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CONTENTS

Research Articles

Research Article

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Gamma knife radiosurgery for central arteriovenous malformations: a single-center experience

Santral yerleşimli arteriyovenöz malformasyonlar için gamma knife radyocerrahisi-tek merkez deneyimi

Burak Karaaslan

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Abstract

Purpose: Arteriovenous malformations with basal ganglia, brainstem, and thalamic locations represent complex vessel anomalies with critical areas. Treatment of these central lesions is related to high morbidity and mortality rates. This retrospective study aims to examine the results of our clinic's patients with basal ganglia, brainstem, and thalamic arteriovenous malformations remedied with gamma knife radiosurgery (GKS).

Material and method: The results of patients with basal ganglia, thalamus, and brainstem located AVM who underwent GKS from May 2005-December 2020 were analyzed in the Gazi University Gamma Knife Unit.

Results: A total of 859 patients with intracranial AVM were treated at the Gamma Knife Unit of the Gazi University Neurosurgery Clinic between May 2005-December 2020. Seventy-three patients with basal ganglia, brainstem, and thalamic locations were recorded. In a total of 73 patients who were included in the study, the lesion was located in the basal ganglia in 14 (19.2%), the brainstem in 13 (17.8%), and the thalamic region in 46 (63%) patients. The mean volume of AVMs was 4565.54 mm^3. The patients who underwent embolization before gamma knife surgery treatment were found to be 19.2%. The mean of AVMs' obliteration time after SRS is 38.4 months. We detected that there was no significant statistical rate relationship between obliteration rate and AVMs volume, Spetzler - Martin Grading Scale, nidus type, gender, and prior embolization (p>0.05).

Conclusions: GKS is one of the effective choice treatment methods used for cerebral AVMs today. Furthermore, it has been accepted as the first choice treatment method, especially in central AVMs, which are risky in terms of mortality and morbidity, and also with good outcomes.

Key words: Arteriovenous malformations, stereotactic radiosurgery, gamma knife radiosurgery, basal ganglia, thalamus.

Karaaslan B. Gamma knife radiosurgery for central arteriovenous malformations: a single-center experience. Pam Med J 2023;16:162-167.

Öz

Amaç: Bazal gangliyon, beyin sapı ve talamik yerleşimli arteriovenöz malformasyonlar (AVM) kritik bölgede yerleşmiş kompleks damar anomalileridir. Bu santral yerleşimli lezyonların tedavisi yüksek morbidite ve mortalite riski taşır. Çalışmamızda bazal ganglion, beyin sapı ve talamik yerleşimli AVM nedeniyle kliniğimizde gamma knife radyocerrahi uygulanmış hastaların sonuçları retrospektif olarak incelenmesi amaçlandı.

Gereç ve yöntem: Mayıs 2005-Aralık 2020 tarihleri arasında Gazi Üniversitesi Gamma Knife Ünitesinde tedavi edilmiş bazal gangliyon, talamus ve beyin sapı yerleşimli AVM hastalarının sonuçları incelendi.

Bulgular: Mayıs 2005-Aralık 2020 tarihleri arasında Gazi Üniversitesi Gamma Knife Ünitesinde toplam 859 intrakraniyal AVM hastası tedavi edildi. Bazal ganglion, beyin sapı ve talamik lokalizasyonu olan 73 hasta çalışmaya dahil edildi. Çalışmamıza dahil edilen toplam 73 hastanın 14'ü (%19,2) bazal ganglionda, 13'ü (%17,8) beyin sapında ve 46'sı (%63) talamik bölgede yer alıyordu. Ortalama AVM hacmi 4565,54 mm^3 olarak hesaplandı. Gamma knife cerrahisi tedavisinden önce embolizasyon uygulanan hastaların oranının %19,2'idi. SRS sonrası AVM'lerin ortalama tam kapanma sürelerinin 38,4 aydı. AVM hacmi, Spetzler Martin derecelendirme ölçeği, nidus tipi, cinsiyet ve önceki embolizasyon ile kapanma oranı arasında istatistiksel olarak anlamlı ilişkisi saptanmadı (p>0,05).

Sonuç: GKS günümüzde serebral AVM tedavisinde kullanılan etkin bir tedavi yöntemidir. GKS özellikle tedavisi yüksek morbidite ve mortalite riski içeren santral yerleşimli AVM tedavisinde başarılı sonuçları ile öncelikli tercih edilen tedavi yöntemlerindendir.

Anahtar kelimeler: Arteriyovenöz malformasyonlar, stereotaktik radyocerrahi, gamma knife radyocerrahisi, bazal ganglionlar, talamus.

Karaaslan B. Santral yerleşimli arteriyovenöz malformasyonlar için gamma knife radyocerrahisi-tek merkez deneyimi. Pam Tıp Derg 2023;16:162-167.

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Introduction

The central location's arteriovenous malformations (AVMs) constitute 3-13% of all intracranial AVMs [1]. Due to the critical area of these AVMs, they must be treated with early intervention [2]. Due to the deep location of the basal ganglia, brainstem, and thalamic AVMs, as well as the narrow study corridors, surgical resections are related to relatively poor obliteration rates and higher persistent neurological morbidity rates [3]. For this reason, stereotactic radiosurgery (SRS) has become the preferred treatment method in this patient group [4]. This study aims to review our experiences with gamma knife radiosurgery (GKS) in treating of basal ganglia, thalamus, and brainstem arteriovenous malformations and to evaluate the results in these patients.

Materials and methods

Patient population

A total of 859 patients with intracranial AVM were treated at the Gamma Knife unit of the Gazi University Neurosurgery Clinic between May 2005-December 2020. The primary patient data included AVM and SRS treatment variables. In addition, patient variables consisted of age, gender, previous AVM bleeding criteria, prior embolization, AVM volume, and The Spetzler - Martin score and Gamma Knife data of each patient were calculated.

The patients were analyzed retrospectively, and 73 patients with basal ganglia, thalamus, and brainstem locations were seen. Patients were included with a follow-up of at least two years in the study.

Radiosurgical procedure

Our SRS technique for AVMs was performed under local or monitored anesthesia, and the patient's calvarium was enclosed in a Leksell frame. SRS was performed using digital cerebral angiography (DSA) and Gamma Knife unit based on angiography of AVM nidus defined by contrast thin slice (1-2 mm) Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) when MRI was contraindicated.

Follow-up

Follow-up with radiological imaging was recommended at intervals of 6 months for clinical evaluation of the patients. Angiography was used to prove the obliteration of the AVM nidus. Control angiograms were planned to be two years after GKS treatment.

Statistical analysis

All factor categories were analyzed using the chi-square and T-test. Statistical analysis was performed using the SPSS 2020 software. A *P*-value of ≤0.05 was considered significant.

Permission was obtained from Gazi University Clinical Research Ethics Committee for the study.

Results

In a total of 73 patients included in our study, the lesion was located in the basal ganglia in 14 (19.2%), the brainstem in 13 (17.8%), and the thalamic region in 46 (63%) patients. The mean age of the patients was 25.14 years. Thirty-seven patients (50.7%) were male, and thirty-six patients (49.3%) were female. There was no noticeable gender difference in terms of AVMs locations (Table 1).

The mean SRS margin doses were 20.2, and the maximum was 39 Gy. The mean number of isocenters was 8.95. The mean volume of AVMs in the basal ganglia was 3885 mm^3, the mean volume of brainstem AVMs was 12300 mm^3, and the volume of thalamic AVMs was 14800 mm^3 (Table 2). According to the Spetzler - Martin grading scale, grade 1 AVMs and grade 5 AVMs ratios were equal to 2.7% (2/73) (Figure 1). Diffuse nidus AVMs were observed more

Table 1. Anatomical locations of 73 patients with central AVMs

Location	Patient (n) – Valid Percent (%)
Basal Ganglion	14-19.2
Brainstem	13-17.8
Thalamic	46-63.0
TOTAL	73-100

frequently in brainstem-located AVMs (23.1% (3/13)), while compact nidus was observed more frequently in basal ganglia-located AVMs (92.9% (13/14)). The patients who underwent embolization before SRS were found to be 19.2%. And, the basal ganglia, brainstem, and thalamus AVMs were found to be 23.6% (4/14), 23.1% (3/13), and 15.2%, respectively (7/46) (Table 3).

In general, the total rate of minor and major complications was 21.9% (16/73), while the highest rate of complications according to the location was found to be in the brain stem at a rate of 28.5% (4/14) (Figure 2).

The most common clinical presentation was a headache at 37% (27/73) in all locations, followed by bleeding at 17.8% (13/46). In follow-up, bleeding was seen at 7.1% in basal ganglia AVMs and 2.2% in thalamic AVMs after gamma knife surgery, while no bleeding was observed in the brainstem during follow-up.

The mean of AVMs' obliteration time after SRS is 38.4 months. There was no significant difference between the obliteration after SRS to the location. Obliteration rates were detected at 64.3% (9/14) in basal ganglia AVMs, 61.5% (8/13) in brainstem AVMs, and 60.9% (18/46) in thalamic AVMs. AVMs were followed without complications and did not obliterate during

Table 2. Age (year), Follow-up (month), Prescription dose, and AVM Volumes (mm^3) tables of patients with central AVMs according to locations

Location		Minimum	Maximum	Mean
BASAL GANGLION	Age	12	36	23.36
	Follow-up	24	71	37.21
	Prescription dose	14	25	21.00
	AVM Volume	16	11170	3885.00
BRAIN STEM	Age	8	50	32.54
	Follow-up	24	77	48.08
	Prescription dose	14	22	17.00
	AVM Volume	184	12300	2680.08
THALAMIC	Age	7	66	23.59
	Follow-up	23	112	37.72
	Prescription dose	16	24	19.52
	AVM Volume	538	14800	5321.96

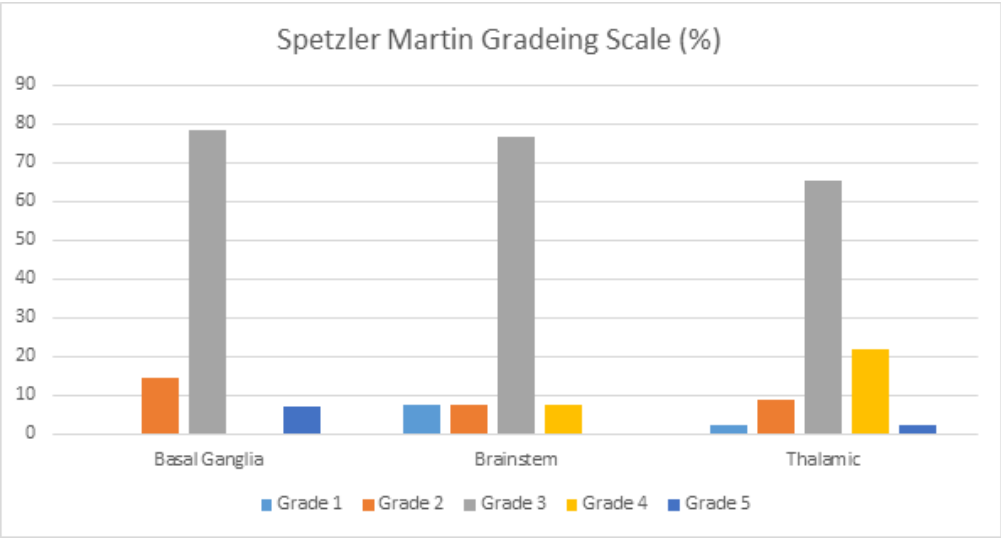

Figure 1. Distribution of central AVMs according to the Spetzler Martin Grading Scale

Table 3. Prior embolization rates of Central AVMs

Location	Embolizatıon	Patient (n) –Valid percent (%)
BASAL GANGLION	+	9-64.3
	-	5-35.7
	Total	**14-100.0**
BRAIN STEM	+	8-61.5
	-	5-38.5
	Total	**13-100.0**
THALAMIC	+	28-60.9
	-	18-39.1
	Total	**46-100.0**

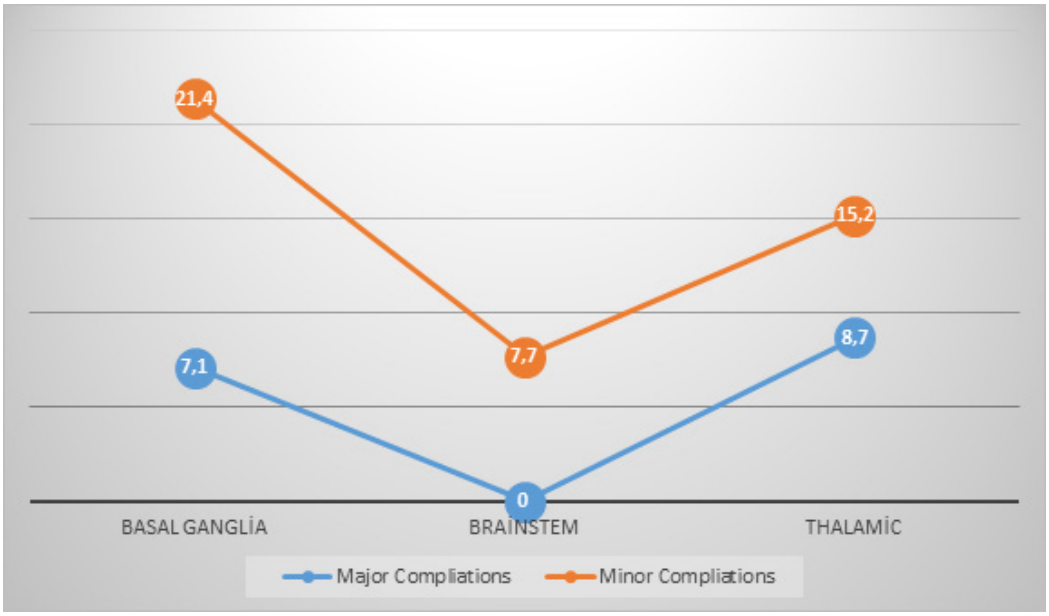

Figure 2. The prevalence value percents of major and minor complications after SRS

follow-up and were seen at 2.7% (2/73). Additionally, our study detected that there was no significant statistical rate relationship between obliteration rate and AVMs volume, Spetzler - Martin grading scale, nidus type, gender, and prior embolization (p>0.05).

Discussion

Cerebral arteriovenous malformations of the central locations denote vascular anomalies that are difficult to manage and carry significant lifelong risks for patients. In addition, they created a very high-risk subgroup with intracranial AVMs due to the very high annual bleeding risks related to increased mortality and morbidity rates.

