptığımız tüm hastalar geriye dönük olarak tarandı. Demografik, klinik ve laboratuvar verileri ile histopatolojik sonuçlar hasta dosyalarından kaydedildi. Altmış yaş ve üzeri hastalar yaşlı olarak kabul edildi. Böbrek hastalıklarının dağılımı yaş grupları arasında karşılaştırıldı.

Bulgular: On beş yılda yapılan 767 BB değerlendirildi. Yüz yetmiş birinde (%22,2) açıklanamayan ABH vardı ve bunların %35'i yaşlıydı. Her iki grupta da erkekler baskındı. En sık tanı glomerüler hastalıklar (%71,9) iken, bunu tubulointerstisyel hastalıklar (%21,7) ve vasküler lezyonlar (%5,3) izledi. Erişkinlerde en sık karşılaşılan patolojik tanı immünoglobulin A nefropatisi iken yaşlılarda pauci-immün glomerulonefrit idi.

Sonuç: Verilerimiz; ABH'de BB sonuçlarının yaşa göre değiştiğini ve BB tanı ve tedavinin yönlendirilmesindeki değerini göstermektedir.

Anahtar Kelimeler: Akut böbrek hasarı, histopatoloji, böbrek biyopsisi

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INTRODUCTION

Acute kidney injury (AKI) is a heterogeneous disorder, manifested by an increase in serum creatinine level with or without reduced urine output for hours to days (1,2). Injury ranges from mild to severe kidney failure, sometimes requiring dialysis. Moreover, AKI is associated with an increased risk of mortality and development of chronic kidney disease (3). The diagnostic evaluation can be used to classify AKI as pre-renal AKI, which results from inadequate perfusion of the kidneys, and post-renal AKI, which results from obstruction to the flow of urine and renal AKI, which can be due to injury or dysfunction of the renal glomeruli, tubules, interstitium, or blood vessels. The majority of AKI cases can be diagnosed by clinical history, physical examination, and investigations of urine, blood, and radiology tests. Kidney biopsy (KB) is a crucial diagnostic tool when pre-renal and post-renal causes have been excluded and the intrinsic cause of AKI is unclear (4). Further, KB is critical for correct treatment and predicting prognosis. Patients undergoing KB for evaluation of AKI had a higher risk for complications due to lower pre-biopsy hemoglobin and higher baseline serum creatinine (5). Therefore, clinicians must consider diagnostic uncertainty and potential therapeutic relevance when deciding to perform a diagnostic KB in patients with AKI and take all precautions to prevent complications. This study aimed to determine the histopathological features in patients presenting with AKI and to compare the frequency of the different causes in patients with different age groups.

METHODS

Our study was retrospective, including all patients who underwent native KB for AKI. All native kidney biopsies performed at University of Health Sciences Turkey Bakırköy Dr. Sadi Konuk Training and Research Hospital between January 2006 and April 2021 were screened. Indications for kidney biopsies are as follows:

1. AKI: Abrupt decrease in renal function, with or without oligoanuria or rapidly progressive renal failure, including worsening of chronic kidney disease

2. Nephrotic syndrome: Proteinuria >3.5 g/24 h, with hypoalbuminemia (serum albumin <3.5 g/L) and edema

3. Nephritic syndrome: Proteinuria (1.5-3.5 g/24 h), hematuria with or without high blood pressure, and edema

4. Urinary abnormalities: Proteinuria <1.5 g/day or hematuria, without impaired kidney function

The renal specimens were collected by biopsy needle guidance technique under ultrasonography. The same pathologist examined the KB specimens and processed them for standard analysis, including light microscopy with hematoxylin-eosin, periodic acid-Schiff, Masson's trichrome, and periodic acid-methenamine silver staining and immunofluorescence. Patients were eligible if aged ≥16 years and submitted to KB for AKI. The exclusion criteria were incomplete records, inadequate biopsies (<8 glomeruli), and transplant kidney biopsies.

For all cases in which the indication for biopsy was AKI, we reviewed the patient's files, including the renal biopsy report. We recorded the following information: Age, sex, comorbidities, laboratory findings at the time of biopsy (serum creatinine, serum albumin, urine microscopy, 24-hour urine protein or spot urine protein/creatinine ratio, ANCA serological results), and histopathological diagnosis. Renal diseases were classified into three major categories: glomerular diseases, tubulointerstitial nephropathies, and vascular lesions. Patients aged 60 years and older were regarded as elderly.

Statistical Analysis

The 2007 NCSS (Number Cruncher Statistical System; Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used for study data evaluation. The suitability of quantitative data to normal distribution was tested by Kolmogorov-Smirnov, Shapiro-Wilk test, and graphical evaluations. Student's t-test was used to determine the significance of differences between normally distributed variables and Mann-Whitney U test for non-normally distributed variables. Pearson chi-square test, Fisher-Freeman-Halton Exact test, and Fisher's Exact test were used to compare qualitative data, where appropriate. $P<0.05$ was considered statistically significant.

RESULTS

Of the 767 kidney biopsies performed at our institution for 15 years, 171 (22.2%) were conducted on patients with unexplained AKI (Table 1). The frequency of biopsy-confirmed AKI in adult patients was 18.5%, increasing to 36% in the elderly ($p=0.001$). As shown in Table 2, no statistically significant differences was observed in sex, serum creatinine, serum albumin, and proteinuria level between the age groups ($p>0.05$).

Table 3 lists the histological lesions encountered in the patients with AKI, subclassified according to age and primary site of involvement within the kidney. Glomerular disease was the most common type of disease observed in both groups [81 (72.9%) in the adults vs 42 (70%) in the elderly group]. The distribution of glomerular lesions

differed between the age groups. Immunoglobulin A nephropathy was the most common cause (36%) in adults, whereas pauci-immune glomerulonephritis (GN) was the most frequent pathology (33.3%) in the elderly (p=0.001 and p=0.001, respectively). Fifty-six (32.7%) patients presented with rapidly progressive GN: 18 had crescentic immunoglobulin A nephropathy, 32 had pauci-immune GN, 4 had anti-glomerular basement membrane disease, 1 had lupus nephritis class IV, and 1 had shunt nephritis. In detail, immunosuppressive treatment was applied to all of these patients, and seven patients with pauci-immune GN who had hemoptysis and/or radiologic evidence of pulmonary involvement were treated with plasmapheresis.

Table 1. Clinical presentations at the time of biopsy according to age

	Total n=767 (100%)	Adult (<60 years) n=603 (78.6%)	Elderly (≥60 years) n=164 (21.4%)	p
Acute kidney injury (n,%)	171 (22.2)	114 (18.9)	57 (34.7)	[d]0.001**
Nephrotic syndrome (n,%)	309 (40.2)	234 (38.8)	75 (45.7)	
Nephritic syndrome (n,%)	113 (14.7)	97 (16.1)	16 (9.7)	
Abnormal urine analysis (n,%)	174 (22.6)	158 (26.2)	16 (9.7)	

[d]Fisher-Freeman-Halton test, **p<0.01

Tubulointerstitial disease (TID) was noted approximately in 20% of the cases in both age groups. The most commonTID was tubulointerstitial nephritis (TIN) in two groups.

Vascular disease was the least common cause of AKI (5.3%). Noticeably, the most common vascular disease was hypertensive nephrosclerosis. All patients diagnosed with hemolytic uremic syndrome (HUS) were adults, and the frequency of renal cortical necrosis (RCN) was similar in the age groups.

About 49.1% of the patients required hemodialysis. Indications of hemodialysis included uremia, oligoanuria, metabolic acidosis, and hyperkalemia. Although we had incomplete data to quantify complications of KB, no major complications such as death and nephrectomy were reported.

DISCUSSION

Several registries have described clinicopathological spectrums of kidney biopsies in adults; however, a limited number of these studies analyzed the primary clinical syndromes that indicate KB in detail. This observational study describes the histopathological profiles of the patients undergoing a KB for AKI and highlights the difference in age prevalence of the different causes.

The incidence of AKI as a clinical syndrome in the study of kidney biopsies ranges from 10% to 25% (6-12). Our data collected over 15 years showed that AKI constitutes 22.2% of all KB; however, this percentage was lower in adult patients than in the elderly. It is crucial to say that structural and functional changes occurring with age make

Table 2. Demographic and biochemical characteristics of the patients with AKI at presentation according to age

	Total n=171 (100%)	Adults n=111 (64.9%)	Elderly n=60 (35%)	p
Age (years) (mean ± SD)	49.39±16.22	40.41±11.61	67.37±5.85	-
Male	47.35±16.75	38.60±11.27	67.77±6.27	-
Female	52.28±15.00	43.30±11.69 (16-59)	66.93±5.42 (60-81)	
Sex (n,%)	-	-	-	[b]**0.272**
Male	100 (58.4)	67 (67)	33 (33)	-
Female	71 (41.5)	44 (62)	27 (38)	
Laboratory on admission (mean ± SD)	-	-	-	-
Creatinine (mg/dL)	4.86±2.76	5.08±2.82	4.42±2.62	[c]**0.107**
Total protein (g/dL)	6.16±0.90	6.20±1.01	6.07±0.73	[a]**0.501**
Albumin (g/dL)	3.27±0.69	3.31±0.72	3.20±0.59	[a]**0.430**
Proteinuria (mg/g)	3148±2847	3274.35±2906.61	2897.86±2734.79	[c]**0.268**

[a]Student's t-test, [b]Pearson chi-square, [c]Mann-Whitney U test, SD: Standart deviation

Table 3. The results of renal pathology according to age

	Total n=171 (100%)	Adults n=111 (64.9%)	Elderly n=60 (35.1%)	p
Glomerular diseases	**123 (71.9)**	**81(72.9)**	**42 (70.0)**	**0.680**
Anti-GBM nephritis	4 (2.3)	3 (2.7)	1 (1.6)	[d]**1.000**
Amyloidosis (AA/AL)	6/3 (5.2)	4/1(4.5)	2/2 (6.6)	[d]**0.697**
Chronic glomerulonephritis	4 (2.3)	3 (2.7)	1 (1.6)	[d]**0.553**
Diabetic nephropathy	6 (3.6)	1 (0.9)	5 (8.3)	[d]**0.186**
Focal segmental GS	8 (4.6)	7 (6.3)	1 (1.6)	[d]**0.263**
IgA nephropathy	49 (28.6)	40 (36)	9 (15)	**0.001***
Lupus nephritis	3 (1.7)	3 (2.7)	0	[d]**0.553**
Membranoproliferative GN	7 (4.1)	6 (5.4)	1 (1.6)	[d]**0.424**
Pauci-immune crescentic GN	32 (18.7)	12 (10.8)	20 (33.3)	**0.001****
Shunt nephritis.	1 (0.5)	1 (0.9)	0	[d]**1.000**
Tubulointerstitial diseases	**37 (21.7)**	**23 (20.7)**	**14 (23.3)**	**0.701**
Tubulointerstitial nephritis	24 (14)	16 (14.4)	8 (13.3)	**1.000**
Acute tubular necrosis	13 (7.6)	7 (6.3)	6 (10)	[d]**0.383**
Vascular diseases	**9 (5.3)**	**6 (5.4)**	**3 (5)**	[d]**1.000**
Hemolytic uremic nephropathy	3 (1.7)	3 (2.7)	0	[d]**0.553**
Hypertensive nephrosclerosis	4 (2.3)	2 (1.8)	2 (3.3)	[d]**0.613**
Acute cortical necrosis	2 (1.1)	1 (0.9)	1 (1.6)	[d]**1.000**
Unclassified	**2 (1.1)**	**1 (0.9)**	**1 (1.6)**	[d]**1.000**
Oxalate nephropathy	1 (0.5)	1 (0.9)	0	[d]**1.000**
Phosphate nephropathy	1 (0.5)	0	1 (1.6)	[d]**1.000**

[b]Pearson chi-square, [d]Fisher-Freeman-Halton test, **p<0.01

GN: Glomerulonephritis, GS: Glomerulosclerosis, IgA: Immunoglobulin A, GBM: Glomerular basement membrane

the elderly more vulnerable to AKI, and once it happened, renal function did not recover fast after the induction of treatment in the older age group.

In both age groups, glomerular diseases accounted for 70% of all diagnoses, similar to the previous studies, in which GN was the primary finding in ≥50% of cases undergoing KB for AKI (8,11,12). The most common diagnosis was immunoglobulin A nephropathy in adults, which accounted for nearly one-third of the cases, and it causes AKI probably by hematuria and/or crescentic proliferation. Consistent with the previous report (8), the most common glomerular disease in elderly patients was pauci-immune GN in our cases. In the current study, 32.7% of the patients showed crescentic proliferation, and approximately 57.1% had pauci-immune GN. Regarding the higher prevalence of crescentic GN among AKI patients, early diagnosis is necessary to prevent irreversible kidney injury by initiating immunosuppressive treatment and plasmapheresis when appropriate.

TID includes TIN and ATN, both of which cause a rapid decline in kidney function. TIN is characterized by interstitial inflammatory infiltrates, edema, and tubulitis in histopathological examination and is usually caused by drugs, infection, and systemic and autoimmune disease and may be idiopathic in origin. It accounts for 2% of all native kidney biopsies and 15-27% of adult cases undergoing KB for unexplained AKI (8,13,14). TIN was the most common type of TID observed in our cohort and accounted for 14% of all biopsy cases. Clinical suspicion, particular attention to extrarenal manifestations, and review of potential risk factors are usually enough for accurate diagnosis of TIN. However, KB should be performed in cases with severe renal dysfunction, lack of an identifiable offending agent, and lack of renal recovery before initiation of immunosuppressive treatment.

ATN is a common etiology of AKI in hospitalized patients and is diagnosed by exclusion of pre-renal and post-renal causes of AKI, examination of urinary sediment, and analysis of urine measures such as fractional excretion of sodium. ATN prevalence in our cohort is low because most cases were diagnosed based on clinical data, and KB was performed only in patients with prolonged or atypical AKI. In fact, histopathological findings of ATN are only diagnostic and do not alter therapy, and its role in predicting renal outcome is unknown.

Vascular diseases are the least common cause of AKI in the present study. Hypertensive nephrosclerosis was present in 2.3% of our cases, which was similar to the findings of a report from Japan (1.3%) (15). HUS characterized by non-immune microangiopathic hemolytic anemia, thrombocytopenia, and AKI was detected in three patients and all were treated with eculizumab. RCN secondary to ischemic necrosis of the renal cortex is a rare disease, accounting for only 2% of all cases of AKI in Western countries and 3.8%-7.1% in patients dialyzed for AKI in developing countries (16,17). In line with the literature, we reported RCN in 1.1% of the cases, and none was associated with obstetrical complications.

Study Limitations

This study had a few limitations. Our study did not include follow-up or outcome data, and these entities must be studied in future studies.

CONCLUSION

The present study provides information about renal histopathology in patients with AKI and reveals the significance of KB for accurate diagnosis of underlying diseases as most of the histopathological diagnoses are entities that are both difficult to recognize without biopsy and are treatable.

ETHICS

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital of Local Ethics Committee (approval no: 214/2021).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: A.Ö., F.S.K.Y., K.G.E., M.Y., Concept: A.Ö., M.Y., Design: A.Ö., F.S.K.Y., M.Y., Data Collection or Processing: A.Ö., F.S.K.Y., K.G.E., Analysis or Interpretation: A.Ö., F.S.K.Y., K.G.E., Literature Search: A.Ö., Writing: A.Ö.

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Research

Evaluation of Serum Amyloid A Levels in Celiac Disease

Çölyak Hastalığında Serum Amiloid A Düzeylerinin Değerlendirilmesi

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ABSTRACT

Objective: In celiac disease (CD), gluten intake triggers the release of T-cell-derived pro-inflammatory cytokines leading to an increase in the level of serum amyloid A (SAA). To confirm inflammation caused by CD in patients, SAA level is expected to be a better biomarker candidate than C-reactive protein (CRP). This study investigated the relationship between clinical and biochemical variables used in the follow-up of CD patients and SAA levels.

Methods: This study is a cross-sectional observational study which was conducted in patients who presented with a diagnosis of CD in a university hospital between June 1, 2019 and December 1, 2019. Patients were categorized into two groups based on the presence of tissue transglutaminase (tTG) immunoglobulin. Statistical analysis of data was performed by considering the age, gender, duration of the disease, frequency of compliance with a gluten-free diet, body mass index, and some laboratory parameters [vitamin D (VD), tTG IgA-IgG, CRP] taken simultaneously with the SAA levels.