Central AVMs constitute about 3-13% of all intracranial AVMs [5]. Nicolato et al. [6] stated that the bleeding rate was 51% in patients with cortical AVMs and said that it increased up to 91% in patients with central AVMs. Even more, Sasaki et al. [7] found that the annual risk of bleeding was higher in patients with central AVMs. A large proportion of patients with central AVMs, such as 86-91%, presented with bleeding [7]. This high incidence of bleeding in central AVMs can be explained by the high perfusion pressure-venous pressure and the higher incidence of related vascular aneurysms in this location [8].

Surgical excision is the complete resection of AVMs by craniotomy, a definitive treatment method [9]. However, both associated with

a greater risk of bleeding associated with conservative treatment the higher the risk of complications as a result of both resection, SRS for brainstem, basal ganglia, and thalamic AVMs has emerged as a therapeutic option, and after one session of 40-86% obliteration rates was provided [2, 10]. Gross et al. [3] detected resection results for the thalamus and basal ganglia AVMs. Overall, they recorded a complete resection rate of 91% and a mortality rate of 2.4% (the deficit rate between 13% and 33% among the reports) [3, 11].

Embolization can be used only with resection or SRS to treat AVMs because embolization can destroy many AVMs and also relate to significant morbidity [12, 13]. For example, persistent morbidity rates after embolization in basal ganglia, brainstem, and thalamic AVMs vary from 11% to 40% [13, 14].

Central AVM obliteration rates of 4 or 5 years were 66% and 86%, and permanent morbidity rates changed between 4% and 17% [13-15]. Pollock et al. [16] reported 10 cases in the basal ganglia, 30 in the thalamus, and 16 in the brainstem. Complete obliteration was reported in 24 (43%) of 56 patients after SRS at an average of 45 months. Koga et al. [15] reported total obliteration rate of thalamic AVMs for five years after SRS treatment was 82%. Sasaki et al. [7] found high morbidity and mortality rates in basal ganglia and thalamic AVMs patients who underwent conservative or only embolization compared to radiosurgery.

The first goal of the SRS is the destruction of the nidus [17]. Many studies have suggested that a higher margin dose increases the obliteration rate [9, 15, 18]. In the current series, it has been noted that in patients who had previous bleeding and had a smaller nidus volume, total obliteration occurred with a high margin dose [18]. But along with this, Choi et al. [19] found that the 20 higher marginal doses (≥20 Gy) were not related to obliteration, as in our study. In addition, it has been said that hormonal differences might contribute to the different hypersensitivity of AVMs to SRS in male and female patients, unlike our study. Some studies have recorded the relationship between progesterone and vascular endothelial growth factor [2, 6]. However, our series observed no practical difference between the genders of AVMs obliteration. Different from our results, fewer obliteration rates after SRS has been reported in bigger AVMs and Spetzler - Martin grade ≥4 AVMs [20]. Again, it has been reported that the incidence of SRS complications is higher in larger AVMs [21]. In brief, although there is no significant relationship between our series data, it has been reported that the dose, the number of isocenters, the previous bleeding, and the compactness of the nidus can be considered a prognostic factor of clinical outcomes for AVMs after SRS.

In conclusion, obliteration rates are poor in the central AVMs. In contrast, the incidence of mortality and morbidity is higher. We believe that SRS has a critical role in AVMs of basal ganglia, thalamic, and brainstem lesions, which are considered to involve extreme risk for surgical removal. The best choice for SRS are patients with a central location and a smaller volume AVM suitable for receiving at least 20 Gy at the margin.

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

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Research Article

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Association between 20 serum mirnas and clinicopathological variables in patients with breast cancer

Meme kanserli hastalarda 20 serum mirna'nın klinikopatolojik değişkenler ile ilişkisi

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Abstract

Purpose: Determining microRNAs in breast cancer pathogenesis suggests that it may be beneficial for diagnosis and treatment.

Materials and methods: Patients serum were collected and microRNAs were isolated. Then microRNAs were converted to cDNA. After that, investigated serum levels of 20 microRNAs (*miR-17, miR-21*, *miR-34a*, *miR-105*, *miR-133a, miR-139-5p*, *miR-141*, *miR-143*, *miR-145*, *miR-155*, *miR-200a*, *miR-200b*, *miR-200c*, *miR-203*, *miR-210*, *miR-299-5p*, *miR-365*, *miR-375*, *miR-411*, *miR-452*) in 39 patients with invasive breast cancer were analyzed before and after treatment.

Results: In the analysis results, it is detected that serum levels of *miR-200c* (p=0.030), *miR-375* (p=0.045), *miR-34a* (p=0.042) were markedly higher in the local advanced/metastatic group. *MiR-141* (p=0.062) levels were lower in patients with positive lymph node involvement, whereas *miR-133a* (p=0.037) levels were higher in the same patient group. *miR-105* (p=0.015), *miR-203* (p=0.015), *miR-375* (p=0.033), *miR-145* (p=0.025) serum levels were markedly higher in the progesterone receptor negative group, likewise *miR-105* (p=0.053) levels were high in the estrogen receptor negative group. The high levels of *miR-375* and *miR-133a* were noticeable in human epidermal growth factor receptor-2 positive patients (p=0.037 and p=0.014, respectively). *MiR-143* (p=0.009) and *miR-145* (p=0.017) levels were observed higher in the patient with a ki-67 index >20% (p=0.007 and p=0.015, respectively). It was found that 2 miRNAs (*miR-133a* (p=0.018) and *miR-139-5p* (p=0.004)) were markedly higher in patients in the luminal B group, which were separated by molecular subgroups. Nine of miRNAs that evaluated (*miR-21* (p=0.001), *miR-34a* (p=0.0001), *miR-105* (p=0.0001), *miR-141* (p=0.041), *miR-200a* (p=0.003), *miR-200b* (p=0.0001), *miR-200c* (p=0.0001), *miR-203* (p=0.0001), *miR-452* (p=0.018)) significantly increased and 5 of the miRNAs (*miR-145* (p=0.0001), *miR-365* (p=0.0001), *miR-155* (p=0.0001), *miR-143* (p=0.0001), *miR-299-5p* (p=0.0001)) were significantly reduced post-treatment.

Conclusion: We think that miRNAs may help in evaluating the follow-up and prognosis of invasive breast cancer.

Key words: Invasive breast cancer, circulating miRNA, molecular subtypes, clinical and pathological variables.

Gokdeniz Yildirim A, Demiray A, Koc AC, Senol H, Yaren A. Associatıon between 20 serum mirnas and clinicopathological variables in patients with breast cancer. Pam Med J 2023;16:168-178.

Öz

Amaç: MiRNA'ların meme kanseri patogenezinde rol oynadığının belirlenmesi, meme kanserinin tanı ve tedavisinde yararlı olabileceğini düşündürmektedir.

Gereç ve yöntem: Toplanan 39 invaziv meme kanserli hasta serumundan mikroRNA'lar izole edildi ve cDNA'lara dönüştürüldü. Hastaların tanı anında ve tedavi sonrasında alınan kanlarından, 20 miRNA'nın (miR-105, miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-210, miR-375, miR-34a, miR-133a, miR-155, miR-139-5p, miR-143, miR-145, miR-365, miR-299-5p, miR-411, miR-452 ve miR-17) serum düzeyleri analiz edildi.

Bulgular: Analiz sonuçlarında, miR-200c (p=0,030), miR-375 (p=0,045), miR-34a'nın (p=0,042) serum düzeyleri lokal ileri/metastatik grupta anlamlı olarak yüksek saptandı. miR-141'in (p=0,062) serum seviyesi lenf nodu tutulumu pozitif hastalarda daha düşük gözlenirken, miR-133a (p=0,037) seviyelerinin aynı hasta grubunda daha yüksek olduğu tespit edildi. MiR-105 (p=0,015), miR-203 (p=0,015), miR-375 (p=0,033), miR-145 (p=0,025) serum seviyelerinin PR negatif grupta belirgin yüksek olduğu, aynı şekilde miR-105 (p=0,053) seviyelerinin ER negatif grupta yüksek olduğu görüldü. Her 2 pozitif hastalarda miR-375 ve miR-133a seviyelerinin yüksekliği dikkat çekti (p=0,037 ve p=0,014, sırasıyla). MiR-143 (p=0,009) ve miR-145 (p=0,017) seviyelerinin ki-67 indeksi >%20 olan hasta grubunda daha yüksek olduğu ve bu miRNA'ların ki-67 indeksi ile korelasyon gösterdiği gözlendi (p=0,007; p=0,015, sırasıyla). Moleküler alt gruplara göre ayrılan hastalardan luminal B grubunda olanlarda 2 miRNA'nın (miRNA-133a (p=0,018) ve miRNA-139-5p (p=0,004)) anlamlı olarak daha

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yüksek olduğu saptandı. Çalışılan miRNA'lardan 9 tanesinin (miRNA-105 (p=0,0001), miRNA-21 (p=0,001), miRNA-141 (p=0,041), miRNA-200a (p=0,003), miRNA-200b (p=0,0001), miRNA-200c (p=0,0001), miRNA-203 (p=0,0001), miRNA-34a (p=0,0001), miRNA-452 (p=0,018)) tedavi sonrasında anlamlı olarak arttığı, 5 tanesinin (miRNA-155 (p=0,0001), miRNA-143 (p=0,0001), miRNA-145 (p=0,0001), miRNA-365 (p=0,0001), miRNA-299-5p (p=0,0001)) tedavi sonrasında anlamlı olarak azaldığı görüldü.
Sonuç: Sonuçlarımızın, invaziv meme kanserinin takibi ve prognozunu değerlendirmede yol gösterici olabileceğini düşünmekteyiz.

Anahtar kelimeler: İnvaziv meme kanseri, dolaşan miRNA, moleküler subtipler, klinik ve patolojik değişkenler.

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Introduction

The most common cancer among women in the world is breast cancer(BC). According to Globocan data, it constitutes 15% of cancer-related deaths in women in 2018 and 2 million 88 thousand new cases have been reported. Unfortunately, despite the increased multimodal treatment options, cure has not been achieved yet. Although most of the cases are diagnosed at an early stage, the risk of recurrence or metastasis is still high. The clinicopathological data such as age, menopause status, tumor size, lymph node involvement(LNI), Ki-67, hormone receptor status and cerBB2/*Her2* (Human epidermal growth factor receptor 2) status and genetic markers have a great influence on determining the prognosis. In recent years, miRNAs have been included as well as many studies on the biological features of BC, the earlier diagnosing of patients and the treatment choices based on the molecular characteristics of the patients.

MicroRNAs(miRs) are RNA regulators that control gene expression at the 20-21 nucleotide length post-transcriptional level and are not encoded. They pair with messenger RNAs (mRNAs) of protein-coding genes, leading to translational inhibition and degradation of mRNA. In recent years, more than 50% of miRs have been shown to be located in cancer-related genomic areas or regions that are easily broken [1]. Moreover, miRs have been reported to play a significant role in the development, differentiation, proliferation, invasion and metastasis biology of various cancer cells [2]. As the role of miRs in BC pathogenesis is clarified by various studies, it is suggested that BC can be new biomarkers to guide clinicians in evaluating the diagnosis, prognosis, and treatment response. It has been shown that miRNA expressions differ between normal and neoplastic breast tissue, and these are associated with tumor size, proliferation index, hormone receptor status and cerBB2 expression, invasion and metastasis invasion [3]. Another role of miRs in tumor biology is that it is effective in the regulation of tumor suppressor genes and oncogenes. Tumor suppressor miRNAs (Ts miR) inhibit the expression of oncogene miRs, while oncogenes (oncomiRs) are responsible for inhibiting the expression of Ts miRs leading to tumor formation [4]. The most interesting feature of miRs is that a single miR can target hundreds of mRNAs, which leads to disruption of expression of many mRNAs and proteins. These act as oncomiR or Ts miR [5]. However, many miRs that predict the treatment response in BC and affect survival have been identified [6].

The fact that the detection of miRs circulating in cancer patients is technically easily applicable and can be used as a new biomarker creates a field of study in this regard. In this study, we aimed to appreciate the serum levels of 20 most frequently studied miRs that are important in patients with just diagnosed BC, before and after treatment. We compared the measured miRs with the patients' clinicopathological features and then we examined their changes with treatment.

Materials and methods

Patients

Thirty-nine serial patients with BC who have been diagnosed invasive ductal carcinoma histologically and started to treat at the Department of Medical Oncology, Pamukkale University, in Turkey, were included in our study. The clinicopathological variables such as age, menopausal status, hormone receptor (estrogen receptor (ER) and progesterone receptor (PR)) and c-erb B2 status, lymph

node involvement, histologic grade, tumor size, staging and types of treatment were enrolled by analyzing all the medical reports. Patients who have inflammatory carcinoma, age <20 or >80 years, and second tumors were excluded. This study has been approved by the local Ethics Committee of Pamukkale University and all patients were informed about the procedure and written consent was obtained. The sign consents of all participants were taken in accordance with the Helsinki Declaration.

Total miRNA isolation

Serum was obtained by centrifuging the blood taken from patients who applied to Pamukkale University Medical Faculty Medical Oncology Department with the ethical committee dated 20.02.2018 and numbered 04 at 4000 rpm for 5 minutes. Serum samples obtained were obtained using the Qiagen miRNeasy Serum / Plasma Kit (qiagen cat: 217184 Hombrechtion, Switzerland). The miRNAs obtained were stocked at -80°C.

miRNA cDNA synthesis

Poly (A) Polymerase Tailing kit (Cat. No: 903 Richmond, Canada) is used to synthezise cDNA with the abm miRNA cDNA Synthesis. The experiment was continued in according to the protocol of this kit. Approximately 75ng was acquired from the total miR to be obtained. 2 µL of 5X Poly (A) Polymerase Reaction Buffer, 1.5 µL of ATP, 1 µL of MnCl2, 0.5 µL of Poly (A) Polymerase were added to 10 µL of RNase-free water. It was incubated at 37ºC for 30 minutes. After standing on ice for a while, 2 µL miRNA Oligo (dT) adapter was added and incubated for 5 minutes at 65ºC. Briefly, it was incubated for 15 minutes at 42ºC and 10 minutes at 70ºC by adding 1 µL of dNTP, 4 µL of 5X RT Buffer, 1 µL of EasyScript RTase and 2 µL of RNase-free water. The cDNAs obtained were stocked at -80°C.

Real-time pcr (qRT-PCR)

The Rotor-Gene 6000 (Corbett Life Science, Australia) device was used to determine the expression levels of mİRs which all its primers were from abm (Richmond, Canada). qRT-PCR was performed using miRNA qPCR MasterMix (abm, Richmond, Canada). Reaction conditions; 5 µL cDNA, 10 µL miRNA Mastermix, 0.5 µL miRNA primer, 0.5 µL Universal primer were performed as 4 µL dH2O. PCR conditions; 10 cycles of 1 minute at 95ºC, 10 seconds at 95, C/15 seconds at 58ºC/5 seconds at 72ºC], and the melting curve analysis at the accuracy of 0.1ºC between 55ºC and 90ºC. For normalization, normal breast cell line and miR-39 miRNA were used. Real-Time PCR analyses were obtained by calculating the number of copies with the standard curve. Calculated copy numbers were converted into numerical data suitable for analysis by 2 ^^ CT method.

Statistical analysis

We analyzed pre-and-post chemotherapy changes in the plasma levels of twenty BCassociated miRs (*miR105*, *-21, -141, -200 (a,b,c), -203, -210, -375, -34a, 133a, -155, -139-5p, -143, -145, -365, -299-5p, -411, -452, -17*) and clinicopathological parameters by using the chi-square test, Mann-Whitney test and Kruskall-Wallis H test. Spearman's test was used to correlate analysis. We compared the plasma levels of twenty miRs between the preand postchemotherapy samples of each patient by using Wilcoxon signedranks test. As a conclusion, we had two-sided tests and all differences we analyzed were considered non-significant when *P* values were greater than 0.05. The statistical analysis was performed using the SPSS 17.0 software package, version (SPSS Inc. Chicago IL).

Results

Thirty-nine patients with BC were evaluated. Eighteen (46.2%) were early stage (I, IIA, and IIB), 21 (53.8%) were local advanced (IIIA, IIIB, IIIC) and metastatic (IV). The median age at disease onset was 51 years (range: 29-79 years). Twenty patients (51.3%) had premenopausal status. Seventeen (43.6%) patients had a tumor size ≤2 cm, and 22 (56.4%) patients had a tumor size >2 cm. The median tumor size was 22mm (range 0.1-9 cm). Twenty-nine (74.4%) patients had lymph node involvement. Seventeen (43.6%) patients had higher Ki-67 levels than 20%. Twenty-two patients (56.9%) had Luminal A, 8 (20.5%) had Luminal B, 3 (7.7%) had *Her2* positive and 6 (15.4%) had triple negative disease. Patients' clinicopathological variables are shown in Table 1.

Table 1. Clinicopathological variables of patients

Clinicopathological variables	All Patients	
	N	%
Median age (range)	**51 (29-79)**	
Menopausal status		
Pre	20	51.3
Post	19	48.7
Tumor size (cm)		
Median (range)	2.2 (0.1-9)	
≤2	17	43.6
>2	22	56.4
Lymph Node involvement		
Positive	29	74.4
Negative	10	25.6
Ki-67 levels (%)		
≤20	22	56.4
>20	17	43.6
Stage		
Early (I, IIA, IIB)	18	46.2
Local advanced (IIIA, IIIB, IIIC)/metastatic (IV)	21	53.8
Molecular subtype		
Luminal A	22	56.4
Luminal B	8	20.5
Her2 positive	3	7.7
Triple negative	6	15.4

Plasma levels of miRNAs according to clinicopathological variables

We compared miR levels in patients with early (n=18) and local advanced/ metastatic (n=21) BC, and as a result of our research, we found that the plasma levels of *miR-200c* (p=0.030), *miR-375* (p=0.045) and *miR-34a* (p=0.042) were higher in local advanced/ metastatic group than in the early-stage patients. The patients with lymph node involvement had lower *miR-141* (p=0.062) and higher *miR-133a* (p=0.037) than lymph node negative patients. In ER negative patients, *miR-105* plasma levels were found higher (p=0.053) than positive patients. In addition, *miR-105* (p=0.015), *miR-203* (p=0.015), *miR-375* (p=0.033) and *miR-145* (p=0.025) levels were higher in patients with PR negative patients. In cerbB2 positive disease, *miR-375* and *miR-133a* levels were higher than cerbB2 negative disease (p=0.037 and p=0.014, respectively). According to Ki-67 levels, *miR-143* (p=0.009) and *miR-145* (p=0.017) levels were higher in patients with >20%. There were no relationships between miRs and age, menopausal status and tumor size.

We detected a correlation between Ki-67 and the levels of *miR-143* (r=+0.433, p=0.007), and also the levels of *miR-145* (r=+0.397, p=0.015). Besides, there was a strong correlation between the levels of *miR-21*0 and Ca 15-3 (r=+0.435, p=0.008). We have not seen any correlation among miR levels and other clinicopathological variables such as age, albumin, CRP.

Plasma levels of miRNAs according to molecular subtype

In Luminal B patients, *miR-133a* (p=0.018) and *miR-139-5p* (p=0.004) levels were higher than non-Luminal B patients. There was no

association between miRs' plasma levels and other molecular subtypes such as Luminal A, Her 2+ and triple negative subgroups.

Pre- and post-treatment plasma levels of miRNAs

We measured pre and post treatment samples of the plasma levels of miRs. After chemotherapy, the plasma levels of *miR-105* (p=0.0001), *miR-21* (p=0.001), *miR-141* (p=0.041), *miR-200a* (p=0.003), *miR-200b* (p=0.0001), *miR-200c* (p=0.0001), *miR-203* (p=0.0001), *miR-34a* (p=0.0001), *miR-452* (p=0.018) were increased, however the plasma levels of *miR-155* (p=0.0001), *miR-143* (p=0.0001), *miR-145* (p=0.0001), *miR-365* (p=0.0001), *miR-299-5p* (p=0.0001) were decreased. Before and after chemotherapy, miR changes are shown in Figure 1.

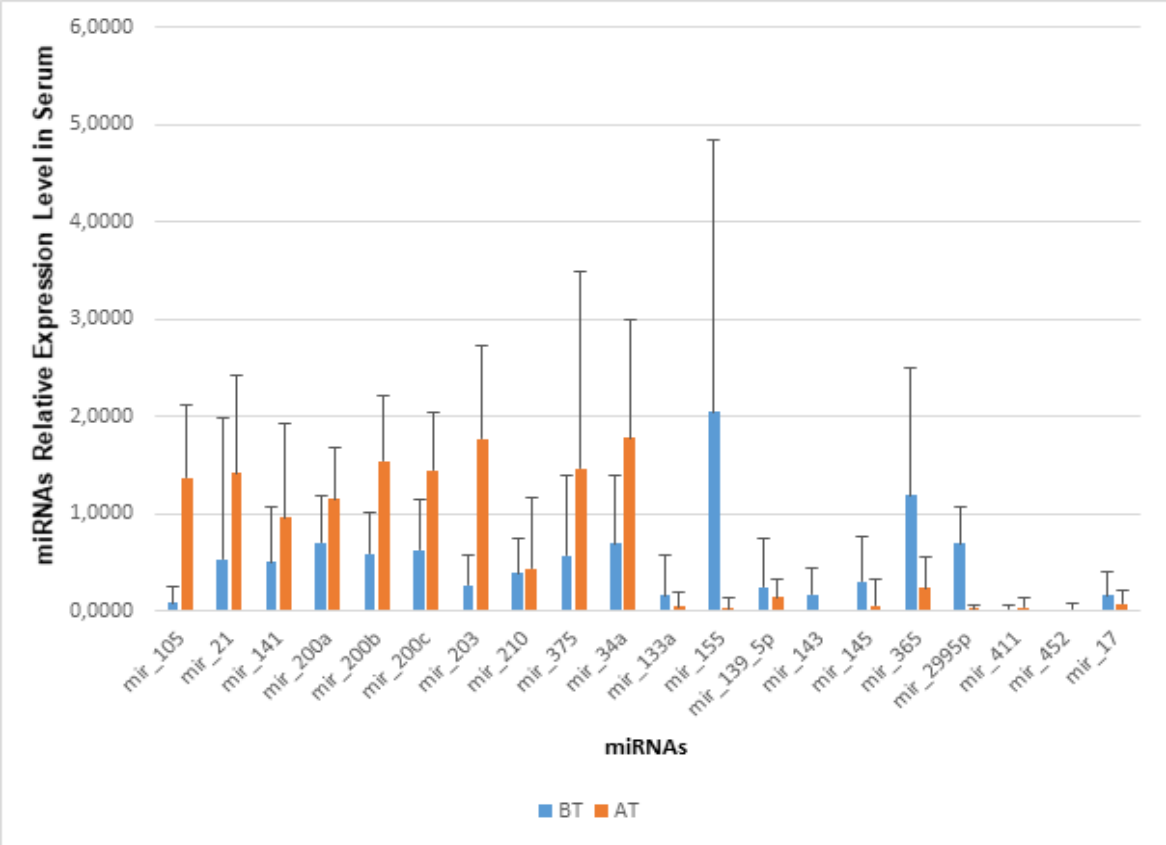

Figure 1. Graphical explanation of miRNA changes before and after treatment. (Before Treatment (BT) After Treatment (AT))

Discussion

In this study, we analyzed 20 miRs (*miR-17, miR-21*, *miR-34a*, *miR-105*, *miR-133a*, *miR-139-5p*, *miR-141*, *miR-143*, *miR-145*, *miR-155*, *miR-200a*, *miR-200b*, *miR-200c*, *miR-203*, *miR-210*, *miR-299-5p*, *miR-365*, *miR-375*, *miR-411*, *miR-452*) expression levels before and after treatment of patients with invasive BC. Serum expression levels of *miR-200c*, *-375, -34a* were markedly higher in the local advanced/metastatic group than in the early stage patients. However, *miR-141* plasma levels were lower in patients with positive lymph node involvement while *miR-133a* levels were higher in this analyze. When evaluated according to hormone receptor states, it was observed that *miR-105* was high in ER and PR negative patients, whereas *miR-203*, *-375, -145* were markedly higher in the PR negative group. Furthermore, *miR-375* and *miR-133a* levels were high in *Her2* positive patients. As the relationship of miRNA with other clinicopathological parameters was examined, we observed that *miR-143* and *miR-145* levels were higher in the group of patients with a Ki-67 index of >20% and these miRs correlated with the Ki-67 index. In patients divided into molecular subtypes, *miR-133a* and *miR-139-5p* levels in luminal B group were markedly higher

than in non-luminal B group. Of the 20 miRs studied before and after treatment, 9 (*miR-105*, *miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-34a, miR-452*) increased markedly after treatment, 5 (*miR-155*, *miR-143, miR-145, miR-365, miR-299-5p*) decreased significantly after treatment.

The most important factors affecting the prognosis of BC are tumor size, lymph node involvement, organ metastasis and molecular subtyping. Circulating miRs have a reliable marker for early detection of lymph node metastasis in invasive BC and staging of the disease has been supported by many studies. In a retrospective study examining miRs associated with distant organ metastasis, it has been shown that *miR-21, miR-184* and *miR-494* are upregulated in patients who develop metastasis and may be useful for future targeted treatments [7]. On the other hand, *miR-199a, miR-29c, miR-424* were found to be higher in patients with invasive BC at an early stage than healthy controls in the study of biomarkers that will facilitate the detection of invasive BC at an early stage [8]. In our study, plasma levels of *miR-375*, *-200c*, *-34a* were markedly higher in the group with local advanced/metastatic disease than in the early stage patients. Madhavan et al. [9] stated that *miR-200c* and *miR-375* levels showed markedly higher expression in BC patients with circulating tumor cells. *MiR-200c* has an important effect on the proliferation, transformation, migration and invasion of the cancer cell. It is also suggested that it regulates epithelial mesenchymal transformation, epidermal growth factor signaling, functions of cancer stem cells, and apoptosis via *p53*. In another study, *miR-200c* and *miR-141* are found higher in metastatic patients than those with localized disease, and it is suggested that *miR-200c* and *miR-141* are regulated by the FOXP3-KAT2B axis [10]. *miR-200c* and *miR-141* were stated that they will be a strong biomarker for determining metastasis for metastatic disease. In a study conducted by Roth et al [11], *miR-10b*, *miR-34* and *miR-155* are higher in advanced BC than early stage. In addition, *miR-200* family (a, b, c) and *miR-210* are higher in patients with metastasis and has been shown to affect survival [12]. However, BC cell cultures examined after *miR-375* inhibition show a decrease in cell proliferation [13]. When *miR-375, -200c, -34a* are evaluated together with other studies that obtained similar results with our study, it may be thought that these miRs may indicate poor prognosis.

Studies have reported that *miR-141*, associated with good prognosis, shows negative correlation with tumor size, lymph node metastasis, cerB-B2 expression levels, and Ki-67 levels. Also, *miR-141* overexpression in vitro has been shown to target ANP32E gene, inhibiting cell growth, proliferation and invasion [14]. In another study, decreased expressions of *miR-141*, *miR-200 family (a, b, c)* levels are detected in BC stem cells. It has also been shown that the *miR-200* family prevents epidermal mesenchymal transformation by suppressing *ZEB1* gene expression, and *miR-200c* also inhibits tumor formation in vivo [15]. However, many studies investigating the *miR-133a* effects on BC pathogenesis have shown the relationship between decreased *miR-133a* levels to advanced clinical stage, lymph node metastasis, and shorter recurrence survival [16, 17]. It has been observed that increased *miR-133a* levels show a Ts effect by decreasing the proliferation, migration and invasion by negative regulation of the *LASP1* gene in vitro [16]. Supporting the studies, in this study, it is predicted that *miR-141*, which is markedly low in patients with positive lymph node involvement, and *miR-133a*, which is found high, may have a positive effect on the course of the disease.