Results: A total of 90 patients, 28 (31.1%) antibody positive and 62 (68.9%) antibody negative, were included in the study. We found out that there was a statistically significant difference between between SAA level, VD levels, and the duration of CD in the patients (p=0.03 and p=0.009, respectively).

Conclusion: VD levels <20 ng/mL, in advanced age, and duration of CD >5 years should be evaluated in the high-risk groups in terms of high SAA levels. If there is a persistent increase in SAA level during follow-up visits of patients with CD, they should be evaluated in terms of complications caused by the chronic inflammation.

Keywords: Celiac disease, serum amyloid A, C-reactive protein

ÖZ

Amaç: Çölyak hastalığında (ÇH) gluten alımı, T-hücre kaynaklı pro-enflamatuvar sitokinlerin salınımını tetikleyerek serum amiloid A (SAA) düzeyinde artışa neden olur. Hastalarda ÇH'nin neden olduğu enflamasyonun değerlendirilmesinde, C-reaktif protein (CRP) yerine SAA seviyesi daha iyi bir biyobelirteç olabilir. Bu çalışmada çölyak hastalarının takibinde kullanılan klinik ve biyokimyasal değişkenler ile SAA düzeyleri arasındaki ilişki araştırılmıştır.

Gereç ve Yöntem: Bu çalışma kesitsel gözlemsel bir çalışma olup, 1 Haziran 2019-1 Aralık 2019 tarihleri arasında bir üniversite hastanesinde ÇH tanılı hastalar ile yapılmıştır. Hastalar doku transglutaminaz (tTG) varlığına göre iki gruba ayrıldı. SAA seviyeleri ile yaş, cinsiyet, hastalık süresi, glutensiz diyete uyum sıklığı, vücut kitle indeksine ve eş zamanlı alınan bazı laboratuvar parametreler arasında [D vitamini (VD), tTG IgA-IgG, CRP] istatistiksel analizler yapıldı.

Bulgular: Çalışmaya 28 (%31,1) tTG antikor pozitif ve 62 (%68,9) tTG antikor negatif olmak üzere toplam 90 hasta dahil edildi. Hastalarda VD düzeyleri ve ÇH süresi ile SAA düzeyleri arasında istatistiksel olarak anlamlı bir fark olduğunu saptadık (sırasıyla p=0,03; p=0,009).

Sonuç: 20 ng/mL'nin altındaki VD düzeyleri, ileri yaş ve 5 yıldan fazla ÇH süresi bulunan hastalarda SAA yüksekliği açısından riskli gruplar olarak düşünülmelidir. Çölyak hastalarının takiplerinde SAA düzeyinde persiste eden bir yükseklik varsa kronik inflamasyonun neden olduğu komplikasyonlar açısından değerlendirilmelidir.

Anahtar Kelimeler: Çölyak hastalığı, serum amiloid A, C-reaktif protein

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INTRODUCTION

Celiac disease (CD), a chronic immune-mediated enteropathy triggered by dietary gluten ingestion in genetically predisposed individuals, is diagnosed by specific serological and histological factors (1). Highly sensitive and specific antibody tests have been developed and applied to screen individuals with CD (2). Specifically, it is shown that tests for the detection of tissue transglutaminase (tTG) antibody in serum have a specificity of 96-100% for the diagnosis of CD (1-3). Till date, the only treatment for CD is based on a gluten-free diet, which targets mucosal healing (Marsh 0 or 1 at follow-up biopsy) (4). However, the response to CD treatment with a gluten-free diet is still under debate. If patients were diagnosed with CD in their adolescence or adulthood, approximately 60% who were on a gluten-free diet in their first year of the treatment achieved mucosal healing (5). In contrast, recent studies indicated that mucosal healing was achieved in 81% of patients with CD if they were under a long-term, gluten-free follow-up treatment (6).

Serum amyloid A (SAA), produced by hepatocytes, are acute-phase reactants that rise rapidly in infections, trauma, and other inflammatory conditions. It is involved in the pathogenesis of many inflammatory diseases (IDs) such as diabetes mellitus, Alzheimer's disease, rheumatoid arthritis, and ulcerative colitis (7-9). It is shown that high levels of SAA and C-reactive protein (CRP) in biological fluids increases cardiovascular risk, and SAA reached a level which was much higher than CRP and then declined rapidly (10). Increased SAA in patients with rheumatoid arthritis has been associated with disease activity, disease-associated autoantibodies, and other acute-phase reactants (9). Amyloidosis, which is a serious complication in chronic IDs, is formed by the accumulation of amyloid A fibrils, which are degradation products of SAA in certain vital organs (11).

This study aimed to determine the correlation between SAA and various clinical and laboratory parameters in CD patients.

METHODS

Patients

This study is a cross-sectional observational study, and the patients who were presented to the University of Health Sciences Turkey, Ankara Keçiören Training and Research Hospital between June 1, 2019 and December 1, 2019 and were diagnosed with CD after the clinical, serological, and histological evaluations, and followed-up, were included in the study. Patients with a serious ID other than CD such as malignancy, active infection, rheumatological and inflammatory bowel disease (IBD), and patients <18 years of age were excluded from the study. Written consent was obtained from all patients who participated in the study. Celiac patients were classified as antibody-positive (tTG Ig A or any antibody from tTG IgG positive) and negative (tTG IgA and tTG IgG antibody negative).

Clinical-Demographic Characteristics

Age, gender, body mass index (BMI), smoking, CD duration, presence of any gastrointestinal symptoms at the time of examination (abdominal pain, diarrhea, constipation, flatulence, dyspepsia, etc.), and dietary compliance were questioned face-to-face by the gastroenterologist.

Laboratory Variables

Along with the laboratory parameters taken during routine controls of the patients, simultaneous SAA levels were measured [complete blood count, tTG IgA-IgG antibodies, vitamin D (VD), CRP, serum iron, ferritin, vitamin B12, folate, alanine aminotransferase (ALT)].

Serum Amyloid A Assay

Venous blood was collected in a red-capped tube from celiac patients who were admitted to the outpatient clinic with fasting for at least 8 hours. The blood was centrifuged for 10 minutes at 4,000 rpm without waiting. Subsequently, serum was stored at -80 °C until the day of analysis. SAA measurements were made for a quantitative determination of SAA (from mg/L level) utilizing N-Latex SAA kit immunnefelometer (OQMP11 Germany/Siemens N-Latex SAA Kit, 105 Test Kit). The upper normal limit of SAA is 6.4 mg/L.

Statistical Analysis

Collected data were analyzed with the SPSS.22 program (Chicago, IL, USA). Descriptive data were presented as the mean ± standard deviation, median, and minimum-maximum values. Chi-square test was used to determine whether there is a relationship between the two variables. When comparing the parameters of more than two patient groups, it was first examined whether the data showed a normal distribution or not with the Kolmogorov-Smirnov tests. For analytical tests, the t-test or Mann-Whitney U test was used to compare the two means. On comparing the three non-parametric analyzed groups, it was tried to be understood between which groups the difference was by performing the dual analyses on data that had a significant difference. Bonferroni correction was made in these analyses. Then, correlation analysis was performed for all parameters in the tTg positive and tTg negative patient group. Non-parametric correlation analysis was used, and $p<0.05$ was considered significant.

RESULTS

Demographic Data

In this study, 90 patients were included, who were subsequently divided into two groups depending on the antibody test results. The mean age of all patients was 36.71 years (Table 1). Summarizes the comparison of clinical-laboratory parameters and SAA levels between the groups according to antibody test results.

Findings on Serum Amyloid A

Although 75.9% of patients with high SAA levels were female and only 24.1% of them were male, there was no statistically significant difference observed between the gender of the patients and SAA groups (high or normal level) (p=0.493). The summary of the relation between laboratory variables and SAA levels are presented in Table 2. The results showed that there was a statistically significant difference between SAA levels and the duration of CD, serum CRP level, and age.

More specifically, high SAA level was found in one (10%) of the patients with a duration of CD for less than one year. High SAA levels were found (mean, 15.25 mg/L) in four (15.4%) of those with durations of CD between 1-5 years, while 24 (44.4%) of the patients with CD for >5 years had a high SAA level (mean, 18.34). Additionally, the results indicated that the duration of CD >5 years in 82.8% of those with a high SAA level, between 1-5 years in 13.8%, and less than one year in 3.4%, which were statistically significant for comparison between CD duration and SAA groups (p=0.009) (Figure 1).

The CRP level was found high (mean, 11.35) in 31% of the patients with high SAA levels. In 77.8% of the patients with high CRP levels, the SAA level was also high. There was a statistically significant relationship observed between CRP and SAA levels (p=0.001). There was no statistically significant relationship found between SAA groups and generalized finite differencing compliance, BMI, tTG IgA or tTG IgG positivity (p>0.05).

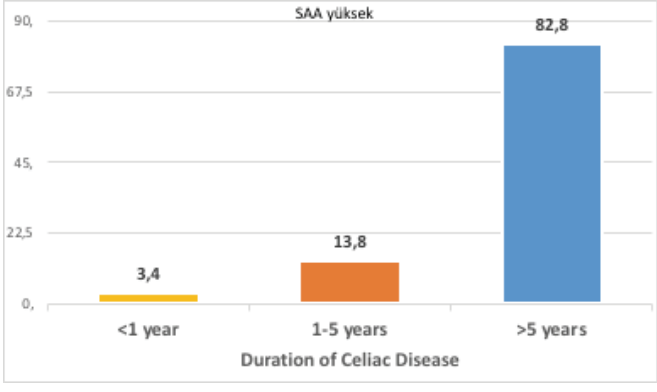

Figure 1. Percentages of higher SAA according to duration of Celiac disease
SAA: Serum Amyloid A

The age was divided into three groups using the k-means cluster method among SPSS analysis programs. SAA levels were found to be higher in 12 of the groups (41.4%) with a median age of 55.11 (37.9%) of those with a median age of 37, and 6 (20.7%) of those with a median age of 23 years. There was a significant relationship found between the age groups and SAA (p=0.032). When the median age of 23 years and the median age of 55 years were compared with the chi-square method, a statistically significant difference was found in terms of SAA levels (p=0.009).

SAA level was also found to be high when VD levels were examined. VD levels in patients with high SAA levels were <20 ng/mL in 16 of patients (42.1%), between 20-30 ng/mL in seven of the patients (18.4%), and >30 ng/mL in six of the patients (54.5%) (p=0.03). The results showed that the

Table 1. Evaluation of clinical-laboratory parameters according tTG* results

Variables	tTG positive (n=28)	tTG negative (n=62)	p
Gender (F/M)	17/11	47/15	0.1442
Presence of the symptoms	14 (50%)	20 (32.3%)	0.0612
Not following gluten-free diet	15 (53.6%)	4 (6.5%)	0.0002
Adherence to gluten-free diet	2 (7.1%)	42 (67.7%)	0.0002
Smoking	17 (60.7%)	24 (38.7%)	0.052
Anemia †	6 (21.4%)	2 (3.2%)	0.012
Duration of celiac disease, years median Duration of celiac disease, mean	4 (0-19) 5.61±1.01	6 (0-21) 7.95±0.77	0.063
Alanine aminotransferase, median (U/L)	21.5 (10-92)	16 (7-54)	0.0023
C-reactive protein, median (mg/L)	2.05 (0.5-16.9)	2.05 (0.5-14.1)	0.8343
Vitamin D, median (ng/mL)	19.5 (5-114)	21 (6-139)	0.4613
Serum Amyloid A (mg/L)	7.65 (3.69-202)	7.22 (3.57-17.9)	0.6263

[1]Independent simple t-test, [2]chi-square test, [3]Mann-Whitney U test. Values p<0.05 are expressed in bold

*tTG: Anti-tissue transglutaminase, †Anemia definition: According to the Turkish hematology society's guideline, hemoglobin limit was accepted as 13 g/dL for men, 12 g/dL for women, and 11 g/dL for pregnant women

patients with a VD level of <20 ng/mL are 3.23 times more likely to have a higher SAA level than those between 20-30 ng/mL.

As a result of the correlation analysis performed to determine the relationship between SAA and study variables in CD patients, a statistically significant positive correlation was found between the SAA level and CRP (r=0.453, p=0.001), ALT (r=0.314, p=0.026) (Table 3).

DISCUSSION

Table 2. Evaluation of laboratory parameters according to SAA results

	High SAA (n=28)	Normal SAA (n=61)	p
Age (years)	40.96±14.02	34.85±12.85	0.046[1]
Duration of celiac disease	8 (0.8-21)	4.75 (0-21)	0.023[2]
Alanine aminotransferase (U/L)	19 (8-36)	16 (7-92)	0.136[2]
B12 (ng/L)	212.5 (81-685)	241 (84-1344)	0.349[2]
C-reactive protein (mg/L)	3.55 (1.2-14.1)	1.7 (0.5-10.4)	0.000[2]
Iron (µg/dL)	80 (28-170)	77.50 (10-235)	0.404[2]
Vitamin D (ng/mL)	17.5 (6-50)	22 (5-139)	0.130[2]
Ferritin (ng/mL)	17 (7-115)	18 (2-93)	0.710[2]
Folic acid (ng/mL)	8 (2-13)	8 (1-23)	0.625[2]
Hemoglobin (g/dL)	14.39±1.57	13.95±1.73	0.262[1]
Lymphocyte (mm^3/L)	1965 (1160-3640)	1910 (900-3900)	0.354[2]

[1]Independent simple t-test, [2]Mann-Whitney U test values p<0.05 are expressed in bold, SAA: Serum Amyloid A

For CD patients' follow-up, there is a high need for a noninvasive marker that may be used to show the active inflammation, disease activity, and further complications. T-cell-derived pro-inflammatory cytokines triggered by a gluten intake in CD patients may increase SAA level. Most of the studies mainly focused on the effect of SAA level on acute or chronic IDs. For example, SAA was compared with other acute-phase reactants such as CRP, and the SAA level was found to be more sensitive than CRP in terms of screening of the inflammation in various diseases, such as rheumatoid arthritis, primary biliary cirrhosis, and chronic active hepatitis (9,12,13). Besides, the SAA levels increase rapidly within a few hours of infection, trauma, cancer, or other inflammatory events (14). Familial Mediterranean fever,

Table 3. Correlation results between SAA and study variables levels

Variables	r	p
Age (years)	0.053	0.718
Alanine aminotransferase (U/L)	0.366	0.01
B12 (ng/L)	0.007	0.964
C-reactive protein (mg/L)	0.418	0.003
Iron (µg/dL)	0.080	0.585
Vitamin D (ng/mL)	-0.177	0.223
Ferritin (ng/mL)	0.251	0.082
Folic acid (ng/mL)	-0.027	0.856
Hemoglobin (g/dL)	0.229	0.113
Lymphocyte (mm^3/L)	-0.003	0.983

Values p<0.05 are expressed in bold, SAA: Serum Amyloid A

chronic respiratory diseases, Hashimoto's disease, and IBDs have been also associated with the increased SAA levels (8,15-19). CD may present with a variety of extraintestinal manifestations (20). Other studies showed the increase in acute and chronic kidney diseases in CD patients who need renal replacement therapy (21,22). However, to the best of our knowledge, there is no information regarding the relation between SAA level and CD in the literature.

In addition, it is observed that VD deficiency causes inflammation. In our study, we found a relationship between low VD levels and high SAA levels. Many studies on VD and some markers of inflammation are available. For example, VD replacement has been shown to reduce the CRP levels in a meta-analysis (23). The effect of VD level on IL-10, IL-6, and tumor necrosis factor -α levels were also examined (24). Although there is no study showing the relation of the SAA level with VD in the literature, there is a study focusing on the relation of VD with serum amyloid p level in the Arabian race, the results of which indicated that serum amyloid p levels decreased with the replacement of VD (25).