Based on the hormone receptor status, BCs are divided into four groups according to molecular classification: luminal A, luminal B, *Her2* (cerB-B2 positive) and triple negative. Although there is little information about these gene receptors yet, they provide significant benefits in the selection of treatment and in monitoring the response to treatment. For this reason, studies are continuing on miRs showing hormone receptor status and biomarker potential associated with molecular subtypes. In a study, 309 miRs have been identified in 93 breast tumors with different molecular subtypes. In this study, differential miR expression provides an accurate classification of basal and luminal subtypes, and it is shown that the 31 miRNA identified can differentiate different subtypes [18]. Similarly, in a study, the relation between estrogen receptor (ER) with *miR-342, miR-299, miR-217, miR-190, miR-135b, miR-218*; and between progesterone receptor (PR) with *miR-*

520g, miR-377, miR-527-518a, miR-520f-520c and *Her2* with *miR-520d*, *miR-181c, miR-302c, miR-376b, miR-30e* are identified by Lowery et al. [19] *miR-342* and miR-520g overexpression are further analyzed in 95 breast tumors and *miR-342* expression is found high in ER and *HER2* positive tumors and low in triple negative tumors. In a recent study, Piasecka et al. [20] have detected an increase *miR-10b*, *miR-21*, *miR-29, miR-9, miR-221/222, miR-373* and a decrease in *miR-145*, *miR-199a-5p, miR-200* family, *miR-203* and have had a prognostic value in triple negative BC. In this study, we found markedly higher levels of *miR-105* in the ER negative patient group and the levels of *miR-105*, *miR-145*, *miR-203* and *miR-375* were found high in PR negative patients. *miR-375* and *miR-133a* expression levels in *Her2* positive patients were significantly higher. Similar to our study, *miR-105* has been suggested to be upregulated in the plasma of ER/PR and *Her2* negative BC patients [21]. It is stated that *miR-105* activates wnt / p-catenin signaling with SFPRI down regulation and decreases survival by promoting metastasis. Unlike our study, Yu et al. [22] determined that *miR-203* levels increase in ER/PR positive patients compared to the control group and suggested that estradiol can control cell proliferation by regulating miR expression. Another example, the study of Han et al. [23], *miR-145* levels are found to be markedly higher in PR positive patients compared to PR negative patients.

It has been emphasized that, in studies investigating the effect of miRs to development of trastuzumab resistance, which is a monoclonal antibody agent developed against each receptor, the decrease in *miR-375* levels may be responsible for resistance. Studies continue to show that *miR-375* response to the treatment of trastuzumab by targeting insulin-like growth factor receptor 1 (IGF1R) [24]. Other miR study has been done on gastric cancer cells, and *miR-133a* has shown that it inhibits proliferation of stomach cancer cells by reducing ERBB2 expression [25]. Our data contains results that contradict the literature regarding the detected miRs based on the low number of studies performed on hormone receptor status and the number of patients included in our study. It is thought that there may be biomarkers that can be used in determining the subtype of miRNAs determined by the results of larger studies to be conducted in the future, and evaluating the response to anti-Her2 treatment.

However, in comparison with molecular subtypes, we found *miR-133a* and *miR-139-5p* miRs markedly higher in patients with luminal B subtype compared to non-luminal B subtype. It has been reported that *miR-133a* and members of the *miR-139-5p* family inhibit invasion and migration in breast cell culture [21]. In both studies, *miR-139-5p* has been shown that it induces apoptosis in BC cells, causes cell cycle arrest in the S phase, thereby inhibiting invasion and metastasis [26]. As a result, it is stated that *miR-139-5p* and *mir-133a* have significant functions in the development of tumorigenesis and BC and may take place in clinical applications.

Today, the fact that CA-15-3 (cancer antigen-15-3) and CEA (carcinoembryonic antigen), which are the biomarkers used for post-treatment follow-up in BC, are seen as valuable in terms of follow-up in long-lasting metastatic breast cancer patients, there is a need of new biomarkers because they can be false positive for 6-12 weeks due to associated drug-related cell death after treatment and their long half-life. As a result of the correlation analysis we conducted in this analyze, it has been shown that there is a strong correlation between *miR-21*0 and ca-15-3 levels. Although it has (been) shown that *miR-21*0 levels decrease in patients with postoperative BC due to the reduction of tumor burden, when meta-analyses showing the relationship between breast tumors with high *miR-21*0 levels and decreased survival are evaluated together, it can be thought to be used in follow-up and prognosis with a high sensitivity and specificity after treatment [6, 27]. In addition to our study, the correlation of another prognosis marker Ki-67 monitored remarkably high in proliferation index with *miR-143* and *miR-145*, and these two miR clusters in patients with a Ki-67 index of >20%. The correlation of *miR-143* and *miR-145* with the Ki-67 proliferation index are associated with poor prognosis, which have been shown to suppress breast proliferation and invasion of BC cells by inhibition of ERBB3 translation, suggest that existing miRs may not have been elucidated yet [28].

In the literature, there are few miR studies in invasive BC that vary depending on the treatment. We think that miRs expressed

especially in serum or plasma are very valuable in terms of guiding clinicians in the diagnosis and follow-up of the disease. In our study, it is detected that 9 of the 20 miRNAs we looked at in patient serum (*mir-21*, *mir-34a*, *mir-105*, *mir-141*, *mir-200a*, *mir-200b*, *mir-200c*, *mir-203*, *mir-452*) increased after treatment. Alike to this study which examines differences in between miRNAs before and after neoadjuvant chemotherapy in the plasma of 25 BC patients, it has noted that the levels of *mir-34a* increase after treatment and this miRNA is particularly high in 7 patients who partially responded to treatment [29]. This increase has been attributed to the release of *mir-34a* from liver tissues and treatment-related DNA damage due to hepatotoxicity caused by anthracycline-based therapies alongside tumor tissue. Considering that the anthracycline group chemotherapy used in the patient group in our study are influenced by *p53* activation, it can be thought that the increase of *miR-34a* after chemotherapy is realized through the *p53* activation mechanism through treatment-related DNA fractures.

Wang et al. [30] showed that cell motility and migration decreased by approximately 50% of *miR-203* in triple negative BC cells. Data have stated that *mir-203* inhibits proliferation and invasion and acted as a Ts by lowering the levels of BIRC5 and LASP1 proteins. However, it has been suggested that estradiol increases the migration and invasion ability of ER positive BC, which is accompanied by a decrease in *miR-203* levels [31]. Few studies on the relationship between *miR-452* and BC showed evidence that *mir-452* acts as a Ts. Less expression of *miR-452* in BC cells compared to healthy tissues is one of the evidence showing tumor suppressor miRNA properties. However, it has also been shown to suppress cell migration and invasion by targeting RAB11A in BC cells in which *miR-452* is transfected [32]. In our study, the increase in tumor suppressor-bearing miRNAs after treatment may be evidence that patients benefit from treatment, and this may be associated with good prognosis. With studies to support this hypothesis, *miR-141*, *-200a*, *-200b*, *-200c*, *-203* and *miR-452* can be useful as predictive and prognostic miRs in BC and can guide clinicians in follow-up to treatment.

Conversely, Zhou et al. [33] have compared MDA-MB-231 BC cells and MCF-10A healthy breast epithelium, the tight binding protein of *miR-105*, which is expressed and secreted by metastatic BC cells, is ZO-1 (zonula occludens-1) has been shown to be a powerful migration regulator by targeting. While overexpression of *miR-105* causes metastasis and vascular permeability in distant organs, these effects have been found to be reduced by inhibition of *miR-105* in metastatic tumors. For this reason, it has been reported that high *miR-105* levels are associated with the development of metastases and may have predictive and prognostic value for metastatic progression in early-stage BC. Another study has been noted that increased *miRNA-21* expression is markedly associated with poor survival of BC patients, and *miR-21* is involved as an oncomiR targeting Ts miR. It is also suggested to be an effective biomarker that controls uncontrolled cell proliferation, BC cell growth and metastasis caused by programmed cell death 4 (PDLD-4) and tropomyosin 1 (TPM-1). In the same study, an average of 3.2-fold reduction has been observed in *miR-21* levels after treatment [34]. In our study, during the time we followed up, although none of our patients had progression, but one, an unexpectedly significant increase was observed in the *miR-105* and *miR-21* levels, which were functioning as oncomiR, after the treatment. Considering that studies on *miR-105* and *miR-21* strongly correlate these two biomarkers with poor prognosis and tumor aggressiveness, it can be interpreted that patients' follow-up time is insufficient to evaluate progression.

In addition, as a result of our study, we detected 5 miRs (*miR-155*, *-365*, *-143*, *-145* and *miR-299-5p*) that decreased after treatment. Similarly, Sun et al. [35] have detected a decrease in serum *miR-155* levels in 79% of 29 patients diagnosed with breast cancer after 4 cycles of adjuvant chemotherapy and associated the reduction in *miR-155* levels with response to treatment and disease remission. The role of *miR-365* has not been clarified yet in BC and there are studies supporting that it is an oncomiR that increases cell proliferation and migration by targeting ADAMTS-1, an anti-angiogenic gene [36].

The tumor suppressor effect of *miR-143* and *miR-145* in BC is thought to be through the suppression of HER receptors [28]. *MiR-143* has also been shown to reduce proliferation

and migration in other types of cancer [37]. This anti-cancer effect is due to Bcl-2, MYO6, ELK1 and ERK5, which play a role in cell proliferation, apoptosis and migration. Cellular mitogens and stress-activated ERK5 targets proteins that regulate cell proliferation, such as the nuclear factor (NF)-kB, c-myc, and cyclin D1. Moreover, mitogen-activated protein acts on 3 kinase 7 (MAP3K7) or transforming growth factor (TGF-beta) -activated kinase-1. In a study conducted by Zhou et al. [38], *miR-143* is decreased in BC tissue, and p-ERK5, ERK5, p-MAP3K7 and MAP3K7 expressions are increased. It has been suggested that ERK5 and MAP3K7 are the targets of *miR-143*, since the expression of ERK5, p-MAP3K7, MAP3K7 and cyclin D1 are found to be reduced by miR143 upregulation. In addition, there are studies showing that *miR-145* induces apoptosis by activating *p53* and reduces estrogen receptor-α [39]. The role of *Mir-299-5p* in BC patients is not known much. Shevde et al. [40] have been reported that a decrease in *mir-299-5p* levels in BC tumor tissue may cause an increase in osteopontin, a glycoprotein associated with radiotherapy and chemotherapy resistance, invasion and metastasis. The roles of *miR-155*, *-365*, *-299-5p* in BC pathogenesis are not known clearly and *miR-143* and *miR-145*, which have been shown Ts properties in many studies, showed a decrease in our study that is contradictory in the literature. The results of the study show that there is a need to organize studies involving a large number of patients and long follow-up.

Consequently, this study contributes to the findings of previous studies on miRNA levels detected in plasma in BC patients. The major limitation of this study is that it contains a relatively small sample that does not provide enough power to evaluate the relationships between circulating miR levels and clinical features. This study is organized as a preliminary study. We think that it will shed light on future studies with larger sample sizes. In addition, short observation time and insufficient time for survival results are deficient in the prognostic values of miRs that change. Further diagnostic studies are needed for miRNAs with longer follow-up time, greater number of patients, and other predictive and prognostic factors evaluated together.

Conflicts of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

A.G.Y. and A.Y. constructed the main idea and hypothesis of the study. A.G.Y. and A.Y. developed the theory and arranged/edited the material and method section. A.G.Y., A.Y., A.C.K., A.D. and H.S. have done the evaluation of the data in the Results section. Discussion section of the article written by A.G.Y. and A.Y. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Comparison of primary suturing and dacron patch in carotid endarterectomy

Karotis endarterektomide dacron yama ve primer kapatma yöntemlerinin karşılaştırılması

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Abstract

Purpose: In carotid artery atherosclerotic disease; indications for intervention are accepted to be over 75% stenosis for asymptomatic patients and over 50% for symptomatic patients. Preferred surgical treatment is carotid endarterectomy. In this study, we compared clinical outcomes and ultrasonographic findings in two different surgical techniques of arteriotomy closure. The first technique consisted of primary suturing and the second is patch angioplasty with a dacron patch. In 6 month follow-up period clinical examinations and dupplex ultrasonography were performed and analyzed statistically.

Material and methods: 60 patients who underwent carotid endarterectomy for carotid artery disease between January 2017 and December 2020 were enrolled in the study. Data were obtained from hospital database and evaluated statistically. 30 arteriotomy incisions were closed primarily and 30 with dacron patch angioplasty. As surgical indication 50% stenosis in symptomatic patients and 70% stenosis in asymptomatic were determined. Postoperative complications were evaluated. After discharge one-week, two-month, six-month clinical examitanions were performed and in six-month follow up dupplex ultrasonography was performed by an independent radiology specialist, and these results were compared statistically.

Results: When the patients were evaluated in terms of postoperative complications, clinical follow-up after discharge, and 6-month Doppler ultrasonography, no significant differences were detected.

Conclusion: No statistically significant difference was found among techniques primary suturing and dacron patch angioplasty for stroke, occlusion and re-stenosis rates. According to short term results both techniques may be feasible for arteriotomy closure during carotid endarterectomy.

Key words: Carotid endarterectomy, stroke, primary suturing, dacron patch angioplasty.

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Öz

Amaç: Karotis arter hastalığında asemptomatik hasta için %75 ve üzeri darlıkta, semptomatik hasta için %50 ve üzeri darlıkta operasyon önerilir. Cerrahi olarak ise karotis endarterektomi yapılır. Yazımızda karotis endarterektomide primer kapatılan ve dacron yama ile kapatılan hastalardaki, postoperatif komlikasyonlar yönünden ve 6 aylık takipler açısından sonuçlarımızı değerlendirdik.

Gereç ve yöntem: 2017 ve 2020 yılları arasında karotis endarterektomi yapılan yapılan 60 hasta (38 erkek, 22 kadın) retrospektif olarak değerlendirildi. Çalışmaya alınan vakaların tamamı tek taraflı opere edilmiş olup, ameliyatların hepsi genel anestezi eşliğinde yapılmıştır. 30 hastanın karotis arteriotomisi primer kapatılmış, diğer 30 hastanın arteriotomileri dacron yama ile kapatılmıştır. Hastalarda ameliyat kriteri olarak semptomatik hastalarda %50 ve üzeri darlık, asmeptomatik hastalarda %75 ve üzeri darlık kabul edilmiştir. Hastalarda postoperatif komplikasyonlara bakıldı. Taburculuk sonrası 1. hafta, 2. ay ve 6. aydaki kontrollerde klinik olarak değerlendirildi. 6. ayda bağımsız bir radyolog tarafından yapılan doppler ultrasonografi sonuçlarındaki stenoz oranlarına bakıldı.

Bulgular: Hastalar da postoperatif komlikasyonlar, taburculuk sonrası klinik takiplerinde ve 6. ay doppler ultrasonografileri darlık açısından değerlendirildiğinde anlamlı farklılıklar tespit edilmedi.

Sonuç: Karotis endarterektomide primer kapatma ve dacron yama ile kapatma yöntemlerinde postop restenoz açısından anlamlı istatiksel farklılıklar tespit edilmedi. Mevcut çalışmamızda iki kapatma yönteminde güvenle tercih edilebileceği düşünülmektedir. Tabi ki daha büyük ölçekli çalışmalarda farklı sonuçlar çıkabileceği de unutulmamalıdır.

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Anahtar kelimeler: Karotis endarterektomi, inme, birincil dikiş, dakron yama anjiyoplasti.

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Introduction

Carotid Artery Disease (CAD) is a commonly encountered disease because of the neurologic symptoms. In guidelines; 5 year stroke rates and mortalities are shown to be 10.9% even with maximal medical treatment [1]. But in symptomatic patients these rates are even higher [2, 3]. In CAD in addition to medical treatments; carotid endarterectomy (CEA) and interventional radiologic carotid artery stenting (CAS) are the options for treatment [4]. But the gold standard treatment method in carotid artery stenosis is still carotid endarterectomy. Patients with high comorbidity and mortality and stenting and endovascular treatment modalities are good alternatives to endarterectomy, but no significant difference was demonstrated in closure technique subclasses [5].

Recent studies proves that gold standard treatment of CAD is still CEA [6]. CAS can be the alternative treatment in selected patients and stenosis without thrombi [7, 8].Main reason behind carotid stenosis is atherosclerosis in 90% of cases. Symptoms may vary according to the zone of stenosis. The most common zones of stenosis is the carotid bifurcation and proximal segment of internal carotid artery [9]. Clinical outcomes are caused by stenosis percentage and ischemia. Stroke, syncope, vertigo, transient ischemic attack are the common presentations of CAD.

Material and method

In our study, 60 patients who underwent carotid endarterectomy for carotid artery disease between January 2017 and December 2020 were evaluated retrospectively. Data was collected from a predetermined database. 60 patients (38 male, 22 female) were enrolled. The age varies from 48-83 years old and mean age of 65.5. Patient demographics are shown in the Table 1.

Table 1. Patient demographics

		Min-Max	Median	Mean±SD/n-%
Age		48.0 - 83.0	68.0	67.2 ± 9.3
Gender	Female			22 36.7%
	Male			38 63.3%
Smoking				31 51.7%
HT				32 53.3%
DM				39 65.0%
HL				31 51.7%
Previous Cerebrovascular Event				48 80.0%
Preoperative Stenosis	50-69%			32 53.3%
	≥60%			28 46.7%
Postoperative HT				52 86.7%
Postoperative Complication	(-)			54 90.0%
	(+)			6 10.0%
	Haematoma			2 3.3%
	Contralateral Weakness			2 3.3%
	Hemiplegia			1 1.7%
	Hoarseness			1 1.7%
6th Month Dupplex Ultrasonography	No Stenosis			56 93,3%
	40% Stenosis			1 1.7%
	50-69% Stenosis			3 5.0%

48 patients had a history of previous cerebrovascular events and 32 of these patients had 50-69% stenosis. All other patients had stenosis over 70%. Totally occluded carotid arteries were not operated and not included to the study. Stenosis percentages were shown in the Table 2. Stenosis rates were determined by dupplex ultrasonography and were verified by contrast enhanced computer tomography.

Surgical technique and medication

All patients were hospitalized 2 days preoperatively. Routine Cardiology, Pulmonology, and Anesthesiology consultations were performed. All operations were performed under general anesthesia. Carotid artery was explored and controlled with vascular tapes. 5000 IU unfractionated heparin was administered intravenously. Vascular clamps were placed in internal carotid, common carotid and external carotid arteries. Clamping time was 16-22 minutes (mean 19 minutes). After performing endarterectomy half of the arteries were sutured primarily (Figure 1) and half were closed with dacron patch angioplasty (Figure 2). After haemostasis hemovac drains were placed. Patients were taken to the ICU unit. As a postoperative medication, all patients received 100 mg acetylsalicylic acid once daily and 4000 IU enoxaparin sodium once daily and were discharged with the same treatment. At 1st week follow up enoxaparin was discontinued. At the 6th month follow up dupplex ultrasonography was performed by an independent radiology specialist.

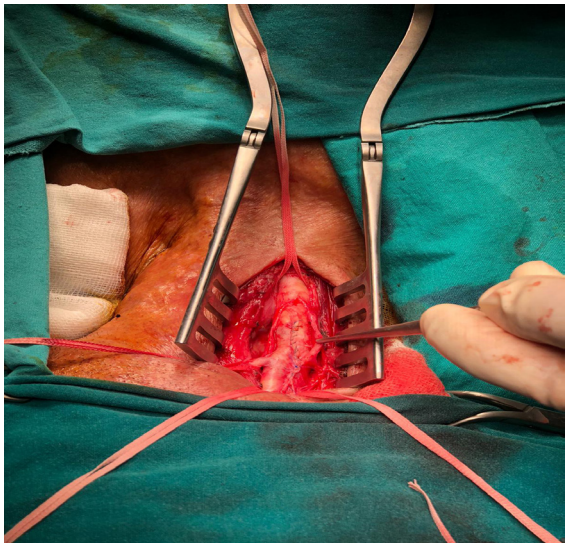

Figure 1. Primary suturing closure technique

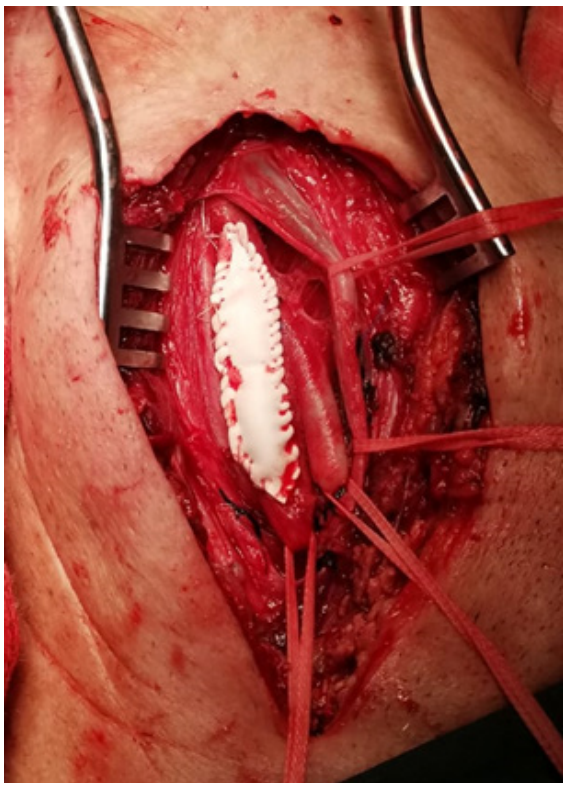

Figure 2. Dacron patch angioplasty closure technique

Statistical methods

Statistical analysis of the descriptive data was presented as mean, standard deviation, median, minimum value (min), maximum value (max), frequency, and percentage. The normality of the numeric variables was checked by using the Kolmogorov-Smirnov test. Continuous independent variables were analysed by the Mann-Whitney U test. Categorical variable analysis was performed by chi-squared test and when chi-squared test was not suitable Fischer test was performed. SPSS 27.0 computer software was used to perform statistical analysis.

The study was approved by the Nigde Omer Halisdemir University Non-Interventional Clinical Research Ethics Committee with its decision.

Results

This study aims to compare results of surgical techniques; primary suturing and carotid dacron patch angioplasty for arteriotomy closure in patients who underwent CEA. The demographic characteristics of the patients were summarized in Table 1. No statistically

Table 2. Comperation of arteriotomy closure techniques

		Primary Suturing		Dacron Patch		*P*	
		Mean±SD/n-%	Median	Mean±SD/n-%	Median		
Age		66.6 ± 9.4	67.5	67.8 ± 9.4	68,5	0,657	m
Gender	Female	11 36.7%		11 36.7%		1,000	X^2
	Male	19 63.3%		19 63.3%			
Smoking		16 53.3%		15 50.0%		0,605	X^2
HT		15 50.0%		17 56.7%		0,176	X^2
DM		22 73.3%		17 56.7%		0,796	X^2
HL		18 60.0%		13 43.3%		0,196	X^2
Previous Cerebrovascular Event		24 80.0%		24 80.0%		1,000	X^2
Preoperative Stenosis	50-69%	16 53.3%		16 53.3%		1,000	X^2
	≥60%	14 46.7%		14 46.7%			
Postoperative HT		24 80.0%		28 93.3%		0,129	X^2
Postoperative Complication	(-)	26 86.7%		28 93.3%		0,670	X^2
	(+)	4 13.3%		2 6.7%			
	Haematoma	1 3.3%		1 3.3%			
	Contralateral Weakness	2 6.7%		0 0.0%			
	Hemiplegia	0 0.0%		1 3.3%			
	Hoarseness	1 3.3%		0 0.0%			
6th Month Dupplex Ultrasonography	No Stenosis	27 90.0%		29 96.7%		0,611	X^2
	40% Stenosis	0 0.0%		1 3.3%			
	50-69% Stenosis	3 10.0%		0 0.0%			

Mann-whitney u test/X^2, Chi-squared test

significant difference was found between two groups. Patient ages and genders showed no significant difference ($p>0.05$). Also no statistically significant difference was observed in Postoperative HT, Postoperative Complication and also in 6th month dupplex ultrasonographic findings ($p>0.05$). Statistical analysis p values, mean±SD values and median values were shown in Table 2.

All patients were extubated in postoperative first 4 hours. 52 patients required antihypertensive medication due to postoperative hypertension. In two patients which were in the primary suturing group experienced contralateral muscle weakness. This weakness resolved by steroid treatment within six hours. One patient in the primary suturing group experienced hoarseness and this symptom regressed in postoperative third day, in first week follow up there were no signs of hoarseness. One patient in the dacron patch angioplasty group experienced hemiplegia at postoperative eighth hour, difficulty in breathing and had to entubated again. In radiologic images of brain diffusion magnetic resonance pathological findings were not present. After consulting neurologist medical treatment was arranged. When patinet was re evaluated after 48 hours there were no pathological findings and hemiplegia was healed. Patient was extubated and discharged at seventh postoperative day. Two patients; one in primary suturing group and one in dacron patch angioplasty group underwent reexploration due to hematoma in surgical site.

After discharge, patients were evaluated at one-week, two-month and six-month. In follow-up examinations no neurologic pathologies were found. As an additional examination at six-month follow-up all patients underwent dupplex ultrasonography. In 3 patients in primary suturing group 50-69% stenosis was observed, in one patient in dacron patch angioplasty group 40% stenosis was present. But none of the patients had clinical symptoms.

Discussion

CEA still is the most accepted treatment in preventing cerebrovascular events in both symptomatic and asymptomatic patients [10, 11]. There are some studies comparing primary suturing and dacron patch angioplasty. In COCHRANE study (Bond et al.[12]) shows that dacron patch angioplasty has statistically significant superiority over primary suturing comparing stroke rates [12-14]. Although randomized clinical trials claim that there is no superiority of the techniques over each other contrary to retrospective studies recent evidence shows that patch angioplasty decreases re-stenosis, occlusion and combined stroke and death rates [15]. Lazarides et al. [16] conducted a meta-analysis study with 4440 patients and compared 7 surgical closure techniques such as primary closure, eversion endarterectomy (EVE), dacron, vein patch, PTFE, bovine pericardium, polyurethane. EVE and patching with bovine pericardium or PTFE is associated with a lower incidence in both short-term and late undesired outcomes following CEA and seems to represent the best choice compared with other carotid closure techniques. And another meta-analysis of relatively small randomized controlled trials suggests that carotid patch angioplasty reduces the combined perioperative and long-term risk of stroke and the risk of restenosis, more data are needed [17]. When materials used for patch angioplasty (dacron, ptfe, saphena patch) there is no statistically significant difference between patch grafts. In our study we preferred dacron material for patch angioplasty.

Marsman et al. [18] studied carotid patients with 1280 patches and 1055 primary surgical closures in a meta-analysis study; the review showed no definite proof of a difference between carotid endarterectomy with patch angioplasty versus primary closure of the arterial wall on all-cause mortality, 30 days mortality, 30 days stroke, or any other serious adverse events. In our study, we compared primary suturing with dacron patch angioplasty in some of its aspects and anticipated patch angioplasty to be a better option for arteriotomy closure. We found no statistically significant difference between primary suturing and patch angioplasty techniques.

Some studies suggest that atrial fibrillation can be observed in 50% of the patients diagnosed with atherosclerotic carotid artery stenosis. Vascular pathologies are observed more often in patients with non valvular atrial fibrillation than in patients without any stroke or transient ischemic attack [19]. In this study none of the patients enrolled had atrial fibrillation. There is

still debate about timing of the surgery. Usually 4 to 6 weeks of delay in surgery is recomended after neurologic event. Because if surgery was performed before that recommended delay; non haemorrhagic infarct zone may turn into haemorrhagic zone. But recent studies show that new cerebrovascular events may happen during the time of this delay [20, 21]. But we respected this delay time when planning the time of surgery and did not experience any neurologic events during that time. Clamping of the carotid artery during endarterectomy may cause varying degrees of brain ischemia. Therefore, there are surgeons who advocate the use of shunts during endarterectomy [22]. However, we did not choose it in our cases because of the difficulty of using shunts and the risks of complications. CEA may be performed under general anesthesia or local anesthesia. When the methods of anesthesia were compared, techniques manifested no superiority over each other [23]. We preferred general anesthesia in our operations. No cardiac or pulmonary complications were encountered during or after anesthesia.

In conclusion, we compared primary suturing with dacron patch angioplasty in some of its aspects and anticipated patch angioplasty to be a better option for arteriotomy closure. We found no statistically significant difference between primary suturing and patch angioplasty techniques. It should be considered that statistical differences can be seen in studies conducted according to the number of patients, postoperative follow-up periods, and early and late complication follow-up processes.

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Authors' contributions to the article

H.O. constructed the main idea and hypothesis of the study. F.S. developed the theory and arranged/edited the material and method section. A.N.A. and A.A.G.R. have done the evaluation of the data in the Results section. Discussion section of the article written by H.O. who also reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Epidemiology, aetiology and clinical characteristics of ischaemic stroke in young adults: a retrospective study from Denizli, Türkiye

Genç erişkinlerde iskemik inmenin epidemiyoloji, etiyoloji ve klinik özellikleri: Denizli ili retrospektif tek merkez verileri

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Abstract

Purpose: The incidence of cerebrovascular disease (CVD) in young adults is approximately 6-26 per 100,000 worldwide, and this numbers are increasing every year. Stroke aetiology among young adults are more diverse than those among older adults and require extensive diagnostic work-up. The aim of our study is to determine risk factors and stroke etiology in stroke patients aged 45 years and younger, followed in our clinic for the last 10 years, and to compare them with literature.