Various cardiac pathologies occur as inflammatory mediators cause atherosclerosis and vascular damage due to their increase by the result of the autoimmune mechanisms triggered in CD (10). It has been shown that high SAA and CRP levels are associated with increased cardiovascular risk (26). In AA amyloidosis, amyloid burden and circulating concentration of SAA was >10 mg/L, and subsequently, a significant decrease in survival of the patients with persistent inflammatory activity was found. During the patients' follow-up, most of the patients with SAA level consistently >50 mg/L have showed an increase of amyloid burden and a deterioration of the organ function (27). Moreover, in some

studies, CD has been connected with both primary and secondary isolated cases of cutaneous amyloidosis (28). Together, there has also been a case report emphasizing the connection between CD and renal amyloidosis with systemic secondary amyloidosis (28). In our study, we found a positive correlation between SAA and CRP as an important inflammation marker. We found a hypothesis that SAA may be an alternative and more sensitive marker compared to CRP to show inflammation, which has not been considered before in the literature. Larger series should be evaluated to reach more precise results.

In this study, we determined the relationship between SAA level and the duration of CD. SAA levels were found to be significantly higher in patients with a duration of CD >5 years, regardless of the current antibody of the disease. Patients with CD should be followed-up in terms of SAA level, and those with persistent inflammatory activity should be further tracked to monitor complications caused by chronic inflammation.

Study Limitations

This study could not include endoscopically evaluated patients with SAA due to the lack of mucosal damage for the comparison. At the time of diagnosis, the patients were seronegative CD, and thus, the initial antibody levels of patients could not be reached.

CONCLUSION

In this study, SAA level might have been a more sensitive marker candidate than the CRP level to show inflammation in patients with CD. Groups at a risk for chronic inflammation (VD level <20 ng/mL, advanced age, CD duration >5 years) should be carefully evaluated. More comprehensive studies on SAA level should be conducted.

ETHICS

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Ankara Keçiören Training and Research Hospital of Local Ethics Committee (no: 2012-KAEK-15/1895, date: 08.05.2019).

Informed Consent: Written consent was obtained from patients who participated in the study.

Authorship Contributions

Surgical and Medical Practices: R.Y.Ç., M.A., N.E., Concept: R.Y.Ç., M.A., M.K., Ö.G., N.E., Design: R.Y.Ç., M.A., M.K., Ö.G., N.E., Data Collection or Processing: R.Y.Ç., M.A., M.K., Ö.G., N.E., Analysis or Interpretation: R.Y.Ç., M.A., M.K., Ö.G., N.E., Literature Search: R.Y.Ç., M.A., M.K., Ö.G., N.E., Writing: R.Y.Ç., M.A., N.E.

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

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Research

Intensive Care Unit Admission Parameters for Patients with COVID-19

COVID-19 Hastalarında Yoğun Bakım Kabulünü Belirleyen Parametreler

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ABSTRACT

Objective: The aim of this study was to determine the factors that may be useful in the early identification of populations that are sensitive to Coronavirus disease-2019 (COVID-19) and the need for intensive care unit (ICU) admission.

Methods: In this retrospective cohort study, patients who were hospitalized with COVID-19 and requested intensive care consultation at University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital between April 1 and July 1, 2020 were analyzed.

Results: Of the 208 patients included in the study, 129 were admitted to the ICU and 79 were not. Patients over the age of 85 were more often admitted to the ICU. The probability of ICU admission increased 2.6 times [odds ratio (OR): 2.687; confidence interval (CI): 95% 1.172-6.164] in patients with a single comorbidity relative to those with no comorbidity, while this risk increased 9.8 times (OR: 9.825, CI: 95% 2.557-37.749) in the presence of four or more comorbidities. In patients admitted to the ICU, the D-dimer and ferritin levels were higher, and the lymphocyte count was lower ($p<0.001$), relative to those who were not. Receiver operator characteristic (ROC) analysis gave cut-off values of 520 ng/mL, 765 ng/mL, and 0.89 109/L for ferritin, D-dimer and the lymphocyte count.

Conclusion: In addition to male gender and advanced age, a higher number of comorbidities is associated with higher disease severity in patients with COVID-19. It is also believed that a low lymphocyte count, as well as high D-dimer and ferritin levels, may guide clinicians in the early diagnosis of patients who require ICU admission.

Keywords: COVID-19, SARS-CoV-2, coronavirus, intensive care units, admission parameters

ÖZ

Amaç: Bu çalışma, Koronavirüs hastalığı-2019 (COVID-19) hastalarında yoğun bakım ünitesine (YBÜ) kabul parametrelerinin belirlenmesi amacı ile planlanmıştır.

Gereç ve Yöntem: Retrospektif tasarıma sahip bu araştırmada 1 Nisan-1 Temmuz 2020 tarihleri arasında Sağlık Bilimleri Üniversitesi, Gaziosmanpaşa Eğitim ve Araştırma Hastanesi'nde COVID-19 tanısı ile hastaneye yatırılan ve yoğun bakım konsültasyonu talep edilen hastalar analiz edildi.

Bulgular: Araştırmaya dahil edilen 208 hastanın 129'u YBÜ'ye kabul edildi 79'unda ise YBÜ'ye gerek görülmedi. Seksen beş yaş üzeri hastaların YBÜ kabulünün daha fazla olması dışında gruplar arasında ortalama yaş benzer bulundu. Komorbiditesi olmayan hastalara göre tek bir komorbiditesi olanlarda YBÜ olasılığı 2,6 kat artarken, bu riskin 4 ve üzeri komorbidite varlığında 9,8 kat arttığı bulundu. YBÜ'ye kabul edilen hastalarda D-dimer ve ferritin değerleri daha yüksek, lenfosit sayısı daha düşük bulundu ($p<0,001$). Ferritin değeri için yapılan ROC analizi sonucunda cut-off değeri 520 ng/ml olarak, D-dimer değeri için cut-off değeri 765 ng/mL olarak, lenfosit sayısı için cut-off değeri 0,89 109/L olarak belirlendi

Sonuç: COVID-19 hastalarında erkek cinsiyet ve ileri yaşa ek olarak daha fazla sayıda komorbidite, daha yüksek hastalık şiddeti ile ilişkilidir. Ayrıca lenfosit sayısı düşüklüğü, D-dimer ve ferritin yüksekliğinin YBÜ kabulü gerekecek hastaların erken tanınmasında klinisyenlere yol gösterebileceği düşünülmektedir.

Anahtar Kelimeler: COVID-19, SARS-CoV-2, coronavirus, yoğun bakım üniteleri, kabul parametreleri

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INTRODUCTION

In December 2019, a disease was reported in the Wuhan State of China caused by Severe Acute Respiratory syndrome-Coronavirus-2 (SARS-CoV-2) (1). The World Health Organization declared a pandemic due to this infectious disease, which was designated Coronavirus disease-2019 (COVID-19) and it had caused more than 225 million cases and 4,636,153 deaths in 220 countries as of September 14, 2021 (2,3). It is expected that this number will continue to increase rapidly for a while and will threaten the lives of more people worldwide, as well as their physical and mental health.

The first investigations of COVID-19 revealed a clinical spectrum ranging from moderate infection to severe complications such as acute respiratory distress syndrome and multiple organ dysfunction syndrome, which resulted in death, following an incubation period of 2 to 14 days (4,5). To date, no specific treatment has been proven for this disease other than supportive care. Recent reports have indicated that approximately 14%-29% of patients with COVID-19 pneumonia required intensive care (IC) (6,7). In this pandemic, which is predicted to cause great pressure and insufficiency in intensive care units (ICUs), early recognition of critical patients and ensuring timely triage is very important (8). Reports from China and Italy demonstrated that the risk factors for severe disease included advanced age and at least one comorbid disease (4,9). Particularly, elderly patients who have underlying comorbidities such as diabetes, hypertension, and coronary heart disease are at greater risk of adverse outcomes (10).

The requirement for ICU admission is primarily determined by the clinical view, peripheral oxygen saturation (SpO_2) levels, and the patient's comorbidities, although specific laboratory markers may aid in determining the severity of the condition. In order to predict the need for ICU admission in hospitalized COVID-19 patients, it is recommended to consider the levels of C-reactive protein (CRP), D-dimer level, ferritin, and serum cardiac troponin, as well as the low lymphocyte count (11). This study was planned to determine the parameters that may be useful in predicting the ICU need of COVID-19 and to identify the factors affecting the progression from mild to severe disease.

METHODS

Subjects and Methods

This retrospective cohort study was conducted at the ICU of University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital, İstanbul, Turkey. This referral hospital, which has 600 beds and 40 different medical departments, includes an ICU consisting of 38 patient beds admitting an average of 3.386 medical, surgical, or trauma patients per year. The ratio of nurses to patients is 1:2 in this unit, which provides IC services as a separate unit, where extracorporeal treatments (ECMO, hemodialysis, plasmapheresis) can be administered 24/7 by the IC specialists, Anesthesiology and Reanimation specialists, and assistants.

Data Collection

The data on patients who were hospitalized for COVID-19 in our hospital between April 1 and July 1, 2020 and were consulted for assessment of the need for ICU admission, were collected using the hospital database. Treatment for COVID-19 was planned according to the guidelines published and updated by the Ministry of Health (12,13). The findings of respiratory rate ≥30 times/min, dyspnea and respiratory distress symptoms, SaO_2 (arterial oxygen saturation) <90% or PaO_2 (partial pressure of oxygen) 70 mmHg despite oxygen support, PaO2/FiO_2 (the fraction of inspired oxygen) 300, 4 mmol/L lactate, bilateral infiltrates or multilobar involvement, on chest X-ray or computed tomography, hypotension (mean arterial pressure 65 mmHg), skin perfusion disorder, kidney or liver function disorder, thrombocytopenia, organ dysfunctions such as confusion, presence of immunosuppressive disease, the presence of multiple and particularly uncontrolled comorbidity, troponin elevation, and arrhythmia were evaluated as the indications for ICU admission (12).

The patients consultation responses were checked for the decision on follow-up in the ICU. Clinical and laboratory data of these patients during consultation were obtained electronically and evaluated retrospectively. Patient data including age, gender, comorbid diseases, respiratory rate per minute, FiO_2, SaO_2, laboratory parameters obtained from blood samples, and the data obtained as a result of examination and recorded on the hospital database consultation form were analyzed.

Sample

All the patients who were hospitalized with COVID-19 and were consulted for assessment of the requirement for ICU admission during the planning period of the study constituted the intended sample. During the study period, 23,269 outpatients presented to our hospital with a pre-diagnosis of COVID-19. A total of 1554 patients with a confirmed diagnosis of COVID-19 were hospitalized. ICU consultation was requested for 396 of these patients, and

212 patients were admitted to the ICU. Following evaluation of the exclusion criteria, a total of 208 patients consulted to the ICU were included in the study.

Inclusion Criteria

It was planned to include all patients over the age of 18 who were followed up at the hospital with a diagnosis of COVID-19 and consulted to evaluate the need for ICU admission.

Exclusion Criteria

Younger than 18 years old (n=1)

Patients with multiple requests for consultation in the ICU (n=62)

Patients referred to an external center (n=81)

Patients referred from an external center (n=31)

Missing data (n=15)

Ethical Considerations

Ethical approval and institutional permission was obtained from the University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital Ethics Committee (decision number: 134, date: 05/08/2020). The Council for Scientific Research Studies of the Directorate General of Health Services, which is associated with the Ministry of Health of the Republic of Turkey, also gave permission. The research conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Brazil 2013).

Statistical Analysis

The data collected in the study were evaluated using SPSS Statistics, version 22.00 (IBM Corp.; Armonk, NY). The Shapiro-Wilk test was performed to determine if the data had a normal distribution. Numerical variables were reported as mean standard deviation or median and interquartile range (IQR) whereas categorical variables were given as frequency (n) and percentage (%) IQR. For numerical data comparisons, the independent samples t-test was employed, and the Mann-Whitney U test was utilized when the assumptions of this test could not be met. For categorical variables, the chi-square test was employed, and when the chi-square test criteria were not satisfied, Fisher's Exact test was used. In addition, a logistic regression model including all the patients was created in order to determine the effect of the comorbid disease burden on ICU admission. Receiver operator characteristic (ROC) analysis was used to determine cut-off values for the laboratory parameters that were assumed to be predictive of ICU admission. A p-value less than 0.05 was identified as the level of statistical significance.

Primary Outcome

The primary aim of the study was to determine the parameters for admission to the ICU in patients who were followed-up due to COVID-19.

Secondary Outcome

The secondary aim of the study was to compare the patients' comorbidities and laboratory values and to determine the cut-off values that could determine the transition from mild to severe disease as well as the need for ICU admission.

Results

The patients' demographics and baseline characteristics are shown in Table 1. Patients who were consulted for ICU follow-up were split into two groups: those who had been admitted to the ICU and those who had not. The mean age was similar among the groups, and the number of patients over the age of 85 who were admitted to the ICU was higher. Male gender (87; 69%, $p<0.05$) and comorbid diseases (111; 85.4%, $p<0.05$) were more common in patients who were admitted to the ICU. The number of comorbid diseases was higher in the group of patients who were admitted to the ICU [2 (1-3), $p<0.001$]. Hypertension was the most common comorbid disease in both groups. Diabetes mellitus (DM) (65; 50.4%), cardiovascular disease (CVD) (37; 28.7%), and chronic obstructive pulmonary disease (COPD) (25, 19.4%) were more common in the patients who were admitted to the ICU ($p<0.05$).

Measurements of fever were found to be similar in all patients during the examination. Dyspnea was found in 91.5% (118) and tachypnea (respiratory rate ≥30 per min) in 85.7% (108) ($p<0.05$) of the patients who were admitted to the ICU. Although patients admitted to the ICU were treated with 5 L/min O_2, SaO_2 was <90% or PaO_2 was <70, PaO_2/FiO_2 was <300, and the rate of circulatory disorders was higher ($p<0.05$). The proportion of patients with hypotension (MAP <65 mmHg), organ dysfunction symptoms, lactate >4 mmol/L, and bilateral or multilobar lung involvement on computed tomography scan were similar between the groups (Table 1).

Considering the laboratory parameters, PaO_2 and SaO_2 were lower in the blood gasses of the patients who were admitted to the ICU. The D-dimer and ferritin levels were higher, and the lymphocyte counts were lower in patients who were admitted to the ICU (Table 2).