Materials and methods: The study is included in the patients between the age of 18-45 years and are followed by Ischemic CVD in Pamukkale University Neurology clinic between January 2010 and November 2020.The clinical and demographic data of the patients were retrospectively analyzed.

Results: The most common risk factor was smoking (31.4%); hypertension (20.2%), diabetes mellitus (14.4%), hyperlipidemia (14.4%) and coronary arterial disease (11.6%) were following it. According to Trial of Org in Acute Stroke Treatment (TOAST)classification there were, large vessel disease in 13.4%, small vascular disease in 19.8%, cardioembolism in 16.7%, other determined aetiology in 11.5% and the most frequently stroke of undetermined etiology in 38.6%. The most common reason in other determined aetiology was Antiphospholipid Antibody Syndrome.

Conclusion: The incidence of young stroke is increasing every year and it is necessary to determine the underlying reasons to prevent and to give treatment for aetiology. Thus this will contribute to head off major health care costs, loss of workforce and to save young lives.

Key words: Young stroke aetiology, stroke in young adults, ischemic stroke.

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Öz

Amaç: Genç erişkinlerde serebrovasküler hastalık (SVH) insidansı dünyada yaklaşık 100.000'de 6-26'dır ve bu oran her yıl artmaktadır. Genç erişkinlerde inme etiyolojisi, yaşlılara göre daha çeşitlidir ve kapsamlı tanısal çalışma gerektirir. Çalışmamızın amacı, kliniğimizde son 10 yıldır takip edilen 45 yaş ve altı inme hastalarında risk faktörlerini ve inme etiyolojisini belirlemek ve literatürle karşılaştırmaktır.

Gereç ve yöntem: Çalışmaya Pamukkale Üniversitesi Nöroloji Kliniği'nde Ocak 2010-Kasım 2020 tarihleri arasında İskemik SVH ile takip edilen 18-45 yaş arası hastalar dahil edilmiştir. Hastaların klinik ve demografik verileri retrospektif olarak incelenmiştir.

Bulgular: En sık görülen risk faktörü sigara (%31,4) iken; hipertansiyon (%20,2), diabetesmellitus (%14,4), hiperlipidemi (%14,4) ve koroner arter hastalığı (%11,6) bunu izlemekteydi. Trial of Org in AcuteStrokeTreatment (TOAST) sınıflamasına göre etiyoloji: %13,4 büyük damar hastalığı, %19,8 küçük damar hastalığı, %16,7 kardiyoembolizm, %11,5 diğer etiyoloji idi ve hastaların çoğunu (%38,6) etiyolojisi belirlenemeyen inmeler oluşturuyordu. Belirlenen diğer etiyolojide en sık neden Antifosfolipid Antikor Sendromu idi.

Sonuç: Genç erişkinlerde inme insidansı her yıl artmakta olup, altta yatan nedenlerin belirlenmesi ve etiyolojisine yönelik tedavi verilmesi gerekmektedir. Böylece, bakım maliyetlerinin, işgücü kaybının önüne geçilmesine ve genç hayatların kurtarılmasına katkıda bulunulacaktır.

Anahtar kelimeler: Genç inme etiyolojisi, genç erişkinlerde inme, iskemik inme.

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Introduction

Stroke in young adults has a significant socioeconomic impact due to high health care costs and loss of workforce, and its incidence is increasing every year [1]. Although there is no official definition of the concept of young stroke, it generally included the ages of 18 to 50 [2]. In different studies, the lower limit was between the ages of 15-18, while the upper limit was taken as 45-55 years [2, 3]. The incidence of cerebrovascular disease (CVD) in young adults has been reported to be approximately 6-26 per 100.000 worldwide [4]. While this rate is 7-8 per 100.000 in Europe, it rises to 100 per 100.000 in Africa [1, 4]. "Young stroke" is more common in women under the age of 35, while it is more common in men between the ages of 35-50. It can be thought that the intensification of gender-specific risk factors such as pregnancy, postpartum period and oral contraceptive use in young women who are in reproductive age and the intensification of vascular risk factors in middle-aged men may lead to this difference in incidence [5, 6]. While unidentified rare causes constitute the majority of stroke causes in young adults, the incidence of large vessel atherosclerosis and small vessel disease increases with age [6]. Although the chance of surviving a stroke is higher in younger patients than in older age, survivors face complications such as recurrent paralysis, neuropsychiatric problems and seizures for a much longer period of time. Therefore, the causes of stroke among young adults are more diverse than those among older adults and require extensive diagnostic work-up [7].

The aim of our study is to determine risk factors and stroke etiology in stroke patients aged 45 years and younger, followed in our clinic for the last 10 years, and to compare them with literature data.

Material and method

The study was approved by the Clinical Research Ethics Committee of Pamukkale University.

In this study, records of 1189 patients who were between the ages of 18-45 and those hospitalized in our clinic with the diagnosis of ischemic CVD between January 2010 and November 2020 were retrospectively analyzed. Intracranial hemorrhage and venous sinus thrombosis were excluded. Demographic characteristics of the patients and risk factors that may cause stroke (hypertension, diabetes, hypercholesterolemia, smoking, hyperhomocysteinemia) were examined. Patients who were previously diagnosed with diabetes, were using antidiabetic drugs at the time of admission, or had a blood glucose level of ≥200 milligram/deciliter (mg/dL) at any time were considered as diabetic. A serum total cholesterol level ≥200 mg/dL was defined as hypercholesterolemia. Smoking cigarette 1 or more per day was evaluated as risk factors [7].

Blood tests to determine the etiology of stroke, hypercoagulability and vasculitis markers (Antithrombin III, protein C, protein S, activated protein C resistance, antinuclear antibody (ANA) profile, antiphospholipid antibodies, homocysteine, peripheral blood smear, serum lactate-pyruvate level, Anti-HIV, VDRL, toxoplasma, brucella antibodies, borrelia burgdorferi IgM-IgG.) Imaging methods (Carotid-vertebral Doppler ultrasonography (CVDUSG), aortic arch, carotid-vertebral CT (Computarized Tomography) angiography, cranial MRI (Magnetic Resonance Imaging), MR or CT angiography, Digital Subtraction Angiography (DSA) for patients deemed necessary) were examined. In addition, electrocardiogram, transthoracic echocardiography and/or transesophageal echocardiography examinations performed on the patients were evaluated.

Patients were divided into CVD subgroups according to the Trial of Org in Acute Stroke Treatment (TOAST) criteria. According to this classification, the following subgroups were determined:

1) Large vessel atherosclerosis

2) Cardiac embolism

3) Small vessel disease (Lacunar infarct)

4) Other determinedaetiology: Hematologic causes, coagulopathies, thrombocytosis, polycythemia, deficiency of coagulation inhibitors, antiphospholipid antibody syndrome, Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) and other causes of CVD not listed above

5) Stroke of undeterminedaetiology [8, 9].

Statistics

Data analysis was performed with SPSS 24.0 package program. Mann Whitney U test was used to compare independent group differences. The relationships between continuous variables were analyzed with Spearman or Pearson correlation analyzes. The differences between categorical variables were analyzed with Chi-square analysis and *p* value below 0.05 was considered statistically significant

Results

A total of 1189 patients were examined and 148 (53%) of 277 patients included in the study were male and 129 (47%) were female. The mean age of the patients was 37.8±7.02 (20-45). Recurrent stroke was observed in 14 (5.1%) patients, and first stroke in 263 (94.9%) patients. Eighty-seven (31.4%) of the patients were smokers. Of 277 patients, 56 (20.2%) had hypertension, 40 (14.4%) had diabetes, 40 (14.4%) had hyperlipidemia, 32 (11.6%) had coronary artery disease. High homocysteine level in 113 patients, normal homocysteine level in 141 patients and low homocysteine level in 8 patients were determined, while homocysteine levels of 15 patients were not studied. According to the TOAST classification large vessel disease in 37 (13.4%), small vessel disease in 55 (19.8%), cardioembolism in 46 (16.7%), stroke due to other determined causes in 32 (11%, 5) and stroke of undetermined causes in 107 (38.6%) were detected among 277 patients (Table 1, 2).

Table 1. Risk factors of stroke in young adults

Risk factors	Number of patients	Percent
Smoke	87	31.4%
Hypertension	56	20.2%
Diabetis mellitus	40	14.4%
Hyperlipidemia	40	14.4%
Coronary artery disease	32	11.6%

Table 2. The subgroups of patients according to TOAST classification

TOAST Classification	Number of patients	Percent
Large vessel disease	37	13.4%
Small vessel disease	55	19.8%
Cardioembolism	46	16.7%
Other causes	32	11.5%
Cause unknown	107	38.6%
Total	277	100%

In the TEE imaging of the patients, PFO was detected in 17 patients (it was associated with ECO pathology in 9 patients). Holter ECG imaging was not performed in 17 (6.1%) of 277 patients, and Atrial Fibrillation was detected in only 7 (2.5%) of the remaining group. Holter ECG imaging of 253 patients was normal (91%).

Carotid vertebral Doppler ultrasonography was performed in 16 of the patients with large vessel disease, and total occlusion in the right Internal Carotid Artery (ICA) was detected in only one. A total of 227 patients underwent MR Angio/Arcus-carotid vertebral-brain CT angio, and thrombus in the vertebral artery in 6 patients, dissection of the vertebral artery in 1 patient, thrombus in the basilar artery in 2 patients, stenosis in the Posterior Inferior Cerebellar Artery (PICA) in 2 patients, dissection in the right ICA in 1 patient, aneurysm in the ICA in 2 patients, total occlusion in the ICA in 8 patients, 50% stenosis in the right ICA in 2 patients, stenosis of 30-60% in the left ICA in 4 patients, stenosis between 70-90% in 4 patients, occlusion in the M2 segment of the Middle Cerebral Artery (MCA) in 2 patients (one on the right , one on the left), vasculitic in 1 patient, 50% stenosis in the celiac artery in 1

patient, and aortic aneurysm in 1 patient were detected. DSA was performed on 34 patients and 22 were found to be normal. Vasculitis findings in 2 patients, ICA dissection in 1 patient, fibromuscular dysplasia in 1 patient, total occlusion in left ICA in 1 patient, 90% stenosis in ICA in 2 patients, thrombus in left MCA M1 in 2 patients, total occlusion in left MCA in 1 patient, thrombus in left ICA distal and MCA M1 in 1 patient were detected.

Considering other determined causes, AFAS in 3 patients, SLE in 6 patients, CADASIL in 3 patients, malignancy in 6 patients, polycythemia vera in 6 patients, Behçet's disease in 2 patients, sjögren's disease in 1 patient, scleroderma in 1 patient, Marfan syndrome in 2 patients, and ARA in 2 patients were detected. In the thrombophilia panel, homozygous MTHFR in 9 patients, homozygous factor V leiden in 3 patients, heterozygous factor V leiden in 3 patients, MTHFR heterozygous in 18 patients, PAI 4G/5G homozygous in 6 patients, PAI 4G/5G heterozygous mutations in 2 patients were detected.

Two hundred thirty-five (84%) of the patients were not using antiaggregant/anticoagulant drugs at the first admission. After treatment adjustment, it was determined that 133 of the patients used acetylsalicylic acid (asa), 57 of them clopidogrel, 24 of them dual asa+clopidogrel, 33 of them warfarin, 23 of them low-molecular-weight-heparin (LMWH),2 of them asa+warfarin, 3 of them Non-vitamin K antagonist Oral Anticoagulant (NOAK), 2 of them asa+dipyridamole.

Discussion

Stroke is a leading cause of death and adult disability worldwide. Early recognition and prompt treatment of stroke is essential to prevent or minimize morbidity and mortality. Every year, approximately 2 million young people worldwide suffer from stroke, which causes serious harm and financial burden to patients and their families [1, 10]. While the incidence of stroke in the elderly decreases from year to year, the incidence of stroke in the young people shows an increasing trend [1, 11, 12].

In the study of Ischemic Stroke in Young Adults aged 18-44 years in Northern Sweden, the incidence of stroke was found to be 11.3 per 100.000, and it was around 5 to 15 /100.000 per year in many European studies [7]. In our study, young age ischemic cerebrovascular disease was detected at a rate of 24% among all CVDs. In addition, the incidence of ischemic stroke is 53% higher in the male population, which is consistent with the literature.