A logistic regression model was created to assess the effect of the comorbidity burden on ICU admission. As a result of this model, the ICU admission risk was 2.6 times higher in patients with one comorbid disease (odds ratio

Table 1. Demographics and clinical characteristics of patients with COVID-19

Parameters	Non-admission to ICU (n=79) n (%)	Admission to ICU (n=129) n (%)	p
Gender			0.010
Male	39 (31.0)	87 (69.0)	-
Female	40 (48.8)	42 (51.2)	-
Age (years, mean ± SD)	64.77±12.96	67.85±14.86	0.118
<55	18 (22.8)	24 (18.6)	0.466
55-64	19 (24.1)	29 (22.5)	0.794
65-74	18 (22.8)	27 (20.9)	0.753
75-84	21 (26.6)	28 (21.7)	0.421
≥85	3 (3.8)	21 (16.3)	0.006
Comorbidity	54 (69.2)	111 (85.4)	0.005
Hypertension	34 (43.6)	67 (52.3)	0.223
Diabetes	23 (29.1)	65 (50.4)	0.003
Cardiovascular disease	10 (12.7)	37 (28.7)	0.007
Chronic obstructive pulmonary disease	5 (6.3)	25 (19.4)	0.009
Cerebrovascular disease	7 (8.9)	24 (18.6)	0.055
Chronic renal failure	11 (13.9)	24 (18.6)	0.381
Dementia	6 (7.6)	11 (8.5)	0.812
Malignancy	3 (3.8)	8 (6.2)	0.539*
Other	5 (6.3)	10 (7.8)	0.700
NIMV	12 (15.2)	33 (25.6)	0.077
Vasoactive drug	2 (2.5)	7 (5.4)	0.488*
Body temperature (°C, mean ± SD)	37.27±1.01	37.41±1.04	0.352
Bilateral infiltration	66 (83.5)	118 (91.5)	0.082
Dyspnea	61 (77.2)	118 (91.5)	0.004
Respiratory rate ≥30 (per min)	18 (22.8)	108 (85.7)	<0.001
PaO_2/FiO_2 <300	42 (53.2)	122 (94.6)	<0.001
SaO_2 <90% or PaO_2 <70 mmHg[a]	37 (46.8)	122 (76.7)	<0.001
Hypotension (MAP <65 mmHg)	4 (5.1)	16 (12.4)	0.081
Organ dysfunction signs	14 (17.7)	36 (27.9)	0.095
Skin perfusion disorder	3 (3.8)	17 (13.2)	0.029
Lactate ≥2 (mmol/L)	23 (29.1)	58 (45.0)	0.023
Lactate ≥4 (mmol/L)	15 (19.0)	30 (23.3)	0.468

NIMV: Non-invasive mechanical ventilation, PaO_2: Partial pressure of oxygen, FiO_2: Fraction of inspired oxygen, SaO_2: Arterial blood oxygen saturation. * means Fisher's Exact test and [a]means ≥5 L/min despite the oxygen therapy ICU: Intensive care unit, COVID-19: Coronavirus disease-2019, SD: Standard deviation

Table 2. Comparison of laboratory findings and clinical outcomes in patients with COVID-19

Parameters	Patients non-admitted to ICU (n=79) (mean ± SD) Median (IQR)	Patients admitted to ICU (n=129) (mean ± SD) Median (IQR)	p
pH	7.42 (7.40-7.43)	7.44 (7.39-7.46)	0.012*
pCO_2 (mmHg)	37 (34-41)	36 (33-42)	0.106*
PO_2 (mmHg)	64 (61-68)	58 (51-66)	0.017*
SaO_2 (%)	90 (89-92)	88 (80-91)	<0.001*
HCO_3 (mEq/L)	24.3±2.5	24.1±4.1	0.758
Lactate (mmol/L)	1.49±0.54	2.14±1.10	0.151
CRP (mg/L)	133 (61-190)	142 (50-252)	0.347*
Procalcitonin (ng/mL)	0.56 (0.35-1.38)	0.49 (0.26-1.10)	0.196*
WBC (10^9/L)	8.8 (6.6-9.2)	8.4 (6.5-10.6)	0.901*
Lymphocyte (10^9/L)	0.86±0.32	0.61±0.20	<0.001
D-dimer (ng/mL)	385 (297-565)	1654 (965-2970)	<0.001*
Ferritin (ng/mL)	488±268	801±473	<0.001
Fibrinogen (mg/dL)	388±30	435±132	0.401
Lactate dehydrogenase (U/L)	418 (398-494)	398 (283-656)	0.447*
Troponin (ng/mL)	10 (9.7-18.9)	17.5 (9.2-62)	0.527*
Aspartate transaminase (U/L)	24 (23-29)	46 (32-59)	0.144*
Alanine transaminase (U/L)	21(18-61)	42 (24-68)	0.139*
LOS-ICU time (day)	-	9 (4-17)	-
LOS-Hospital time (day)	13 (10-15)	16 (12-22)	<0.001*

pH: Potential of hydrogen, pCO_2: Partial pressure of carbon dioxide, PO_2: Partial pressure of oxygen, SaO_2: Arterial blood oxygen saturation, HCO_3: Bicarbonate, CRP: C-reactive protein, WBC: White blood count, LOS-ICU: Length of stay in the intensive care unit, LOS-Hospital: Length of stay in the hospital, SD: Standard deviation, ICU: Intensive care unit, COVID-19: Coronavirus disease-2019, *Mann-Whitney U test

(OR): 2.687; confidence interval (CI) 95%: 1.172-6.164), 2.7 times higher in patients with two comorbid diseases (OR: 2.774, CI 95%: 1.212-6.348), 3.1 times higher in patients with three comorbid diseases (OR: 3.174, CI 95%: 1.318-7.642), and 9.8 times higher in patients with four or more comorbid diseases (OR: 9.825, CI 95%: 2.557-37.749) compared to the patients with no comorbid diseases (Table 3).

ROC analysis was performed for the ferritin, D-dimer, and lymphocyte counts, which differed significantly between groups in laboratory parameters, in order to determine a cut-off value to predict ICU admission. As a result of the ROC analysis performed for the ferritin value, the cut-off value was determined as 520 ng/mL according to the Youden J index [area under curve (AUC): 0.761, p<0.00]). It was determined that values above 520 required ICU admission in patients with COVID-19, with 66.9% sensitivity and 67.9% specificity (Figure 1). The cut-off value for the D-dimer value was determined as 765 ng/mL (AUC: 0.947, p<0.001). Accordingly, it was found that values above 765 ng/mL required ICU admission in patients with COVID-19, with 91.1% sensitivity and 88.5% specificity (Figure 2). As a result of the ROC analysis performed for the lymphocyte count, the cut-off value was determined as 0.89 109/L (AUC:

Table 3. Logistic regression of the effect of comorbid disease burden on the admission of patients with COVID-19 to the intensive care unit

Parameters	OR	CI 95	p
Comorbidity			
Non	Reference		
1	2.687	1.172-6.164	0.020
2	2.774	1.212-6.348	0.016
3	3.174	1.318-7.642	0.010
≥4	9.825	2.557-37.749	0.001

OR: Odds ratio, CI: Confidence interval, COVID-19: Coronavirus disease-2019

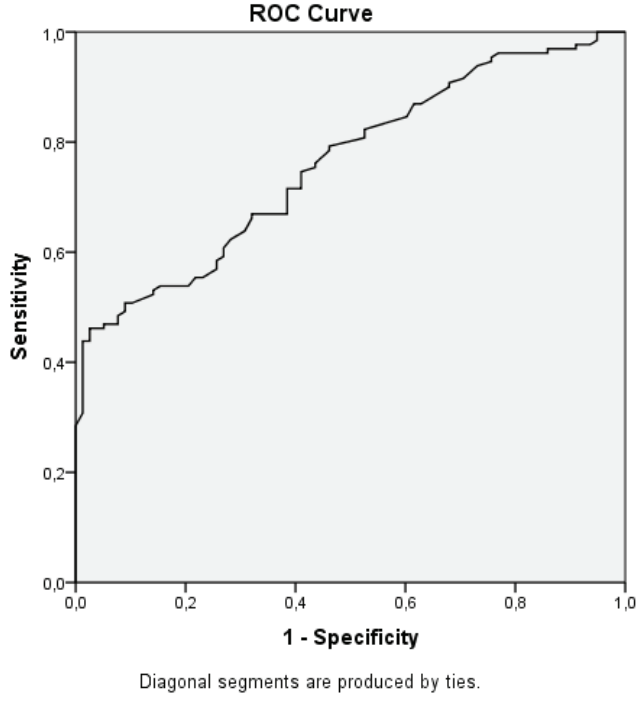

Figure 1. ROC curves for ferritin
ROC: Receiver operator characteristic

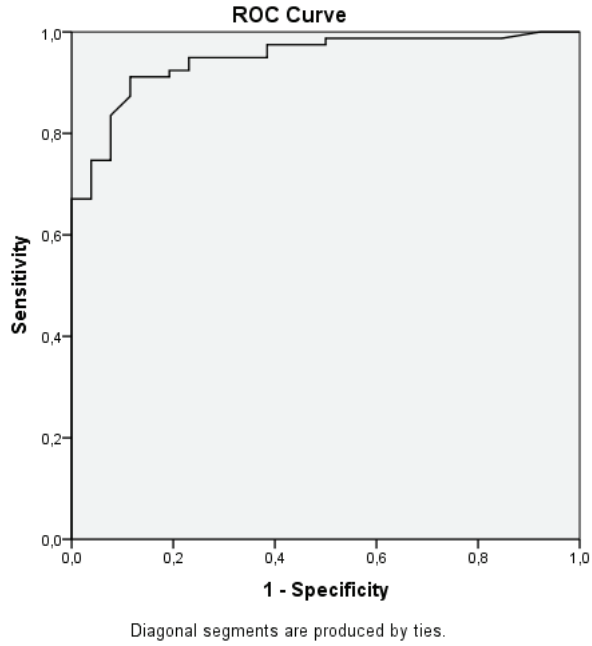

Figure 2. ROC curves for D-dimer
ROC: Receiver operator characteristic

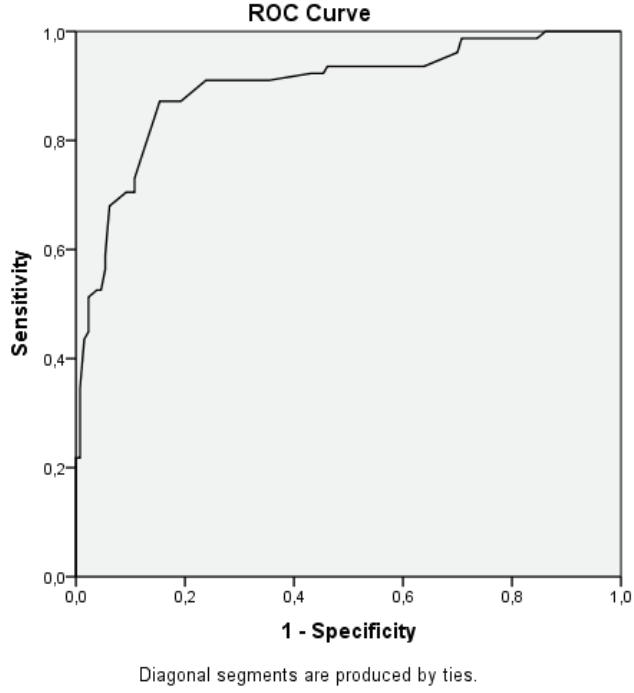

Figure 3. ROC curves for lymphocyte count
ROC: Receiver operator characteristic

0.899, p<0.001), and the values below 0.89 109/L were found to require ICU admission in patients with COVID-19 (Figure 3), with 87.2% sensitivity and 84.6% specificity (Figure 3).

Finally, the duration of ICU hospitalization in patients admitted to the ICU was 9 (4-17) days, the need for invasive mechanical ventilation was 72.9% (94), and the mortality was found to be 55.8% (72/129). The length of hospitalization was higher in the ICU group (p<0.001).

DISCUSSION

In this study, which was planned to guide the early identification of populations sensitive to the COVID-19 outbreak, it was determined that the rate of critical disease development increased in the male gender at the age of 85 and over. In our study, the need for more IC in patients over the age of 85 was consistent with previous studies. However, contrary to some studies, which indicated an increased need for ICU admission over the age of 65, we were not able to determine an effect of age on the need for ICU in patient groups below the age of 85 (14). In many similar studies, it was determined that the male gender and advanced age had higher severity of disease and higher rates of admission to the ICU (15,16). In a study conducted with 1591 ICU patients in Italy, the prevalence rate of males was 82.0% (17). This suggests that hospitalized geriatric COVID-19 patients of the male gender may have an increased risk of clinical deterioration that required ICU admission.

Comorbidities, in addition to epidemiological variables, are potentially significant factors that might influence the severity and prognosis of COVID-19. In favor of the results obtained in similar studies reporting that circulatory and endocrine comorbidities are common in COVID-19 patients, it was determined in our study that at least one comorbidity was observed in 79% of hospitalized patients due to COVID-19 and the presence of comorbidity increased the need for ICU admission (18). In a similar study previously conducted on 5279 patients, it was found that the 80% of hospitalized patients had at least one comorbidity (16). In our study, the most common comorbidity was determined as hypertension. This finding supports the observations of previous studies that determined hypertension as the most common comorbidity (6,14,17). It is believed that the most common comorbidity, hypertension, is related to the angiotensin converting enzyme receptor, which is an important regulator of blood pressure and also the binding site of SARS-CoV-2 (19). In our study, no relationship was found between hypertension and ICU admission. By contrast, it was determined that the presence of any of the DM, CVD, and COPD increased the risk of critical disease development and ICU hospitalization in patients with COVID-19. In terms of diabetes, a meta-analysis of six studies (1.527 patients) found that the prevalence was twice as high in the IC group compared with the patients with mild COVID-19 (20). Another study showed diabetes to be 3.6 times more common in ICU patients compared to COVID-19 patients who were not admitted to the ICU (21). The worse outcomes of patients with both COVID-19 and diabetes may be attributed to the additional underlying comorbidities associated with diabetes. According to our findings, it was determined that COPD, although it was rarer than other comorbidities, increased the rate of ICU admission. In a similar study, it was found that COPD was an extremely powerful indicator of both disease severity and ICU admission in COVID-19, contributing to the admission of patients with COVID-19 to the ICU as well as invasive ventilation. According to our results, a study conducted for CVD, which was another comorbidity that increased the ICU admission rate, determined that patients with CVD were 3.4 times more likely to be hospitalized in the ICU than patients without any comorbidity (15). In addition, our study determined that multiple comorbidities were associated with higher disease severity of COVID-19. It was found that the probability of ICU admission increased 2.6 times in patients with a single comorbidity compared to the patients without any comorbidity, while this risk increased 9.8 times in the presence of four or more comorbidities. Supporting our findings, previous studies demonstrated that multiple comorbidities were associated with higher disease severity of COVID-19, increasing the probability of ICU hospitalization (18-22). Appropriate triage and careful examination of the medical history will help to identify patients with COVID-19 who are more likely to develop critical disease. In addition, better protection should be provided to patients with COVID-19 and comorbidities following diagnosis.

In the present study, findings of dyspnea, an increased respiratory rate (≥30 breaths/min), and hypoxia were more common in patients who were admitted to the ICU. In another study, it was determined that dyspnea increased the need for ICU 6.6 times (21). Considering the importance of dyspnea in predicting admission to the ICU, attention should be paid to early hospitalization, clinical intervention, and close monitoring in patients with dyspnea. It is an expected situation that patients with markers of hypoxemia (SaO_2 <90% or PaO_2 <70 mmHg, PaO_2/FiO_2 300 despite oxygen support) have a greater need for IC. It is not surprising that patients who are followed in the ICU have lower SaO_2 and PO_2 values. In another study, the ratio of PaO_2/FiO_2 rates were also found to be lower in patients, who were admitted

to the ICU (23). In another study, patients with oxygen saturation <88% were associated with critical illness, and it was determined that hypoxia was an important marker of severe disease despite oxygen support (16).

Considering the lack of definitive treatment and vaccine despite the recent advances, there is a need for rapid and reliable biomarkers for the early diagnosis of patients with COVID-19 who would require ICU admission. Certain biomarkers have been proposed to determine the severity of the disease (11). In the present study, significant differences were found in the lymphocyte count, D-dimer, and ferritin values among the laboratory parameters that could reflect the severity of COVID-19 during the early period. A lymphocyte count below 0.89 109/L was determined to be predictive of critical disease. Because the targeted invasion of SARS-CoV-2 virus particles destroys the cytoplasmic component of the lymphocyte and causes its death, lymphocytopenia is a notable finding among hematological parameters in critically ill patients with SARS-CoV-2 infection (24). Significantly lower lymphocyte counts were found in patients who died from COVID-19 compared with survivors (11,25,26). In a study, it was determined that lymphocytopenia occurred in more than 80% of critically ill patients (10). Low lymphocyte values can be used in the clinic to diagnose novel coronavirus infections or to predict the clinical course.

COVID-19 infection presents with a coagulopathy problem that increases mortality, which is characterized by predominantly high D-dimer levels (5). Coagulopathy and disseminated intravascular coagulation seem to be linked to a high risk of death. In our study, a D-dimer value over 765 ng/mL was found to be associated with ICU admission. In a similar study, D-dimer levels higher than 1 ug/L were found to be the strongest independent predictor of mortality (26). Ferritin was determined as another parameter related to the severity of the disease. In our study, it was determined that a ferritin level higher than 520 ng/mL was associated with critical COVID-19 illness that would require ICU admission. In individuals with severe COVID-19, high ferritin levels have been documented due to secondary hemophagocytic lymphohistiocytosis (sHLH) and cytokine storm syndrome. A predictive H-score has been proposed based on body temperature, organomegaly, blood cell cytopenia, lipids, fibrinogen, AST, and ferritin levels to predict the probability of developing secondary HLH (27). As the COVID-19 pandemic intensifies, the need for biomarkers that can identify the progression of this disease to serious and deadly forms will increase. Therefore, clinicians should consider a low lymphocyte count and high D-dimer and serum ferritin levels.

Study Limitations

Our study has certain limitations in addition to its strengths, such as the administration of the same treatment protocol to all patients and avoiding data loss due to the inclusion of patients who spent the entire disease process in our hospital. First, it lacks dynamic clinical and laboratory data due to its retrospective design. The information was gathered from a database of electronic health records. This precluded the intended level of detail, which could be obtained with manual review of the medical records. All our patients were from a single geographical region, and they were treated in the same health center. Therefore, despite the diversity in our patient population, the factors associated with the results may differ in other geographic regions. Some of the patients in the group who were not admitted to the hospital may have been transferred to another facility. Certain factors, such as secondary infection, that may have an impact on prognosis could not be evaluated. The likelihood of obesity, which contributes to death in patients with COVID-19, was not included in the study due to the lack of data on BMI. In addition, the underreporting of comorbidities that may have arisen from the lack of awareness in patients and/or the lack of diagnostic tests during the reporting of comorbid diseases, could have caused the overestimation of the relationship with the negative result in terms of its strength.

CONCLUSION

In conclusion, the male gender, advanced age, and concurrent comorbidities in patients with COVID-19 largely determine the need for ICU admission. In addition, multiple comorbidities are associated with higher severity of COVID-19. Triage, which could be administered by careful review of the patient's medical history, would help to identify patients with comorbidities, who are more likely to develop critical disease. In addition, it is believed that low lymphocyte count, and high D-dimer and ferritin levels may guide clinicians in early and rational triage of patients who require ICU admission.

ETHICS

Ethics Committee Approval: Ethical approval and institutional permission was obtained from the University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital Ethics Committee (decision number: 134, date: 05/08/2020).

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: M.S.S., F.Ö., O.Ö., Ü.A.T., V.D., Concept: M.S.S., F.Ö., O.Ö., Ü.A.T., V.D., Design: M.S.S., F.Ö., O.Ö., Ü.A.T., V.D., Data Collection or Processing: M.S.S., F.Ö., O.Ö., Ü.A.T., V.D., Analysis or Interpretation: M.S.S., Ü.A.T., Literature Search: M.S.S., F.Ö., O.Ö., Ü.A.T., V.D., Writing: M.S.S., F.Ö., O.Ö., Ü.A.T., V.D.

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Research

The Effect of PEEP Adjusted by Automatic Recruitment Maneuver on Total and Dynamic Mechanical Power in Bariatric Surgery

Bariyatrik Cerrahide Otomatik Rekruitment Manevrası ile Ayarlanan PEEP Düzeyinin Total ve Dinamik Güç Üzerine Etkisi

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ABSTRACT

Objective: Mechanical power is a predictor of ventilator-induced lung damage. An automatic recruitment maneuver (ARM) is used during anesthesia to adjust the optimal positive end-expiratory pressure (PEEP) level. This study compared the total mechanical and dynamic power of patients who underwent sleeve gastrectomy due to morbid obesity who are ventilated with PEEP adjusted according to the anesthesiologists' decision and those adjusted using ARM.

Methods: Patients between the ages of 18 and 65 years, with American Society of Anesthesiologists (ASA) III and body mass index of >40, were divided into two groups: group 1 with PEEP adjusted using the ARM and group 2 without the ARM. Total mechanical power and dynamic power in both groups were calculated using respiratory mechanics measured at 10-min intervals. Patient averages of the calculated power values of both groups were compared with the Mann-Whitney U test.

Results: Simplified mechanical power of pressure control ventilation ($MP_{pcv(simpl)}$) (dynamic) resulted 2.9 (2.4-3.4) in group 1 and 7.9 (6.4-9.6) in group 2 (p<0,0001). $MP_{pcv(simpl)}$ (total power) resulted 8.1 (7.4-8.9) in group 1 and 12.8 (9.9-15.2) in group 2 (p<0.0001).

Conclusion: Optimal PEEP adjustment by ARM reduced the lung elastance and DP, resulting in less dynamic power applied to the respiratory system.

Keywords: Obesity, mechanical power, PEEP, recruitment maneuver

ÖZ

Amaç: Mekanik güç, ventilatörün yol açtığı akciğer hasarını öngörmede kullanılan bir prediktördür. Otomatik rekruitment manevrası (ARM), anestezi sırasında optimal pozitif ekspirasyon sonu basıncı (PEEP) düzeyini tayin etmek amacıyla kullanılmaktadır. Bu çalışmada morbid obezite nedeniyle sleeve gastrektomi uygulanan hastalarda, sabit PEEP uygulanan hastalar ile ARM ile optimal PEEP düzeyi tayin edilen hasta gruplarında hesaplanan total mekanik güç ve elastik güç değerleri karşılaştırıldı.

Gereç ve Yöntem: Vücüt kitle indeksi >40 üzerinde olan Amerikan Anestezi Uzmanları Derneği (ASA) III, 18-65 yaş aralığındaki hastalar iki gruba ayrıldı. Birinci grupta PEEP ayarlaması ARM ile yapıldı. İkinci grupta sabit PEEP değerleri kullanıldı. Her iki grupta 10 dakika aralıkla ölçülen solunum mekanikleri kullanılarak total mekanik güç ve elastik güç değerleri hesaplandı. Hesaplanan mekanik güç değerlerinin hasta ortalamaları Mann-Whitney U testi ile karşılaştırıldı.

Bulgular: Basınç kontrollü havalandırmanın basitleştirilmiş mekanik gücü ($MP_{pcv(simpl)}$) (dinamik) birinci grupta 2,9 (2,4-3,4) j/dakika ve ikinci grupta 7,9 (6,4-9,6) j/dakika (p<0,0001). Total güç $MP_{pcv(simpl)}$ (total power) birinci grupta 8,1 (7,4-8,9) j/dakika ve ikinci grupta 12,8 (9,9-15,2) j/dakika (p<0,0001) olarak hesaplandı.

Sonuç: ARM kullanılarak yapılan optimal PEEP tayini, akciğer elastans ve sürücü basıncını azaltarak, solunum sistemine uygulanan elastik güç değerini düşürmektedir.

Anahtar Kelimeler: Obezite, mekanik güç, PEEP, rekruitment manevrası

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INTRODUCTION

Postoperative pulmonary complications are associated with increased morbidity and mortality in patients undergoing surgery (1). Growing evidence showed that lung-protective strategies including low tidal volume, the use of optimal positive end-expiratory pressure (PEEP), and recruitment maneuvers during intraoperative mechanical ventilation reduce postoperative pulmonary complications (2). Mechanical power is an evolving concept in lung-protective ventilation, associated with increased mortality in acute respiratory distress syndrome and non-injured lungs (3).

Nowadays, the number of patients who undergo bariatric surgery is increasing due to increasing morbid obesity. Patients with obesity are at risk for developing ventilator-induced lung injury (VILI) since they are ventilated at high pressures due to a stiff chest wall and abdominal fat mass. Applying PEEP in patients with obesity avoids alveoli closure by keeping the alveolar pressure above the closing pressure. Alveolar closing pressures are high due to the chest wall pressure on the pleural space (4).

Mechanical power, which gathers different variables such as tidal volume, driving pressure (DP), flow, respiratory rate (RR), and PEEP in a single parameter, is related to VILI in various studies, offering new possibilities in VILI prediction at the bedside (5-8). The formula mechanical power (MPrs) developed by Gattinoni et al. (8) is based on the motion equation, although with all VILI variables, is too complex for practical use and is only used in volume-controlled ventilation. Becher et al. (9) proposed a simplified equation (MPpcv(simpl)) and a more comprehensive formula (MPpcv(slope)) to calculate the mechanical power in pressure control ventilation (PCV).

Recently, mechanical power showed an association with VILI and mortality (8,10,11). DP is considered the most important factor in VILI (12-14). However, mechanical power is more important as it takes other respiratory mechanics (PEEP, RR, and time) into account (5). The contribution of DP to power is also calculated as dynamic power (15). The negative and positive contribution of PEEP to power is currently discussed. Beyond applying and not applying PEEP, concepts of good PEEP (best PEEP) and bad PEEP (worst PEEP) were introduced (4,16,17). Thus, how to use PEEP? How to distinguish between good PEEP and bad PEEP? Particularly, can anesthesia safety be increased with the best PEEP in anesthesia applications?

This study aimed to evaluate the use of automatic recruitment maneuver (ARM) to set an optimal PEEP level to reduce the mechanical power, VILI, and postoperative pulmonary complications. The study was conducted in patients with obesity who underwent bariatric surgery. Total mechanical and dynamic power were compared between patients who were ventilated with PEEP set without ARM and patients whose optimal PEEP level were adjusted using ARM.

METHODS

Adult patients aged 18-65 years, with American Society of Anesthesiologists (ASA) III and body mass index (BMI) of >40, signed a voluntary informed consent form and were included in the study. Inclusion criteria are as follows: Without alcohol or drug addiction, without chronic obstructive pulmonary disease diagnosis by a pulmonogist during the preoperative routine evaluation, with normal FEV1/FVC ratio, FEV1, and FVC values, and without history of abdominal surgery. STOPBANG assessment test was performed on each pre-op patient. Spirometry was performed by a single anesthesiologist on the day of surgery and FEV1, FVC, and FEV1/FVC ratio of all patients were recorded. Patients with normal FEV1 and FVC were included in the study.

Three-lead electrocardiogram, pulse oximetry, non-invasive blood pressure, bispectoral index (BIS) and train of four monitoring were applied to all patients after placing them on the operating table in a ramp position.

Propofol between 2 and 3 mg/kg was administered according to the ideal weight and in a manner BIS score at 40-60 and rocuronium at 0.5 mg and fentanyl at 150 mcq were intravenously administered according to the ideal weight. Patients were ventilated with 6 L/min of 60% oxygen (O_2) for 2 min. Patients were endotracheally intubated using the Macintosh Laryngoscope.

After intubation, 50 mg of ranitidine and 8 mg of ondansetron were routinely administered. The remifentanil dose was kept and infusion was performed at a rate of 0.1 mcg/kg/min. Sevoflurane percentage was maintained by changing the minimum alveolar concentration (MAC) value from 0.6 to 1.1 in a way that the BIS score was between 40 and 60.

Patients were divided into two groups: group 1 applied with PEEP adjusted using ARM and group 2 without ARM. Both groups were given 3 L/min of fresh gas flow (50% O_2, 50% air) and ventilated in pressure-regulated volume control (PRVC) mode with Maquet Flow-i (Sweden). Tidal volume was set at 6-8 mL according to the ideal weight, FiO_2 at 40%, and RR according to end-tidal carbon dioxide ($EtCO_2$).

Group 1 received incremental ARM twice after insufflation and desufflation so that the target peak pressure (Ppeak)

reached 40 cm H_2O and the target PEEP at 20 cm H_2O. The PEEP value at which the highest dynamic compliance (Cdyn) was measured during ARM was considered the ideal PEEP for the patient, and PEEP after ARM was set at this value. PEEP set without ARM was set according to the anesthesiologists' decision in group 2. MAC, BIS, mean arterial pressure (MAP), heart rate, oxygen saturation (sPO_2), $EtCO_2$, RR, Ppeak, PEEP, and compliance (C) values were recorded for both groups every 10 min peroperatively. Elastance (ELrs) was measured with inspiratory and expiratory hold maneuvers simultaneously (every 10 min) and values were recorded.

Remifentanyl was discontinued 10 min before the end of the operation in both groups. Afterwards, 1 g of paracetamol and 100 mg of tramadol were intravenously administered. Sugammadex at 2 mg/kg was administered according to the ideal weight at the end of the surgery. When the trigger was zero, patients were extubated when they achieved sufficient tidal volume, with a BIS score between 80 and 100. Patients with an ARISCAT Risk index of 9≥9 in the review were sent to the floor.

Vital signs, including MAP, were monitored postoperatively. Tenoxicam at 20 mg was administered intravenously in the first hour. The patient-controlled analgesia device was set in a way that tramadol at 300 mg/100 mL: Bolus as 10 mg, lock time as 12 min, and no basal infusion is administered. Patients with Numeric Rating scale scores of ≥4 were intravenously given with 4 mg of morphine as a salvage dose.

Analgesia was provided 24 hours after surgery, maintaining the visual analog scale (VAS) score at <4, and spirometry was repeated by the same doctor before the surgery.

Patient Randomization

In the study, random numbers were generated to prevent selection bias. Of the produced numbers, 0 was predetermined as control and 1 was predetermined as the experimental group, and patients were assigned to groups in this direction. Random numbers were made with the program MedCalc 18.2.1. (MedCalc Statistical Software version 18.2.1 MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).

Group 1 was defined as patients who received PEEP with ARM and group 2 as patients who received PEEP without ARM.

Mechanical Power Calculation:

The power calculations in this study were also calculated with Becher's simple formula (pressure control simplified mechanical power equation, $MP_{pcv(simpl)}$) (9).

$MP_{pcv(simpl)}$ (total power) (J/min) = 0.098 × RR × TV × (DP + PEEP)

$MP_{pcv(simpl)}$ (Dynamic power) = 0.098 × RR × TV × DP

DP = Ppeak-PEEP and 0.098 is the conversion factor to Joule

Since the tidal gas flow and inspiratory resistance (Ri) cannot be measured in PRVC mode, the resistive component of the mechanical power between the two groups is not calculated.

PRVC mode is a PCV mode. Assuming no intrinsic PEEP, airway pressure in PCV was considered constant at the end of inspiration, with equal Ppeak and alveolar pressure $_{(Pplateau)}$ (Pplateau = Ppeak) (18-21).

If DP = $P_{plateau}$-PEEP = P_{peak}-PEEP, then C = ΔV/DP

Total MPrs and the dynamic component of power were calculated using respiratory mechanics measured at 10-min intervals. Patient averages of the calculated power values were statistically analyzed.

Statistical Analyses

GraphPad Prism (v 5.01) program was used for statistical analysis of obtained study findings. Results of the Shapiro-Wilk normality test did not conform to the normal distribution of numerical variables in the two groups, thus the Mann-Whitney U test was used for binary variable comparison. Categorical variables were evaluated using the chi-square and Fisher's Exact test. Median and interquartile range (IQR) values were taken as a basis for statistical evaluation. Statistical values of $p<0.05$ were considered significant.

The necessary data were recorded peroperative for each patient for 90 min and once every 10 min. A total of 954 measurements were made for a total of 106 patients, wherein 56 are from group 1 and 50 from group 2. Data were saved based on excel. Statistical analyzes were made according to patient averages.

Total mechanical power values are calculated as MPpcv (simpl) formula, which is the primary outcome of the preliminary study (first 10 patients). Mean and standard deviation values for group 1 were calculated as 8.1±3.2 J/min and 10.5±3.1 J/min for group 2. The standard error margin (Alpha error) was calculated as 0.05 and 95% and the effect size was calculated as 0.6 in the power analysis made according to the power difference, the number of required patients in the study was calculated as 50 patients for each group (G*Power 3.1.9.4).

Institutional permission and ethical approval from the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinical Research

Ethics Committee were obtained from where the study was conducted before it begins (protocol code: 2018/474, decision number: 2018-23-21 and approval date: 17.12.2018). Written, informed consent was obtained from all patients. All methods were carried out by relevant guidelines and regulations.

RESULTS

Group 1 includes 50 patients and group 2 had 56 patients. Patient characteristics revealed no statistically significant difference between gender, age, BMI, duration of surgery, MAP, heart rate, Ariskat score, and postoperative 1, 6, and 24-hour VAS scores ($p>0.05$). STOPBANG scale was significantly higher in group 2 than that of group 1 ($p=0.004$) (Table 1). The respiratory parameters evaluated three stages (after induction of anesthesia, before insufflation, after desufflation) in the peroperative process: Average during the operation (Perop. mean). Compliance, ELrs, PEEP, Ppeak, SpO_2, DP, and total and dynamic mechanical power Perop. mean values were statistically significant (Table 2). The difference of PEEP, DP, and total and dynamic power values was statistically significant between the two groups ($p<0.0001$).

DISCUSSION

This study observed that the ARM, which improves respiratory mechanics and oxygenation, did not significantly change the hemodynamic parameters. However, without difference between the pulmonary function tests in both groups, the dynamic mechanical power values were lower in patients where PEEP was adjusted with an ARM than in patients without ARM. The dynamic power difference between the two groups was dependent on the difference in PEEP. Energy applied (dynamic) to the respiratory system was significantly less in the group in which PEEP was applied with ARM. No difference was found between the two groups in terms of RR and tidal volumes; however, PEEP was higher in the group with ARM, which was due to low Ppeak and much lower DP values. Lower DP values mean higher compliance and naturally lower lung ELrs.