The etiology and risk factors of young patients with ischemic stroke are in a wide and complex spectrum, such as migraine with aura, hereditary thrombophilia, hyperhomocysteinemia, cardiovascular risk factors and malignancy [5]. The prevalence of standard modifiable vascular risk factors in young stroke patients is different from that in older patients. Although modifiable risk factors are the same for both young and advanced age groups, the prevalence of these risk factors is not the same in these two age groups [13, 14]. In a study that collected data from hospital records of fifteen European cities, in young strokes, rare causes were found in 22%, cardioembolic causes in 17.3% (8.6% high-risk, 8.6% low-risk), small vessel disease in 12%, large vessel disease in 9% etiologically and the cause could not be determined in 40% of the patients. Among the known etiologies, the most common single cause was found to be cervical artery dissection [3, 4]. In our study, however, cervical artery dissection was never detected. The most common causes detected among other causes were Malignancy, SLE and polycytemia vera. Antiphospholipid Antibody Syndrome (APS) was also a common cause among the known other etiologies if it is taken into account that the most of the lupus patients have secondary APS due to Antiphospholipid Antibodies. Stroke, the cause of which cannot be determined according to TOASTcriteria, is the most common type in young patients [15]. Large vessel disease is observed less frequently in young stroke patients than in the elderly, and it has been replaced by other causes in the TOAST scoring [16]. In our study, large vessel disease in 37 (13.4%), small vessel disease in 55 (19.8%), cardioembolism in 46 (16.7%), stroke due to other causes in 32 (11.5%) and stroke of which the cause could not be found in 107 (38.6%) was detected. The most common cause is the group whose cause is unknown, which is compatible with the literature. The second cause was detected as small vessel disease, followed by cardioembolic causes. The reason for cardioembolic stroke is less frequent in this study may be the ethnicity and the

population has more vascular risk factors. Even the most of the classifications based on Europian population some specific ethnicities such as Asian population has adverse vascular risk factors in younger age. That can be explain the greater distribution of small and large vessel disease as ischemic stroke etiology in young patients. Although large vessel disease is the least common group in line with the literature, it was detected at a higher rate compared to the literature. Patients with stroke due to cardioembolism or large vessel atherosclerosis have the highest risk of recurrent stroke compared to other TOAST stroke subtypes, and secondary prevention is very important in these patients [17, 18]. Hereditary thrombophilias are one of the important risk factors. In a study performed by Mialovytska et al. [19] which included young ischemic stroke patients, it was found that increase was detected in the development of ischemic brain lesions with MTHFR gene mutation positivity, and there was a correlation between MTHFR gene mutation and high homocysteine. Also in our study, a gene mutation affecting the treatment regulation was found in 3.2% of the patients, in 9 patients, 5 of whom were homozygous for MTHFR and 3 of them were homozygous for factor V leiden. In the obtained data, high homocysteine level in 113 patients, normal homocysteine level in 141 patients and low homocysteine level in 8 patients were determined. The understanding that a high level of homocysteine in plasma may predispose to arterial or venous thromboembolism emerged more than 40 years ago when it was determined that patients with homocysteinuria are at high risk of early vascular disease. A meta-analysis by Holmen et al. [20] also points out the relationship between homocysteine levels and ischemic stroke risk. It was found that risk estimates reported in studies were significantly higher when homocysteine levels above 15 μmol/L were reached, indicating a possible nonlinear relationship between homocysteine and ischemic stroke. It is known that vasculitic processes are risk factors in young strokes, and many rheumatological diseases such as Behçet's, Sjögren's and SLE may cause stroke. In a cohort study performed by Hanly et al. [21], it was observed that neuropsychiatric findings in patients with SLE can often manifest themselves with stroke and TIAs. Hypercoagulability conditions caused by antiphospholipid hyperhomocysteinemia, high factor VIII, protein C deficiency and mutations such as methylenetetrahydrofolate reductase C677T, prothrombin G20210A and factor V Leiden (FVL) mutations are considered among possible causes despite different opinions. FVL is the most common hereditary coagulation disorder in many parts of the world. The role of this mutation in ischemic stroke patients is controversial. It demonstrated the function of FVL as an independent etiologic factor, especially in younger patients. On the other hand, there are many studies suggesting that FVL is a predisposing factor for ischemic stroke only when accompanied. Therefore, screening for coagulopathy is generally recommended in young stroke patients, especially in those whose disease origin is unknown [22]. In our study, polycythemia vera patients were found to be common among other causes, and the relationship between thrombosis and JAK 2 mutation is known even if there are no distinct myeloproliferative neoplasms in myeproliferative diseases. In addition, it should be kept in mind to consider screening for JAK 2 mutations in a young patient with cryptogenic stroke with or without polycythemia or thrombocytosis [23]. Although craniofacial dysplasia is rare, it is among the risk factors. Craniofacial fibrous dysplasia can cause various cerebrovascular diseases by causing narrowing of intracranial vessels and alteration of general hemodynamics of intracranial vessels. A possible mechanism considered here is that it causes neurovascular compression, resulting in hemodynamic changes and decreased cerebral perfusion [24]. Regarding cardiovascular risk factors, Sub-stenotic atherosclerosis, left atrial dysfunction, and occult atrial fibrillation are probably the most common causes, but undetected malignancy, hypercoagulable states, and PFO-related stroke are alternative causes to be considered. Many studies performed with patent foramen ovale (PFO) have been shown to be associated with stroke. It suggests a potential etiological link, by increasing over 40% in the Cryptogenic stroke population; however, specific clinical and cardiac structural variants that conclusively indicate causation are not yet known [25]. The most relevant complication of AF is cardioembolic stroke. Thrombus often form in the left atrial appendage (LAA) due to abnormal blood flow in the left atrium associated with erratic electrical signals and lack of coordinated atrial contractions, in addition to

endothelial dysfunction and other prothrombotic conditions. Once 'released' this thrombus could embolize into peripheral or (more commonly) cerebral artery beds. In particular, patients with AF-related embolic stroke are thought to have worse outcomes than those with non-AF-related strokes [26, 27]. Both North American and European guidelines support the use of the CHA2 DS2 -VASc score to classify patients with AF according to the risks of stroke and systemic thromboembolism. This scoring is very important in determining the risk of AF [28, 29]. Other causes include IS arterial dissection in Young adults, Moyamoya disease (MMD), primary and secondary central nervous system vasculitis, antiphospholipid syndrome (APS), reversible cerebral vasoconstriction syndrome (RCVS), cerebral vein thrombosis (CVT), and genetic diseases. Cerebral autosomal dominant arteriopathies with subcortical infarcts and leukoencephalopathy (CADASIL) and Fabry disease should be kept in mind [30]. Considering the treatment arrangement in a study performed by Kunt et al. [31], examining the clinical and demographic characteristics of patients with stroke, the relationship between medicine use and cerebrovascular events was investigated. It was observed that stroke occurred in 527 patients (46.3%) while using antiaggregant or anticoagulant, and when the medicines used were examined, it was found that there was no significant difference between the patient groups in terms of the use of acetylsalicylic acid, clopidogrel and warfarin.

In summary, the incidence of stroke in young people is increasing every year, and it is very important to determine the underlying etiology and to give treatment for the exact reason. The causes of stroke among young adults are more diverse than those among older adults and require extensive diagnostic work-up. Although the chance of surviving a stroke is higher in younger patients than in older age, survivors face complications such as recurrent paralysis, neuropsychiatric problems and seizures for a much longer period of time. Therefore, preventing stroke in young adults will contribute to head off major health care costs, loss of workforce and to save young lives.

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

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Authors' contributions to the article

S.B.A.: study design, data collecting, data analysis.

Z.Ü.: data analysis, writing.

Ç.H.Ö.: data analysis, writing, supervisor.

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Comparison of photoscreeners and hand-held autorefractometer with cycloplegic autorefractometry in children with newly diagnosed attention deficit hyperactivity disorder

Yeni tanı konmuş dikkat eksikliği hiperaktivite bozukluğu olan çocuklarda fotoscreener ve el tipi otorefraktometrenin sikloplejik otorefraktometri ile karşılaştırılması

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Abstract

Purpose: To compare non-cycloplegic refraction measurements of two photoscreeners and the hand-held autorefractometer with cycloplegic measurements of the autorefractometer in patients with attention deficit hyperactivity disorder (ADHD).

Meterials and methods: This cross-sectional, comparative study consisted of 53 children who were newly diagnosed with ADHD. We compared spherical, cylindrical, cylindrical axis and spherical equivalent (SE) measurements in Plusoptix A12, Spot Vision Screener, and Retinomax K-plus Screen with Tonoref II. Reliability was analyzed by using the interclass correlation coefficient (ICC) and Bland-Altman plot was used to evaluate the agreement between devices.

Results: The mean age of children was 9.45±1.68. All of the devices measured spherical power and SE significantly more myopic than the Tonoref II. While The Spot Vision Screener, PlusoptiX A12, and Tonoref II provided similar cylindrical power, Retinomax K-plus Screen measured significantly lower than the Tonoref II. The excellent reliability was detected in spherical power, cylindrical power, SE and J0 between Tonoref II and PlusoptiX A12 (ICC:0.930, 0.921, 0.927 and 0.920, respectively. All of the hand-held devices showed excellent reliability in terms of cylindrical power and J0 (ICC>0.90, for all) and good reliability for J45 (ICC:0.75-0.90 for all).

Conclusion: Despite all devices having advantages or disadvantages, Plusoptix A12 showed excellent reliability for detecting refractive errors in children with ADHD.

Key words: Attention deficit hyperactivity disorder, photoscreener, autorefractometer, children, cycloplegia.

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Öz

Amaç: Dikkat eksikliği hiperaktivite bozukluğu (DEHB) olan hastalarda iki fotoscreener ve el otorefraktometresinin sikloplejik olmayan refraksiyon ölçümlerini sikloplejik otorefraktometre ölçümleriyle karşılaştırmak.

Gereç ve yöntem: Bu kesitsel, karşılaştırmalı çalışmaya yeni DEHB tanısı konan 53 çocuk dahil edildi. Tonoref II ile Plusoptix A12, Spot Vision Screener ve Retinomax K-plus Screen cihazlarında sferik, silindirik, silindirik eksen ve sferik eşdeğer (SE) ölçümlerini karşılaştırdık. Güvenilirlik, sınıflar arası korelasyon katsayısı (SKK) kullanılarak analiz edildi ve cihazlar arasındaki uyumu değerlendirmek için Bland-Altman grafiği kullanıldı.

Bulgular: Çocukların yaş ortalaması 9,45±1,68 idi. Tüm cihazlar sferik gücü ve SE'yi Tonoref II'den önemli ölçüde daha miyop ölçtü. Spot Vision Screener, PlusoptiX A12 ve Tonoref II benzer silindirik güç tespit ederken, Retinomax K-plus Screen, Tonoref II'den önemli ölçüde daha düşük silindirik güç ölçtü. Tonoref II ve PlusoptiX A12 (sırasıyla SKK:0,930, 0,921, 0,927 ve 0,920) arasında sferik güç, silindirik güç, SE ve J0'da mükemmel güvenilirlik tespit edildi. Elde taşınan cihazların tümü, silindirik güç ve J0 (tümü için SKK>0,90) ve J45 için iyi güvenilirlik (tümü için SKK:0,75-0,90) açısından mükemmel güvenilirlik gösterdi.

Sonuç: Plusoptix A12, tüm cihazların avantaj ve dezavantajlarına rağmen DEHB'li çocuklarda kırma kusurlarını saptamada mükemmel güvenilirlik göstermiştir.

Anahtar kelimeler: Dikkat eksikliği hiperaktivite bozukluğu, fotoscreener, otorefraktometre, çocuklar, siklopleji.

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Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most prevalent neurodevelopmental disorder among children and adolescents aged between 6-17 years. The prevalence of ADHD was reported as 2%-18% in different studies. The characteristics of ADHD are inattention, increased hyperactivity, impulsivity, and lack of controlling inappropriate behaviors [1]. To date, several studies have been conducted in children with ADHD in terms of ocular abnormalities such as refractive errors, ocular pathologies, and ocular side effects of agents, methylphenidate, and atomoxetine used for the treatment of ADHD [2, 3].

It is known that refractive errors and amblyopia were higher in children with ADHD. Visual problems such as refractive errors may lead to the diminution of visual acuity and concentration and contribute symptoms of ADHD [3]. Thus, it is essential to have an eye examination as a part of complete physical and psychological evaluation in children with ADHD and rule out underlying ocular disorders that may affect children's attention [4]. However, children diagnosed with ADHD may have difficulties and limitations in adapting to the measuring devices used for the ophthalmologic examination which starts with refraction assessment due to the symptoms of inattention, mobility, and impulsivity, and therefore inappropriate measurements may be obtained [5, 6].

Accurate measurement of the refraction values is crucial in determining the refractive errors, appropriate treatment, and prevention of amblyopia. The cycloplegic refraction, which eliminates accommodation, is the gold standard for detecting refractive errors in children [7]. However, cycloplegic medications can cause unwanted side effects and may be difficult to administer to children each time. Moreover, it takes about 30-45 minutes to reach full cycloplegia, making the process time consuming [8]. Automated refractometers need cycloplegia in children, and table-mounted design can make it challenging to maintain proper position, and providing visual fixation on a target for a sufficient period may be arduous in uncooperative patients [7]. Although the main measurement methods for detecting refractive errors in children are cycloplegic assessments, both photoscreeners and hand-held autorefractometers are alternatives for vision screening in a short time for physically and mentally disabled, non-cooperative patients such as children with ADHD [6, 7].

In this context, the aim of our study is to compare non- cycloplegic refraction measurements of two photoscreeners (Plusoptix A12 and Spot vision screener) and the hand-held autorefractometer (Retinomax K-plus Screen) with cycloplegic measurements of the autorefractometer (Tonoref II).

Materials and methods

Study population and study design

This cross-sectional, comparative study consisted of 53 children aged 6-12 years who were newly diagnosed with ADHD in our university Child and Adolescent Psychiatry Department. The Local Ethics Committee approved the study design and procedures (Sivas Cumhuriyet University School of Medicine, Ethics Committee, which were conducted in accordance with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants and parents of children.

Attention deficit hyperactivity disorder was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) criteria. Also, all children were evaluated by a child psychiatrist with a semi-structured interview to confirm the diagnosis of ADHD and to determine whether the child had any other psychiatric disorder. After the diagnosis of ADHD, these children were referred to the ophthalmology department for an ophthalmological examination. Children with pure ADHD and drug-naive, and without any ocular pathologies except refractive errors were included in the study. Patients were excluded from the study if they had mental retardation, specific learning disabilities, and autism spectrum disorders accompanying ADHD that

could adversely affect visual examinations. Additionally, children with ptosis, strabismus, corneal and lens opacities, retinal abnormalities, and refractive errors beyond measurements according to manufacturers' recommendations were excluded. All of the patients underwent a complete ophthalmic examination, including pupil reactions, Hirschberg's test, cover-uncover, alternate cover tests, slit-lamp biomicroscopy, and dilated fundus examination.

Measurements

The refraction measurements of 106 eyes of 53 children were obtained in the same semi-lit room and the following order, and an average of three consecutive measurements for each device was recorded. Firstly, without cycloplegia, PlusoptiX A12 (Plusoptix GmbH, Nuremberg, Germany), Spot Vision Screener (Welch Allyn, Skaneateles Falls, NY), and Retinomax K-plus Screen (Righton, Tokyo, Japan) were performed respectively. Then, cycloplegia was induced by using cyclopentolate 1% (Sikloplejin; Abdi Ibrahim, Istanbul, Turkey) eye drops two times with an interval of 5 minutes. After 45 minutes, pupillary light reactions were checked, and complete cycloplegia was accomplished. The cycloplegic measurements were performed by Tonoref II (Nidek Co. Ltd, Gamagori, Japan). The accuracy of the cycloplegic refractive measurements in children via table-mounted autorefractometers has been demonstrated previously [9, 10].

We also evaluated the amblyogenic risk factors according to the American Association for Pediatric Ophthalmology and Strabismus (AAPOS) referral criteria for ages >48 months, published in 2013 [11].