PEEP significantly contributes to lung-protective ventilation by reducing lung ELrs in patients with obesity. Particularly,

Table 1. Patient characteristics

Patient median (IQR)	Group 1 (PEEP with ARM) (n=56)	Group 2 (PEEP without ARM) (n=50)	p
Gender, female no (%)	54 (96%)	37 (74%)	0.47
Age median (year)	39 (31-47)	41 (35-48)	0.40
BMI (kg/m^2)	45.2 (41.8-50.2)	46.2 (43.2-50.5)	0.49
Operating time (min)	58 (53.7-70)	56 (50-65)	0.426
MAP Perop, mean (mmHg)	84.1 (75.2-92.8)	85.2 (77.7-95.4)	0.3189
HR Perop, mean (1/min)	84.8 (78.8-97.3)	88.2 (80.8-95.4)	0.3876
Ariskat score	15 (15-15)	15 (15-17)	0.162
Postop 1 hour VAS score	3 (2-4)	3 (2-4.2)	0.255
Postop 6 hour VAS score	2 (1.2-3)	3 (2-3)	0.306
Postop 24 hour VAS score	1 (0-2)	2 (0-2)	0.078
STOPBANG score	3 (3-4)	4 (3-5)	0.004
FEV1 preoperative (mL)	84 (77-95)	85 (76-92)	0.8
FEV1 postoperative (mL)	72 (64-85)	71 (68-83)	0.9
FVC preoperative (mL)	81 (71-90)	80 (70-90)	0.8
FVC postoperative (mL)	75 (64-83)	72 (67-79)	0.3
FEV1/FVC preoperative (%)	1.10 (1.01-116)	1.06 (1.01-1.14)	0.4
FEV1/FVC postoperative (%)	1.03 (0.97-1.12)	1.05 (0.98-1.11)	0.6

The Mann-Whitney U test was used to analyze the parameters shown in the table between the groups and the chi-square test was used to determine the percentage and significance level of gender difference. PEEP with ARM: The group upon whom PEEP is applied with ARM PEEP without ARM: The group upon which positive end-expiratory pressure is applied according to the anesthesiologists' decision. IQR: Interquartile range, BMI: Body mass index, MAP: Mean arterial pressure, HR: Heart rate, VAS: Visual analog scale, STOPBANG score: Score of obstructive sleep apnea score, FEV_1: Forced expiratory volume in one second, FVC: Forced vital capacity

Table 2. Respiratory parameters

Patient Median (IQR)	Group 1 (PEEP with ARM) (n=56)	Group 2 (PEEP without ARM) (n=50)	p
Cstatik perop. mean	44.1 (36.0-51.4)	38.2 (33.6-44.8)	**0.0422**
Elastance perop. mean	22.7 (19.4-27.7)	26.1 (22.2-29.7)	**0.0421**
Cdynamic perop. mean	40.0 (32.8-48.3)	36.07 (31.6-40)	**0.0248**
PEEP perop. mean	8 (7-10.2)	7 (6-8)	**0.0049**
Ppeak perop. mean	19.8 (18.6-22.1)	21.7 (20.5-23.7)	**0.0005**
RR perop. mean	14 (13-14)	14 (13-14)	**0.0647**
TV perop. mean	440 (402-480)	470 (400-520)	**0.2440**
$EtCO_2$ perop. mean	40.7 (38.1-42.5)	41.07 (38-42.5)	**0.7928**
SpO_2 perop. mean	97.7 (96.7-98.5)	96.5 (95.3-97.2)	**<0.0001**
Pplato perop. mean	18.4 (16.6-20.6)	20.3 (18.2-23.5)	**0.0008**
DP perop. mean	**11.14 (9.3-13.7)**	**14.43 (12.8-16.7)**	**<0.0001**
$MP_{pcv(simpl)}$ (total power) perop. mean	8.1 (7.4-8.9)	12.8 (9.9-15.2)	**<0.0001**
$MP_{pcv(simpl)}$ (dynamic component) Perop. mean	2.9 (2.4-3.4)	7.9 (6.4-9.6)	**<0.0001**

Mann-Whitney U test was used to analyze the parameters shown in the table between the groups. IQR: Interquartile range, (Cstatic: Static compliance (mL/cmH_2O), Elastance (cmH_2O/L), Cdynamic: Dynamic compliance (mL/cmH_2O), PEEP: Positive end-expiratory pressure (cmH_2O), Ppeak: Peak pressure (cmH_2O), RR: Respiratuar rate (1/minute), TV: Tidal volume (mL) $ETCO_2$: End-Tidal CO_2 (mmHg), SpO_2: Oxygen saturation (%), Pplato: Peak pressure (cmH_2O), DP: Driver pressure (cmH_2O), $MP_{pcv(simpl)}$ (J/minute): Simplified mechanical power of pressure control ventilation)

PEEP reduces lung damage by decreasing DP (8,17). Patients with PEEP adjusted according to ARM were ventilated with higher PEEP values than patients with PEEP set without ARM, thus lower ELrs values were calculated. PEEP reduces DP, one of the important components of mechanical power, which is the cause of VILI, thus decreases the dynamic component of mechanical power. Administration of PEEP with ARM reduces VILI in patients with obesity. Therefore, optimal compliance values are obtained with a more open lung, and patients are operated on with less peroperative and postoperative lung complications (less atelectasis) (4). Peroperative optimal PEEP application with ARM in patients with obesity who are prone to atelectasis is a feasible solution to achieve a lung-protective ventilation strategy.

Appropriate PEEP usage (higher PEEP) and lower power values, similar $EtCO_2$, and better SpO_2 values were obtained in group 1 than that in group 2. A recent study comparing patients with obesity, morbidly obesity, and non-obesity in intensive care observed that patients with morbidly obesity had higher DP and dynamic power but lower PaO_2/FiO_2 ratios compared to other patients (22).

Dynamic power is an indicator of the combined effect of all variables involved in the pathogenesis of VILI, thus adjusting PEEP in patients with obesity with ARM in bariatric surgery reduced lung damage. Patients with obesity developed atalectasis and alveolar closure due to a stiff chest wall, and individualized PEEP adjustment with ARM increased the anesthesia safety by reducing the energy applied to the respiratory system and lung damage. An analysis of patients with obesity who underwent elective laparoscopic surgery compared the fixed and individualized PEEP determined by electrical impedance tomography. Better oxygenation, lower DP, and redistribution of ventilation toward dependent lung areas were found in patients with individualized PEEP (23).

Study Limitations

Patients were not screened for postoperative complications, such as atelectasis, pneumonia, or pulmonary embolism. In addition, the effect of PEEP levels on arterial blood gases was not evaluated.

CONCLUSION

Appropriate PEEP adjustment with ARM reduced the lung ELrs and DP, and less dynamic power was applied to the respiratory system in patients with obese who were prone to atelectasis due to a stiff chest wall. This approach provides a safer ventilation strategy and contributes to the prevention of VILI and postoperative pulmonary complications.

ETHICS

Ethics Committee Approval: Institutional permission and ethical approval from the University of Health Sciences

Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinical Research Ethics Committee were obtained from where the study was conducted before it begins (protocol code: 2018/474, decision number: 2018-23-21 and approval date: 17.12.2018).

Informed Consent: Written, informed consent was obtained from all patients.

Authorship Contributions

Surgical and Medical Practices: S.A., E.K.T., M.S.S., Concept: S.A., E.K.T., M.S.S., Design: S.A., E.K.T., M.S.S., Data Collection or Processing: S.A., E.K.T., M.S.S., Analysis or Interpretation: S.A., E.K.T., M.S.S., Ö.A., Literature Search: S.A.,Ö.A., E.K.T., M.S.S., Writing: S.A., E.K.T., M.S.S., Ö.A.

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Research

Clinical Profile and Predictors of Severe Malaria: A Study from Two Tertiary Care Centers in İstanbul

Şiddetli Sıtmanın Klinik Profili ve Göstergeleri: İstanbul'da İki Üçüncü Basamak Hastaneden Çalışma

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ABSTRACT

Objective: This study aimed to determine discriminating findings of severe malaria cases compared with other cases.

Methods: Epidemiological, laboratory, and clinical features of the patients were collected retrospectively from two tertiary centers. The diagnosis was established in all cases by the detection of trophozoite and/or gametocyte forms of the parasite in the microscopic examination. "Severe malaria" with a serious clinical course was defined according to 2015 World Health Organization data.

Results: This study included 94 patients with imported malaria composed of 85 male (90.4%) and 9 female (9.6%) patients with a mean age of 32.4±4.56 years. *P. falciparum* (n=72, 76.6%) and *P. vivax* (n=15, 15.9%) were the most frequently identified agents. Of all cases, 12.7% (n=12) were defined as severe malaria. The frequency of abdominal pain, diarrhea, splenomegaly, and deep thrombocytopenia were significantly higher in severe malaria cases than in other cases (p=0.002; p=0.001; p=0.046; p=0.041, respectively).

Conclusion: The frequencies of abdominal pain, diarrhea, splenomegaly, and deep thrombocytopenia were significantly higher in severe malaria cases than in other cases. These factors can be accepted as predictors of severe malaria.

Keywords: Malaria, *Plasmodium, Plasmodium falciparum*, severe malaria

ÖZ

Amaç: Bu çalışma, klinik seyri ciddi olan sıtma olgularının diğer olgulara göre ayırıcı bulgularına dikkat çekmeyi amaçlamıştır.

Gereç ve Yöntem: Hastaların epidemiyolojik, laboratuvar ve klinik özellikleri retrospektif olarak toplandı. Tüm olgularda mikroskobik incelemede parazitin trofozoit ve/veya gametosit formlarının saptanmasıyla tanı konuldu. Dünya Sağlık Örgütü 2015 verilerine göre ciddi klinik seyir gösteren "şiddetli sıtma olguları" tanımlanmıştır.

Bulgular: Çalışmamıza yaş ortalaması 32,4±4,56 yıl olan 85'i erkek (%90,4) ve 9'u (%9,6) kadın olmak üzere 94 ithal sıtma olgusu dahil edildi. *P. falciparum* 72 (%76,6) ve *P. vivax* 15 (%15,9) en sık saptanan etkenlerdi. Tüm olguların; %12,7'si (12 olgu) "şiddetli sıtma olgusu" olarak tanımlandı. Klinik seyri ağır olan "şiddetli sıtma" olgularında karın ağrısı, ishal, splenomegali ve derin trombositopeni sıklığı diğer olgulara göre istatistiksel olarak anlamlı derecede yüksek bulundu (p=0,002; p=0,001; p=0,046; p=0,041, sırasıyla).

Sonuç: Çalışmamızın sonuçlarına göre karın ağrısı, ishal, splenomegali ve derin trombositopeni sıklıkları diğer olgulara göre anlamlı derecede yüksek bulundu ve bu faktörler şiddetli sıtmanın göstergesi olarak kabul edilebilir.

Anahtar Kelimeler: Sıtma, *Plasmodium, Plasmodium falciparum*, şiddetli sıtma

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INTRODUCTION

Malaria is a major pandemic disease caused by parasitic protozoa of the genus Plasmodium. The disease name "malaria" means "bad air" as it occurs by inhalation of bad air from marshlands. It is a parasitic disease transmitted through the bite of female Anopheles mosquito that usually appears between dusk and dawn. The rare mechanisms of transmission include congenitally acquired disease, blood transfusion, shared use of contaminated needles, organ transplantation, and nosocomial transition (1-3). Of the five Plasmodium species that infect humans, P. falciparum and P. vivax are responsible for most of the cases (4). The World Health Organization (WHO) has reported that approximately 238 million and 229 million malaria cases occurred in 2010 and 2019 worldwide, respectively, and that these cases were most commonly found in Africa (94%), Southeast Asia, and East Mediterranean regions. Moreover, approximately 409,000 deaths are caused by malaria annually, and >90% of those deaths occurred in the sub-Saharan African region (4).

Malaria may present different clinical pictures as well as nonspecific and classical symptoms. Mild symptoms are observed in individuals with asymptomatic parasitemia, while the clinical course may range from moderate to comatose states and ends in patient death with severe anemia, metabolic acidosis, cerebral malaria, and multiorgan involvement. Severe malaria is mostly caused by P. falciparum (90%); however, P. vivax and P. knowlesi may cause serious disease (4-7).

WHO has defined severe malaria as the occurrence of unconsciousness or comatose, inability to stand without support, having more than two convulsion attacks within 24 h, acidosis, hypoglycemia (blood glucose <40 mg/dL), serious malarial anemia (hemoglobin <7 g/dL), renal failure, hyperbilirubinemia (total bilirubin >3 mg/dL), pulmonary edema, abnormal spontaneous bleeding, shock, and hyperparasitemia (8).

No new endemic malaria cases originating from the southeast Anatolia region in Turkey were detected since 2010. By contrast, cases observed in the recent 10 years were relapse cases caused by hypnozoites (9). However, imported malaria cases observed after travels to tropical regions, primarily Africa, have threatened the health of our citizens who frequently travel to high-risk regions for business, tourism, humanitarian aid, etc. Thus, this study aimed to examine discriminating findings of severe malaria cases compared with other cases and to determine the clinical profile and predictors of severe malaria by retrospectively analyzing cases followed in two centers.

METHODS

This study included imported malaria cases followed up between 2011 and 2019 in two infectious diseases and clinical microbiology clinics in Istanbul, Turkey. Epidemiological, clinical, and laboratory data and treatment-related and clinical course features of the patients were collected retrospectively. The study was approved by the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethics Committee (2019-04-15/2019-78). Informed consent forms were signed by the patients.

The diagnosis was established in all cases by the detection of trophozoite and/or gametocyte forms of the parasite during the microscopic examination performed after staining of thick-drop and peripheral smear slides by Giemsa stain prepared from peripheral blood samples obtained during the feverish period. The diagnosis of imported malaria was also confirmed by the Department of Malaria Control of Istanbul Province Public Health Directorate. Patients with severe malaria were treated by oral or parenteral administrations of antimalarial medicines (Artemether-lumefantrine, quinine, primaquine, doxycycline, etc.) supplied by the Department of Malaria Control. For monitoring of the parasitemia load, thick-drop and peripheral smear examinations were performed at baseline and daily or 8-h intervals when needed during the hospitalization process until finalization of the parasitemia. "Severe malaria" with a serious clinical course was defined according to 2015 WHO data (8). Clinical and laboratory findings of patients with and without severe malaria were compared.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS version 18.0 software. Descriptive statistics were reported as number (n) and percentage (%) for categorical variables and as mean ± standard deviation values for continuous variables. In the group comparisons, the chi-square test and Fisher's Exact test were applied to categorical variables. Student's t-test was used for the comparison of two groups of continuous variables. A value of $p<0.05$ was accepted as significant.

RESULTS

This study included 94 patients with imported malaria, composed of 85 male (90.4%) and 9 female (9.6%) patients with a mean age of 32.4±4.56 (range, 18 and 62) years. Of the patients, 85% and 15% reported trips to African and Southeast Asian regions in their medical history, respectively. Chemoprophylaxis was not received by 94.7%

of the patients before their trips. The clinical and laboratory findings of the patients at initial application are summarized in Table 1.

P. falciparum (n=72, 76.6%) and P.vivax (n=15, 15.9%) were the most frequently identified agents in our cases. The most commonly seen symptoms at baseline physical examination were high fever (100%), chills/shivering (78.7%), and headache (64%), whereas the most frequent findings were thrombocytopenia (90.5%), anemia (73.4%), and splenomegaly (67%). Severe thrombocytopenia (<50.000 10^3/μL) was present in approximately 40% of the patients.

Of all the patients, 12.7% (n=12) were diagnosed with severe malaria with a serious clinical course. Cerebral involvement and jaundice were monitored in 10 (83.3%) and 5 (41.7%) of those patients, respectively, whereas no hypoglycemia, acute pulmonary edema, and hemorrhage were recorded among them. Moreover, 4 (33.3%) patients with severe malaria with a serious clinical course caused by *P. falciparum* died. Table 2 presents the comparison between severe malaria with a serious clinical course and other cases regarding clinical and laboratory findings.

The frequency of abdominal pain, diarrhea, splenomegaly, and severe thrombocytopenia was significantly higher in patients with severe malaria with a serious clinical course than in other cases (p=0.002; p=0.001; p=0.046; p=0.041, respectively).

Artemisinin-based antimalarial treatment and quinine-based antimalarial treatment were administered in 86% and 6% of the patients, respectively. Since adequate treatment response to standard treatment could not be achieved in 8% of the patients, a combined or consecutive treatment using artemisinin-derived and quinine-derived antimalarial agents were needed.

DISCUSSION

Malaria is an infectious disease that may present a fatal progression unless diagnosed and treated early. According to a WHO report, malaria takes the second and fifth places in Africa and the world, respectively, as the cause of deaths from infectious diseases. The malarial load is disproportionally high in Africa, and more than 90% of malaria cases were reported in Africa in 2019 (4).

Turkey is still under the threat of malaria because of increased travel opportunities, increased migration, and labor force mobility among countries as well as uncontrolled migration

Table 1. Clinical and laboratory findings of the patients at initial presentation

Demographic and clinical features	(n=94, %)	Laboratory findings	(n=94, %)
Male	85 (90.4)	*P. falciparum*	72 (76.6)
Female	9 (9.6)	*P. vivax*	15 (15.9)
Age (mean)	32.4±4.6 years	*P. falciparum* + *P. vivax*	6 (6.4)
Fever	94 (100)	*P. falciparum* + *P. ovale*	1 (1.1)
Chills and shivering	74 (78.7)	Leukocyte (<5000 10^3/μL)	58 (61.7)
Headache	60 (63.8)	Thrombocyte (10^3/μL)	-
Sweating	47 (50)	>150,000	9 (9.5)
Fatigue	39 (41.5)	50,000-150,000	48 (51)
Nausea and vomiting	25 (26.6)	<50,000	37 (39.3)
Abdominal pain	25 (26.6)	Hemoglobin (<13 g/dL)	69 (73.4)
Diarrhea	11 (11.7)	Hemoglobin (<7 g/dL)	8 (8.5)
Altered consciousness	10 (10.6)	Creatinine (>1 mg/dL)	19 (20.2)
Splenomegaly	63 (67)	Renal failure (>3 mg/dL)	6 (6.4)
Hepatomegaly	29 (30.8)	LFT (AST, ALT) (>40 IU/L)	48 (51)
Cerebral involvement	10 (10.6)	T. bilirubin (>3 mg/dL)	5 (5.3)
Use of prophylaxis	5 (5.3)	-	-
ICU admission	3 (3.2)	-	-
Mortality	4 (4.2)	-	-

LFT: Liver function test, ICU: Intensive care unit, LFT: Liver function test, AST: Aspartate aminotransferase ALT: Alanine aminotransferase

Table 2. Comparison of regarding clinical and laboratory findings between severe malaria cases with a serious clinical course and other cases of malaria

		Non-severe (n=82, %)	Severe (n=12, %)	p
Age mean ± SD		31.8±3.49 years	34.2±9 years	[a]0.380
Min-max (median)		18-60 years (31.62 years)	25-62 years (32.34) years	
Gender	Male	74 (90.2)	11 (91.6)	[b]0.678
	Female	8 (9.8)	1 (8.4)	
Chills and shivering		66 (80.4)	8 (66.7)	[b]0.229
Headache		50 (60.9)	10 (83.3)	[b]0.116
Sweating		39 (47.5)	8 (66.6)	[b]0.216
Fatigue		33 (40.2)	6 (50)	[b]0.368
Nausea		22 (23.4)	3 (25)	[b]0.601
Abdominal pain		17 (20.7)	8 (66.6)	[b]0.002**
Diarrhea		5 (6.1)	6 (50)	[b]<0.001**
Splenomegaly		52 (63.4)	11 (91.7)	[b]0.046**
Hepatomegaly		23 (28)	6 (50)	[b]0.116
Leukocyte (<5000 10^3/μL)		52 (63.4)	6 (50)	[b]0.279
Thrombocytopenia (10^3/μL)		73 (89.1)	12 (100)	[b]0.276
50,000-150,000 (mild)		44 (53.7)	4 (33.3)	[b]0.188
<50,000 (severe)		29 (35.4)	8 (66.6)	[b]0.041**
Hemoglobin (<13 g/dL)		64 (78)	12 (100)	[b]0.065
LFT (AST, ALT) (>40 IU/L)		44 (53.6)	4 (33.3)	[b]0.188

**p<0.05, [a]Student's t-test, [b]Pearson chi-square test, LFT: Liver function test, AST: Aspartate aminotransferase ALT: Alanine aminotransferase, SD: Standard deviation

from Syria to Turkey. All cases followed up for malaria by our institute in the recent 10 years were imported cases. A study reported that no indigenous malaria case was detected since 2010 in Turkey and that *P. vivax* malaria cases identified in the recent 10 years were hypnozoite relapse of previous malaria (9). Hence, only imported cases were determined in recent years (9-13). The eradication of malaria cases through the years in Turkey indicates the success of malaria control practices. The increased number of imported cases can be explained by increased travel opportunities to high-risk regions with higher business opportunities. However, the risk of re-emergence of indigenous malaria continues owing to the presence of female Anopheles mosquitoes, the infectious agent for malaria, in our country, irregular migration, subtropical region location of Turkey where malaria can be transmitted through, and increased mean air temperature observed because of climate change.

Malaria may be seen in both genders; however, most of the patients were male. This can be explained by the higher frequency of travel and labor force mobility by men. Although malaria may occur in every age, malaria was reported in patients aged 20-40 years (10-13). The mean age of patients hospitalized in our clinic was 32.4±4.56 years. This may explain the finding that malaria more commonly occurred in young adults because this group travels more frequently for business and tourism. In our analysis of the anamnesis of our patients regarding their previous trips, patients diagnosed with P. falciparum and *P. vivax* malaria had traveled to Africa and the Far East (Afghanistan, Pakistan, India), respectively. This data distribution is consistent with epidemiological data of malaria reported by WHO (4).

In this study, severe malaria was found in 12 (12.76%) patients. Studies from different countries have similarly reported a severe malaria rate of 15%-17.3% (14,15). Fever was the most frequently seen finding in these studies. The lack of a typical malaria course accompanied with shivering in 33.3% of the patients with severe malaria may be attributed to weak immunity, so malaria may become severe in these patients. Physical examination may reveal hepatomegaly, splenomegaly, abdominal tenderness, and altered intestinal habits.

During the disease couse, anemia develops because of the abnormal degradation of erythrocytes, increased amounts of pigments released from the degraded erythrocytes lead to jaundice, and deposition of these pigments in the reticuloendothelial system results in hepatosplenomegaly (16). Splenomegaly is caused by increased erythrocyte degradation and indicates the stage of the disease. In this study, splenomegaly was found in 11 (91.6%) and 52 (63.4%) patients with severe malaria and mild malaria, respectively. Since splenomegaly is accepted as a parameter that indicates the disease stage, a delay in diagnosis and initial presentation to the hospital may be considered in severe malaria.

A broad spectrum of changes in complete blood count may be present. In this study, thrombocytopenia (<50,000 10^3/μL) was detected in 66.6% of patients with severe malaria and was considered associated with disease severity (p=0.041). Similarly, Hanson et al. (17) and Rao et al. (18) have demonstrated that severe thrombocytopenia is an indicator of disease severity in adults.

Studies on adult patients have demonstrated varying frequencies in diarrhea (1.1%-43.8%), and diarrhea was

found in 11.7%, 50%, and 6.1% in all patients, patients with severe malaria, and patients with other cases of malaria, respectively (11,19-24). Although the incidence of diarrhea in the present study was higher in patients with severe cases than in patients with other cases ($p<0.001$), the frequency of diarrhea was not associated with severe cases in the study by Arnold et al. (19). Furthermore, they have reported a lower rate of diarrhea. Such differences may be considered; findings of abdominal distension such as abdominal pain and diarrhea indicate gastrointestinal tract insufficiency, which is a symptom of multiorgan failure in the pathogenesis of severe malaria and is associated with the differences in the abundance of gut microbiota. Different studies have reported that P. falciparum infection was associated with increased intestinal permeability and that parasites may be identified even in the intestinal mucosa of patients who had severe malaria (25-27). In our study, abdominal pain was also linked with disease severity, just as diarrhea, and it may be due to splenomegaly, which is similarly associated with disease severity, aside from diarrhea.

The management of patients with severe malaria presents a broad spectrum of clinical challenges because of the complicated pathophysiology and involvement of multiple organ systems. The most important aspect of treatment is rapid administration of effective antimalarial treatment and provision of concurrent supportive care to manage life-threatening complications. Supportive precautions (e.g., oxygen, ventilator support, and cardiac monitoring) should be taken properly. Intravenous catheters should be placed, and blood samples should be immediately obtained for necessary laboratory tests. Central nervous system pathologies such as intracranial hemorrhage and bacterial meningitis should be ruled out in unconscious patients. The differential diagnostic tests and antimalarial treatment should be performed concurrently with supportive treatments including anticonvulsants, intravenous administration of glucose and fluids, antipyretics, and antibiotics, and blood transfusion. The adequacy of antimalarial chemoprophylaxis depends on antibiotic resistance and compliance with the recommended duration and dosage. Individuals who will travel to other countries should be given recommendations on the contagious disease state of the related country, required vaccinations, drugs for antimalarial prophylaxis, and pretreatment protective precautions by relevant authorities (28).

CONCLUSION

Delays in the diagnosis and treatment of malaria increase morbidity and mortality. In this study, the incidence of abdominal pain, diarrhea, splenomegaly, and deep thrombocytopenia was significantly higher in severe malaria cases than in other cases, and these factors can be accepted as predictors of severe malaria. Therefore, all patients diagnosed with malaria had imported P. falciparum malaria cases, and patients with altered consciousness state, abdominal pain, diarrhea, and renal dysfunction need closer monitoring and observation, considering the risk of these patients to complications and a severe clinical course. Patients with splenomegaly and deep thrombocytopenia need close monitoring considering their risks of complications such as spontaneous splenic rupture and hemorrhage as well as a severe clinical course. The risk for bacteremia secondary to increased intestinal changes should be also considered, including the fact that hypotension may develop because of dehydration in patients with abdominal pain and diarrhea. Pre-travel information and chemoprophylaxis may be life saving for individuals who will travel to endemic regions. Patients who presented to the hospital with a feverish picture should be assessed about their history of foreign travel, and malaria should be included in the differential diagnosis of every feverish disease to obtain an opportunity for early diagnosis and treatment.

ETHICS

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethics Committee (2019-04-15/2019-78).

Informed Consent: Informed consent forms were signed by the patients.

Authorship Contributions

Concept: R.K., Ş.N.K., N.D.S., K.K.Y., Design: R.K., Ş.N.K., N.D.S., K.K.Y., Data Collection or Processing: R.K., N.D.S., S.Ş., E.C.Ü., Analysis or Interpretation: R.K., N.D.S., S.Ş., H.K., K.K.Y., Literature Search: R.K., E.C.Ü., S.Ş., K.K.Y., Writing: R.K., Ş.N.K., N.D.S., S.Ş., K.K.Y.

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Research

Low-Density Lipoprotein Cholesterol Levels and Disease Severity in COVID-19 Pneumonia

COVID-19 Pnömonisinde Düşük Yoğunluklu Lipoprotein Kolesterol Düzeyleri ve Hastalık Şiddeti İlişkisi

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ABSTRACT

Objective: Pneumonia and acute respiratory distress syndrome are observed as major complications in Coronavirus disease-2019 (COVID-19). Cholesterol is a principal lipid component of the cell membranes. Lipoproteins have a fundamental role as the first line of defense against microbes. Lipoprotein levels are altered during viral infections. This study aimed to investigate the association between low-density lipoprotein cholesterol (LDL-C) levels and disease severity of patients hospitalized with COVID-19 pneumonia.

Methods: This is a retrospective and observational study of 817 patients with Severe Acute Respiratory syndrome-Coronavirus-2 (SARS-CoV-2), who are diagnosed with COVID-19 using the real-time polymerase chain reaction and are hospitalized due to moderate and severe COVID-19 pneumonia.

Results: Among 817 patients with COVID-19 pneumonia, 347 (42.5%) were moderate and 470 (57.5%) were severe. Total cholesterol (TC) and LDL-C levels reduced in patients who have severe condition than those with moderate condition (p=0.02, p=0.03, respectively). TC and LDL-C were negatively correlated with the hospitalization duration (r=-0.163, p=0.02, r=-0.154, p=0.03, respectively).

Conclusion: Our results suggest a decreased LDL-C levels in patients with COVID-19 in relation to disease severity. Therefore, a strong link was found between lipid metabolism and SARS-CoV-2, which allow us to suggest LDL as a marker indicating COVID-19 severity in the near future.

Keywords: COVID-19 pneumonia, low-density lipoprotein cholesterol, disease severity

ÖZ

Amaç: Pnömoni ve akut solunum sıkıntısı sendromu, Koronavirüs hastalığı-2019 (COVID-19) majör komplikasyonlar olarak karşımıza çıkmaktadır. Kolesterol, hücre zarlarının başlıca lipid bileşenidir. Lipoproteinlerin mikroplara karşı ilk savunma hattı olarak temel bir role sahip olduğu bilinmektedir. Viral enfeksiyonlar sırasında lipoprotein seviyeleri değişir. Bu çalışmada, COVID-19 pnömonisi ile hastaneye yatırılan hastaların düşük yoğunluklu lipoprotein kolesterol (LDL-C) seviyeleri ile hastalık şiddeti arasındaki ilişkiyi araştırdık.

Gereç ve Yöntem: Polimeraz zincirleme reaksiyonu testi ile COVID-19 tanısı konan ve orta ve şiddetli COVID-19 pnömonisi nedeniyle hastaneye yatırılan Şiddetli Akut Solunum sendromu-Koronavirüs-2 (SARS-CoV-2) 817 hastanın retrospektif ve gözlemsel bir çalışmasıdır.

Bulgular: COVID-19 pnömonisi olan 817 hastanın 347'si (%42,5) orta, 470'i (%57,5) şiddetli klinik tabloda idi. Ağır hastalarda toplam kolesterol (TC) ve LDL düzeyleri orta şiddette hastalarla karşılaştırıldığında anlamlı olarak daha düşüktü (sırasıyla p=0,02, p=0,03). TC ve LDL-K ile hastanede yatış süresi arasında negatif korelasyon saptandı (sırasıyla r=-0,163, p=0,02, r=-0,154, p=0,03).

Sonuç: Sonuçlarımız hastalığın ciddiyeti ile ilişkili olarak COVID-19 hastalarında LDL-C düzeylerinde azalma olduğunu göstermektedir. Lipid metabolizması ile SARS-CoV-2 arasında yakın gelecekte LDL-C COVID-19'un şiddetini gösteren bir belirteç olarak önermemize izin verebilecek güçlü bir bağlantı olduğu sonucuna varılmıştır.

Anahtar Kelimeler: COVID-19 pnömonisi, düşük yoğunluklu lipoprotein kolesterol, hastalık şiddeti

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INTRODUCTION

Severe Acute Respiratory syndrome-Coronavirus-2 (SARS-CoV-2), possibly leading to poor outcomes, is known as a single origin of coronavirus disease-19 (COVID-19) pandemic. Pneumonia, acute respiratory distress syndrome, and septic shock are observed as major complications of the disease, leading to death.

Previously, SARS-CoV-1 caused "SARS," in 2002 (1). Thereafter, the "middle east respiratory syndrome coronavirus (MERS-CoV)" caused the second outbreak in the past two decades (2).

Some common features were found among SARS-CoV-1, MERS-CoV, and SARS-CoV-2 from the epidemiological, clinical, molecular, and infectious aspects (3).

The onset of infections starts with a viral entrance to the host cell. Coated viruses, including CoVs, initially activate the plasma membrane fusion or endocytosis processes for the entrance. SARS-CoV-2, similar to SARS-CoV-1, employs human angiotensin converting enzyme 2 (ACE2) as a receptor to successfully enter the cell (4). ACE2 molecules located in the lipid rafts are widely available in alveolar type II epithelial cells (5).

Studies confirmed that the lipoproteins served as the initial defense barrier against the invading microbes (6), whose levels change during viral infections (7). Cholesterol known as a major lipid in the cell membrane is also an important component within this context (8,9).

Some rare findings were reported that dyslipidemia arises from SARS. Specifically, patients with SARS were reported to have reduced total cholesterol (TC) levels compared with healthy participants (10). Alterations in lipoprotein levels during COVID-19 infection were also reported (11-15).

Lipid profiles of patients with COVID-19 and their possible relation with disease severity were retrospectively investigated in our study.

METHODS

Study Design and Cohort

A total of 1,509 adult patients with COVID-19 pneumonia diagnosed using the polymerase chain reaction technique and radiologic involvement of computed tomography (CT) scan and admitted to University of Health Sciences Turkey, Bakirköy Dr. Sadi Konuk Training and Research Hospital in Istanbul, level-3 pandemic, from September 01, 2020, to December 31, 2020, were retrospectively observed. The hospital electronic database was screened and patients without clinical or laboratory data or with pneumonia arising from other causes were excluded from the study. Other criteria for exclusion, to reduce the confounding effects, includes the existence of dyslipidemia, use of lipid-lowering therapy, end-stage renal failure, chronic dialysis, nephrotic syndrome, obesity, cholestatic liver disease, chronic use of corticosteroids, immunosuppressive conditions, human immunodeficiency virus (HIV) infection, hypothyroidism, diabetes mellitus, chronic alcoholism, terminal conditions due to cancer, serum albumin level measurement of <3 g/dL, and pregnancy or breastfeeding, as well as chemotherapy for cancer treatment.

After the exclusion, 817 patients were enrolled, all of whom were over 18 years old and none was taken to the intensive care unit (ICU). Demographic data, comorbidities, COVID-19-related examinations, respiratory rate, oxygen saturation by pulse oximetry (SpO_2), and mean oxygen requirement during hospitalization were recorded. Our hospital has accredited laboratories standardized for internal and external quality assurance measures to monitor the precision and accuracy of the performed tests. In all cases, blood samples of fasting were obtained from the peripheral vein within 24 hours of hospitalization. Data were categorized as moderate or severe according to the severity classification of the "Chinese Guidelines for Diagnosis and Treatment of Novel Coronavirus Pneumonia (Trial Version 7)" (16). Patients with moderate COVID-19 had a fever (>37.3 °C) and respiratory symptoms identified with radiological findings for pneumonia. COVID-19 cases were considered severe with any of the following criteria: "(1) respiratory distress (≥30 breaths/min), (2) oxygen saturation of ≤93% at rest, (3) arterial partial pressure of oxygen/fraction of inspired oxygen of ≤300 mmHg (l mmHg=0.133 kPa)." All patients were scanned with spiral CT on admission. Radiologist-evaluated CT results were classified into three categories: mild, moderate, and severe involvement (17). All study participants were managed according to the "COVID 19 Treatment Protocol of Turkish Health Ministry" (18). The research was first registered in the data of the "Turkish Health Ministry Scientific Research Committee" and then reviewed and approved by the "Local Ethics Committee."

Serum Lipids Measurement

The dataset consisting of TC, high-density lipoprotein cholesterol (HDL-C)-, and LDL-C were analyzed using the modular dual-phase extraction system "(COBAS-C 501, Roche Diagnostics, Basel, Switzerland)."

Statistical Analysis

Mean ± standard deviation values were estimated as descriptive statistics. Deviations from normality were

assessed using the median and percentage values in the distributions. The Student's t-test was used for continuous variables with normal distributions. Categorical data were analyzed using the chi-square test. Continuous variables having abnormal distribution were evaluated by the Mann-Whitney U test. A p-value of <0.05 was accepted as statistically significant. Commercially available "Statistical Package for the Social Sciences software v.21 (Statistical Package for the Social Sciences Inc., Chicago, IL, USA)" was used for all the statistical analyses.

RESULTS

A total of 817 patients with COVID-19 pneumonia participated in this study, wherein 347 were moderate and 470 were severe. Baseline parameters and comorbidities showed no difference in sex. Patients with severe conditions were older than those with moderate ones. Hypertension and heart failure were more frequent (p=0.003, p<0.001, respectively) in patients in severe condition than those in moderate condition. SpO_2, in baseline and under oxygen support, was lower in severe than in the moderate group. The respiratory rate was higher in the severe group (Table 1). Lymphopenia was lower, whereas neutrophil and platelet counts were higher in the severe than in the moderate group. Glucose, urea, transaminases (alanine aminotransferase and aspartate transaminase), magnesium, and potassium levels were also higher in the severe than in the moderate group. In addition, patients in severe conditions were linked with stronger inflammatory responses, which yielded poor prognostic laboratory findings, such as lactate dehydrogenase, C-reactive protein, troponin I, D-dimer, fibrinogen, and ferritin. Albumin and calcium were lower in patients with severe conditions; however, no difference was found in the procalcitonin between groups. CT revealed a severe involvement in the severe than the moderate group. Thyroid-stimulating hormone and free triiodothyronine values were lower in the severe than in the moderate group, within the reference range. No difference was found in the free thyroxine value between groups. The hospitalization duration was higher in the severe than in the moderate group. TC and LDL-C levels were lower in the severe group than in the moderate group (p=0.02, p=0.03, respectively). A total of 48 patients who died from respiratory failure were all from the severe group. The mortality rate was 10.2% in the severe group (Table 2). TC and LDL-C have a negative correlation with the hospitalization duration (r=-0.163, p=0.02; r=-0.154, p=0.03, respectively) (Figure 1, 2).

DISCUSSION

Lipid profiles of patients hospitalized with COVID-19 pneumonia were retrospectively analyzed based on the clinical laboratory reports of lipid measurements on a large patient population.

Previous studies showed that patients with community-acquired pneumonia had a higher risk of death with reduced LDL-C levels (19). Thus, the risk of sepsis for patients who are hospitalized also increased (20,21). One particular study showed that reduced levels of LDL-C were followed by a higher likelihood of future sepsis, which implied a direct influence of LDL-C on sepsis risk (22).

Table 1. Evaluation of baseline characteristics and comorbidities for patients with moderate and severe conditions

	Moderate (n=347)	Severe (n=470)	p
Age, years	53.89±16.34	59.38±14.84	<0.001
Female, n (%)	140 (40.34%)	169 (35.95%)	NS
Respiratory rate, per minute	16.39±2.52	23.5±4.37	<0.001
SpO_2¶	95.13±1.47	93.91±2.1	<0.001
Body temperature, oC	36.93±0.68	37±0.74	NS
Heart rate, per minute	82.34±13.54	83.05±15.39	NS
Systolic blood pressure, mmHg	123.63±17.87	124.69±16.8	NS
Diastolic blood pressure, mmHg	70.25±10.18	70.29±10.48	NS
Arterial hypertension on treatment	90 (25.9%)	159 (33.8%)	0.03
Heart failure	6 (1.7%)	15 (3.2%)	<0.001
COPD¶¶	10 (2.9%)	20 (4.2%)	NS
Asthma bronchiale	27 (7.8%)	43 (9.1%)	NS

¶SPO_2: Median; under oxygen support, ¶¶ COPD: Chronic obstructive pulmonary disease

Table 2. Laboratory test evaluation, CT results, and mortality of patients with moderate and severe conditions

Characteristics count	Moderate (n=347)	Severe (n=470)	p
Neutrophil, cells/mL	4.24±2.34	6.05±2.93	<0.001
Lymphocytes, cells/mL	1.34±0.6	1.06±0.51	<0.001
Platelets, cells/mL	237.31±96.19	251.7±112.57	0.05
Hematocrit, %	38.07±4.55	38.25±4.39	NS
Glucose, mg/dL	121.66±34.45	134.8±48.32	<0.001
Urea, mg/dL	30.5±13.65	37.88±18	<0.001
Creatinine, mg/dL	0.79±0.24	0.84±0.55	NS
ALT, U/L	39.28±32.7	51.17±45.74	<0.001
AST, U/L	39.04±24.49	49.33±34.05	<0.001
Lactate dehydrogenase, U/L	298.99±103.21	415.06±180.9	<0.001
Potassium, mEq/L	4.15±0.42	4.21±0.51	0.05
Sodium, mEq/L	138.05±3.49	137.36±3.71	0.007
Magnesium, mg/dL	2.05±0.24	2.09±0.28	0.04
Calcium, mg/dL	8.87±0.57	8.59±0.55	<0.001
C-reactive protein, mg/L	71.04±62.95	121.66±80.72	<0.001
Procalcitonin, ng/mL	0.2±0.81	0.43±3.18	NS
Ferritin, mcg/L	377.09±399.98	668.43±664.67	<0.001
D-dimer, mcg FEU/mL	0.63±1.05	0.96±1.23	<0.001
Fibrinogen, mg/dL	477.38±122.24	531.47±139.97	<0.001
INR	1.05±0.2	1.07±0.18	NS
Troponin I, ng/mL	7±17.88	21.66±73.38	<0.001
Albumin, g/dL	38±5.22	34.53±4.82	<0.001
Free T3 (pg/mL)	2.12±0.21	2.78±0.57	<0.001
Free T4 (ng/dL)	1.07±0.23	1.09±0.37	NS
TSH (μIU/mL)	1.03±2.28	1.53±2.46	<0.001
TC (mg/dL)	159.59±42.57	150.97±42.99	0.02
Triglyceride	145.83±75.65	144.22±77.3	NS
HDL-C (mg/dL)	33.97±9.84	33.25±11.59	NS
LDL-C (mg/dL)	96.36±34.37	89.72±35.1	0.03
CT results (n, %)			<0.001
Mild involvement	126 (36.3%)	42 (8.9%)	
Moderate involvement	187 (53.9%)	212 (45.1%)	
Severe involvement	34 (9.8%)	216 (46%)	
Mortality	0	48 (10.2%)	<0.001
Duration of hospitalization, day	7.95±3.59	12.84±6.98	<0.001

TC: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, ALT: Alanine transaminase, AST: Aspartate transaminase, INR: International normalized ratio, CT: Computed tomography, TSH: Thyroid-stimulating hormone

Contrarily, altered lipid profiles in patients arise from the inflammation due to viral infection. For instance, patients with HIV have reduced HDL-C and increased LDL-C levels (23,24). Patients with dengue fever disease have reduced LDL-C serum (25), and patients with cirrhotic hepatitis B also have reduced HDL-C and LDL-C levels (26).

Lipid metabolism changes 12 years after recovery in patients infected with SARS (27), thus dyslipidemia is possible to develop in patients with COVID-19-related diseases. However, several published investigations in the literature that examine the cholesterol levels with COVID-19 were reported.

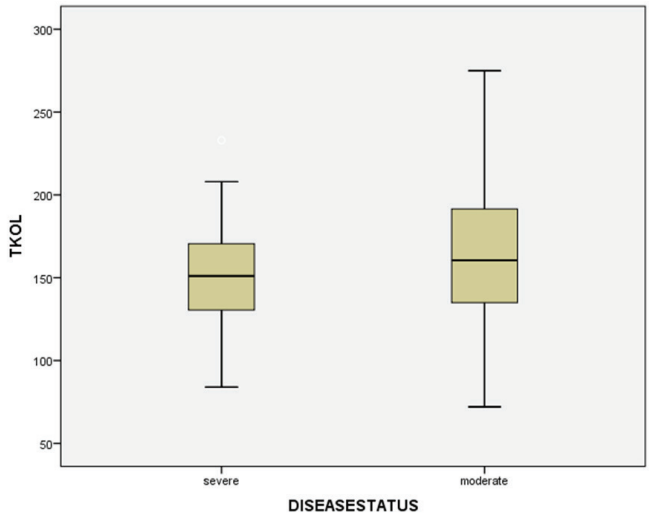

Figure 1: Patients in severe condition show significantly lower TC levels than those in moderate conditions (150.97±42.99 vs. 159.59±42.57 mg/dL, p=0.02)
TC: Total cholesterol

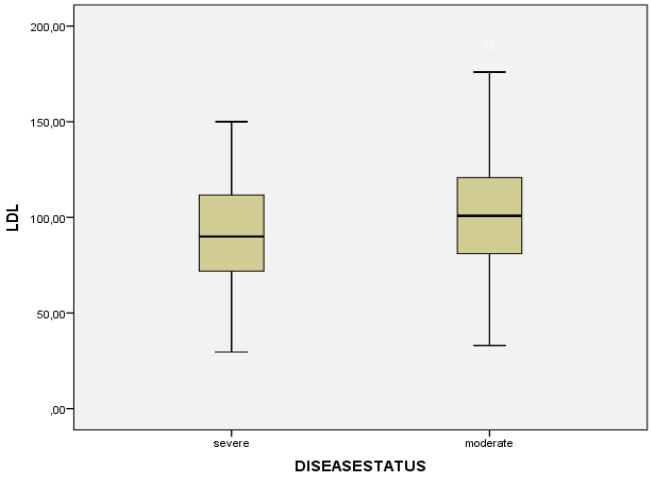

Figure 2. Patients in severe condition show a significantly lower LDL-C level than those in moderate condition (89.72±35.1 vs. 96.36±34.37 mg/dL, p=0.03)
LDL: Low-density lipoprotein

Generally, published studies reported a reduced level of TC, triglyceride, HDL-C, and LDL-C (11). The severity level in both hypolipidemia and COVID-19 showed a positive correlation (12). In addition, severe consequences emerge more likely in reduced levels of HDL-C in COVID-19 (13). Contrarily, ApoCOVID study showed that HDL-C and LDL-C concentrations upon ICU admission are low in patients with severe COVID-19 pneumonia but are not associated with poor outcomes. However, low lipoprotein concentrations in the case of bacterial superinfection during ICU hospitalization are associated with mortality, which reinforces the potential role of these particles during bacterial sepsis (14). Another recent study demonstrated that LDL-C levels declined perpetually until death in patients hospitalized for COVID-19, though the cohort had a small sample size, which makes one think that LDL-C could potentially predict the disease progress and poor prognosis (15).

Various plausible mechanisms are responsible for dyslipidemia. COVID-19 gave possible harm to liver functions thereby reducing the LDL-C biosynthesis. An acute severe inflammation triggered by COVID-19 changes lipid metabolism or vascular permeability, which in turn leads to the entrance of cholesterol molecules into the alveoli forming exudate and causing a deficit in the plasma LDL-C and cholesterol (28). Another possibility is that the COVID-19-activated sterol regulatory element-binding protein-2 disturbs the cholesterol biosynthesis, which usually ends up with a cytokine storm (29).

As lipopolysaccharide creates a reaction with systemic viral infection and/or entrance of a toxic substance, the cholesterol counters the emerging toxic disturbance under selective interaction.

Moreover, LDL-C levels are assumed to be linked to the interplay between dyslipidemia and vasculopathy during a pathological process in patients with COVID-19 (30).

Our results showed that reduced levels of TC and LDL-C were linked with the severity of patients hospitalized with COVID-19 pneumonia. Reduced concentrations of TC and LDL-C most likely resulted from complicated biological and pathological processes caused by COVID-19. Obtained data suggested a decreased LDL-C concentration in patients with COVID-19 is related to disease severity.

Study Limitations

Several exclusion criteria were used to reduce the confounding effect; however, our findings, showing a correlation between the inferior LDL-C concentrations and poor outcomes of patients hospitalized with COVID-19 pneumonia, had its limitations. The baseline cholesterol levels before the start of the infection were unavailable, which made the precise emergence of reduced LDL-C concentrations uncertain. In addition, the LDL-C level during the recuperation was unrecorded due to the retrospective nature of our design, which resulted in a more pronounced account of the link between the variability of LDL-C concentrations and COVID-19 outcomes.

CONCLUSION

The variability of LDL concentrations in the vascular system of patients with COVID-19 indicated a strong relationship with the disease severity. Several researchers already demonstrated the strong link between lipid metabolism and COVID-19 in a way that corroborates our observations.

Therefore, a foreseeable LDL as a marker for predicting COVID-19 severity is not fortuitous.

ETHICS

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Bakirköy Dr. Sadi Konuk Training and Research Hospital of Local Ethics Committee (no: 2021/127-2021-06-05 date: 15.03.2021).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: M.B., Concept: M.B., Design: M.B., Data Collection or Processing: M.B., I.K.A., Analysis or Interpretation: M.B., I.K.A., Literature Search: M.B., I.K.A., Writing: M.B.

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