PlusoptiX A12 (Plusoptix GmbH, Nuremberg, Germany)

PlusoptiX A12 is a hand-held photorefractor, particularly for infants, children, and uncooperative patients, to measure refractive errors without cycloplegia. The device provides measurements of both eyes simultaneously within one second from a one-meter distance. The device uses sound and lights for fixation target and measures pupil size, interpupillary distance, and ocular alignment. Besides, Plusoptix offers a referral recommendation for ophthalmologic examination based on pre-determined referral criteria [8]. The device has a measurement range from -7.0 to 5.0 D for both spherical and cylindrical measurements.

Spot Vision Screener (Welch Allyn, Skaneateles Falls, NY)

The Spot Vision Screener is a hand-held photorefractor that provides measurements of both eyes simultaneously within two seconds from a distance of 1 meter without cycloplegia. The Spot Vision Screener uses random LED visual patterns and audible sound for fixation target and provides a report including pupillary diameter, eye alignment, and estimated refraction errors. The Spot Vision Screener also gives a warning for referral a complete eye examination according to the preprogrammed referral criteria [6]. The device can make spherical measurements at a range of -7.50 to 7.50 D and cylindrical measurements at a range of -3.00 and 3.00 D.

Retinomax K-plus Screen (Righton, Tokyo, Japan)

The Retinomax K-plus Screen is a portable, hand-held autorefraction keratometer with a 3.5-inch monitor. The device provides a monocular measurement of refractive errors from a distance of 5 cm. Retinomax is both an autorefractor and a keratometer and uses a fogging mechanism to control accommodation [12]. The device also provides a quick mode in which measurements are taken in one second, and a melody is played to catch children's attention. The constantly changing color screen inside and outside of the device involves children in the examination throughout the process. The device can make spherical measurements at a range of -20.0 D to 23.0 D and cylindrical measurements at a range of 0 D to ±12 D.

Tonoref II (Nidek Co. Ltd, Gamagori, Japan)

Tonoref II is an automatic non-contact tonometer and autorefractometer. The device is mounted on the table, and the patients' forehead must be placed on the forehead of the device to take measurements. The measurement range of the device is from -30.00 to +25.00 D for spherical and from 0 D to ±12 D for cylindrical measurements.

Study outcomes

Spherical, cylindrical, cylindrical axis, and spherical equivalent (SE) measurements acquired from four devices were evaluated. While calculating SE with [sphere+(cylinder power/2)] formula, the cylindrical and axis components were transformed into vectorial representations due to the problems related to the analysis of the astigmatic component in the traditional form with the following formulas [13]:

Jackson cross-cylinder at axis 0°: (J0) = (-[cylinder (D)/2] cos [2 × α]);

Jackson cross-cylinder at axis 45°: (J45) = (-[cylinder (D)/2] sin [2 × α]).

The C and α represent the negative cylindrical value, and axis in the radians, respectively. The J0 value identifies the powers at 90° and 180°. Negative J0 values indicate against-the-rule astigmatism, whereas positive J0 values indicate with-the-rule astigmatism. The J45 value defines oblique astigmatism. Positive J45 values identify the astigmatic component at 135°, and negative values do so at 45°.

Statistical analysis

The normality of the data was evaluated with the Kolmogorov-Smirnov test. Age and refractive measurements were presented as mean ± standard deviation. Categorical variables were stated as number (n) and percentage (%). The difference between measurements was assessed by the one-sample t-test. About 95% confidence interval of the difference between the compared methods was also calculated. Bland-Altman plot was used to evaluate the agreement between devices, and 95% limits of agreement (LoA) were determined for spherical equivalent, J0, and J45 values. Reliability was analyzed by using the interclass correlation coefficient (ICC). Based on the 95% confidence interval, reliability was divided into four groups as follows: values less than 0.5 as low, between 0.5-0.75 as moderate, 0.75-0.9 as good, and higher than 0.9 as excellent reliability. Data obtained in the study were analyzed using Statistical Package for the Social Science software version 20 (IBM, SPSS 20 for Windows. Armonk, NY, USA). A value of $p<0.05$ was considered statistically significant.

Results

106 eyes of 53 children who completed all of the measurements were taken into the analysis. The mean age of children was 9.45±1.68 years. Eleven (21%) of the children were female, and 42 (79%) of those were male. We evaluated the measurements in Table 1 and Table 2, which were designed to define and compare the spherical power, cylindrical power, axis, spherical equivalent, J0 and J45 values between devices. Also, Bland-Altman plots for comparing devices in terms of spherical equivalent, J0, and J45 values are shown in figure 1. Also, the ARF rate in the study group was detected to be 24.5%.

The mean spherical power was 0.5±1.25, 0.46±1.35, 0.04±1.48, and 0.78±1.72 in Spot Vision Screener, PlusoptiX A12, Retinomax K-plus Screen, and Tonoref II, respectively. All of the devices measured spherical power significantly more myopic than the Tonoref II ($p<0.001$ for PlusoptiX A12 and Retinomax K-plus Screen, and $p=0.003$ for Spot Vision Screener).

The mean cylindrical power was -0.89±0.97, -0.7±0.65, -0.69±0.81 and, -0.80±0.83 in Spot Vision Screener, PlusoptiX A12, Retinomax K-plus Screen, and Tonoref II, respectively. Only Retinomax K-plus Screen measured cylindrical power significantly lower than the Tonoref II ($p=0.001$).

The mean axis measurements were 81.9±73.8, 82.3±74.07, 98.2±69.61 and, 101.1±72.89 in Spot Vision Screener, PlusoptiX A12, Retinomax K-plus Screen, and Tonoref II, respectively. The Spot Vision Screener and the PlusoptiX A12 measured axis significantly lower than the Tonoref II ($p=0.008$ and $p=0.02$).

The distributions of differences between the three devices in terms of SE, J0, and J45 values were shown in figure 2. The mean SE, J0, and J45 measurements were 0.05±1.14, 0.36±0.51 and 0.018±0.2 in Spot Vision Screener, 0.1±1.28, 0.28±0.35 and 0.011±0.18 in PlusoptiX A12, -0.3±1.41, 0.24±0.43 and -0.02±0.22 in Retinomax K-plus Screen, and 0.38±1.65, 0.31±0.42 and -0.026±0.21 in Tonoref II respectively. All of the devices measured SE significantly more myopic than the Tonoref II ($p<0.001$ for PlusoptiX A12 and Retinomax K-plus Screen, and $p=0.002$ for

Table 1. Mean refraction values obtained with Spot Vision Screener, PlusoptiX A12, Tonoref II, and Retinomax K-plus Screen

Group	Mean±SD	Range
Spot Vision Screener (non-cycloplegic)		
• Spherical power (D)	0.5±1.25	-4 to 3.5
• Cylindrical power (D)	-0.89±0.97	-4.25 to 0
• Axis (Degrees)	81.9±73.8	1 to 180
• SE (D)	0.05±1.14	-4.5 to 2.63
• J0 (D)	0.36±0.51	-0.61 to 2.12
• J45 (D)	0.02±0.2	-0.76 to 0.51
PlusoptiX A12 (non-cycloplegic)		
• Spherical power (D)	0.46±1.35	-3.75 to 4.75
• Cylindirical power (D)	-0.7±0.65	-3 to 0
• Axis (Degrees)	82.3±74.07	1 to 180
• SE (D)	0.12±1.28	-4.12 to 3.5
• J0 (D)	0.28±0.35	-0.25 to 1.49
• J45 (D)	0.01±0.18	-0.47 to 0.66
Retinomax K-plus Screen (non-cycloplegic)		
• Spherical power (D)	0.04±1.48	-4 to 5.25
• Cylindirical power (D)	-0.69±0.81	-3.5 to 0
• Axis (Degrees)	98.2±69.61	1 to 183
• SE (D)	-0.3±1.41	-5 to 3.63
• J0(D)	0.24±0.43	-0.35 to 1.71
• J45(D)	-0.02±0.22	-0.91 to 0.86
Tonoref II (cycloplegic)		
• Spherical power (D)	0.78±1.72	-3.75 to 7.5
• Cylindirical power (D)	-0.80±0.83	-4 to 0
• Axis (Degrees)	101.1±72.89	1 to 180
• SE (D)	0.38±1.65	-4.75 to6
• J0 (D)	0.31±0.42	-0.36 to 1.83
• J45 (D)	-0.026±0.21	-0.92 to 0.8

SD: Standard deviation, D: Diopter, SE: Spherical equivalent, J0: Jackson cross-cylinder power at axis 90° and 180° J45:Jackson cross-cylinder power at axis 45° and 135°

Table 2. Comparison of refraction values obtained with Spot Vision Screener, PlusoptiX A12, Tonoref II, and Retinomax K-plus Screen

Group	Mean difference±SD	95% CI of the difference	*p* value
Tonoref II (cycloplegic) vs. Spot Vision Screener (non-cycloplegic)			
Spherical power (D)	0.30±1.03	0.106 and 0.509	0.003
Cylindrical power (D)	0.041±0.35	-0.028 and 0.110	0.2
Axis (Degrees)	20.8±88.7	2.628 and 38.989	0.02
SE (D)	0.33±1.08	0.120 and 0.540	0.002
J0 (D)	-0.015±0.25	-0.063 and 0.033	0.5
J45 (D)	-0.041±0.166	-0.073 and -0.009	0.01
Tonoref II (cycloplegic) vs. PlusoptiX A12 (non-cycloplegic)			
Spherical power (D)	0.39±0.76	0.23 and 0.54	<0.001
Cylindirical power (D)	-0.062±0.35	-0.134 and 0.009	0.08
Axis (Degrees)	21.25±71.2	5.781 and 36.718	0.008
SE (D)	0.36±0.74	0.211 and 0.510	<0.001
J0 (D)	0.022±0.19	-0.015 and 0.06	0.2
J45 (D)	-0.031±0.13	-0.059 and -0.003	0.02
Tonoref II (cycloplegic) vs. Retinomax K-plus Screen (non-cycloplegic)			
Spherical power (D)	0.74±1.05	0.534 and 0.946	<0.001
Cylindirical power (D)	-0.099±0.28	-0.155 and -0.043	0.001
Axis (Degrees)	5.53±86.99	-13.342 and 24.414	0.6
SE (D)	0.69±1.08	0.481 and 0.903	<0.001
J0 (D)	0.075±0.2	0.034 and 0.115	<0.001
J45 (D)	-0.006±0.14	-0.006 and -0.033	0.7

SD: Standard deviation, CI: Confidence Interval, D: Diopter, SE: Spherical equivalent, J0: Jackson cross-cylinder power at axis 90° and 180° J45:Jackson cross-cylinder power at axis 45° and 135°

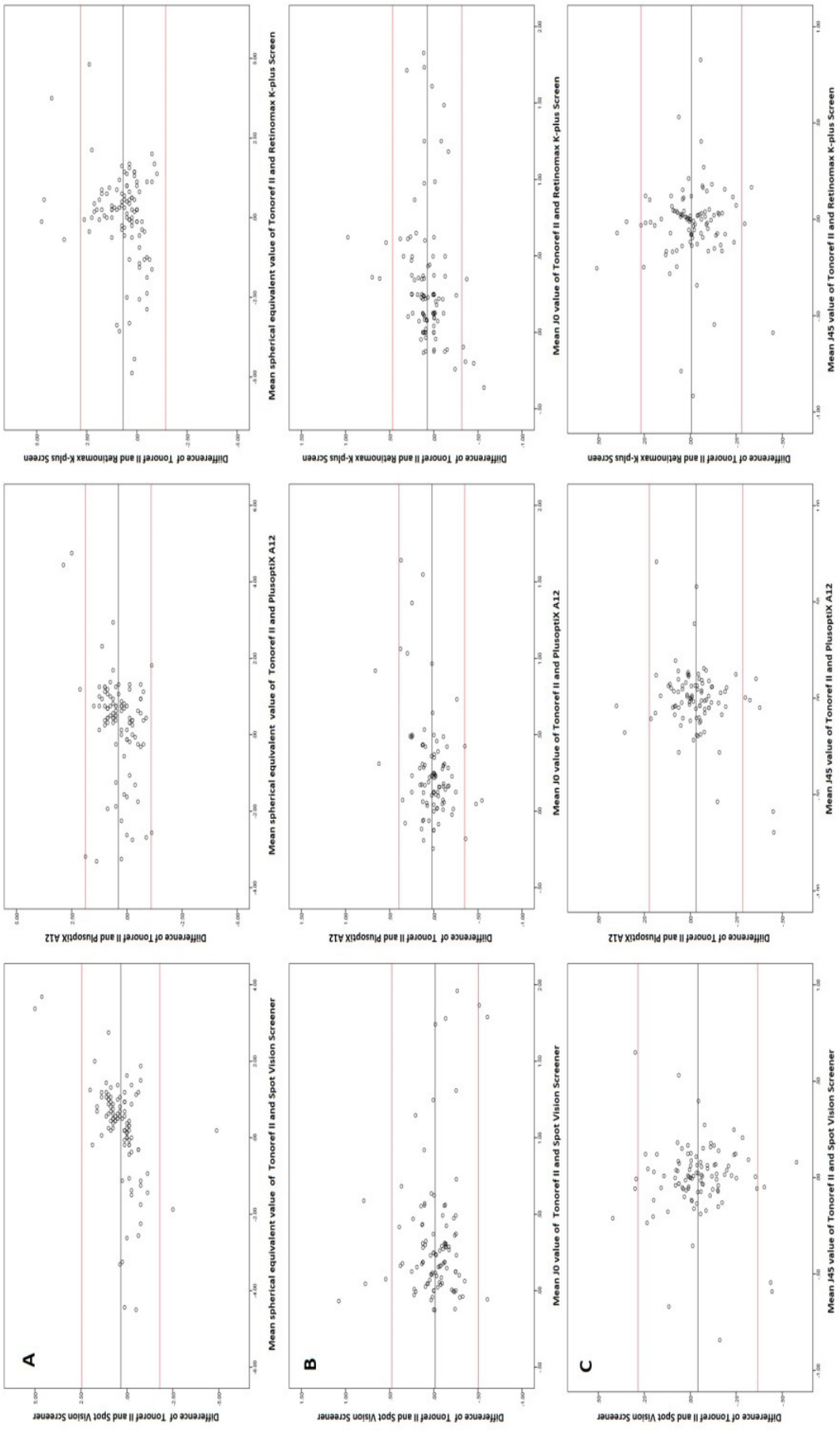

Figure 1. Bland-Altman plots showing agreement between devices in terms of spherical equivalent (A), J0 (B) and J45 (C) values

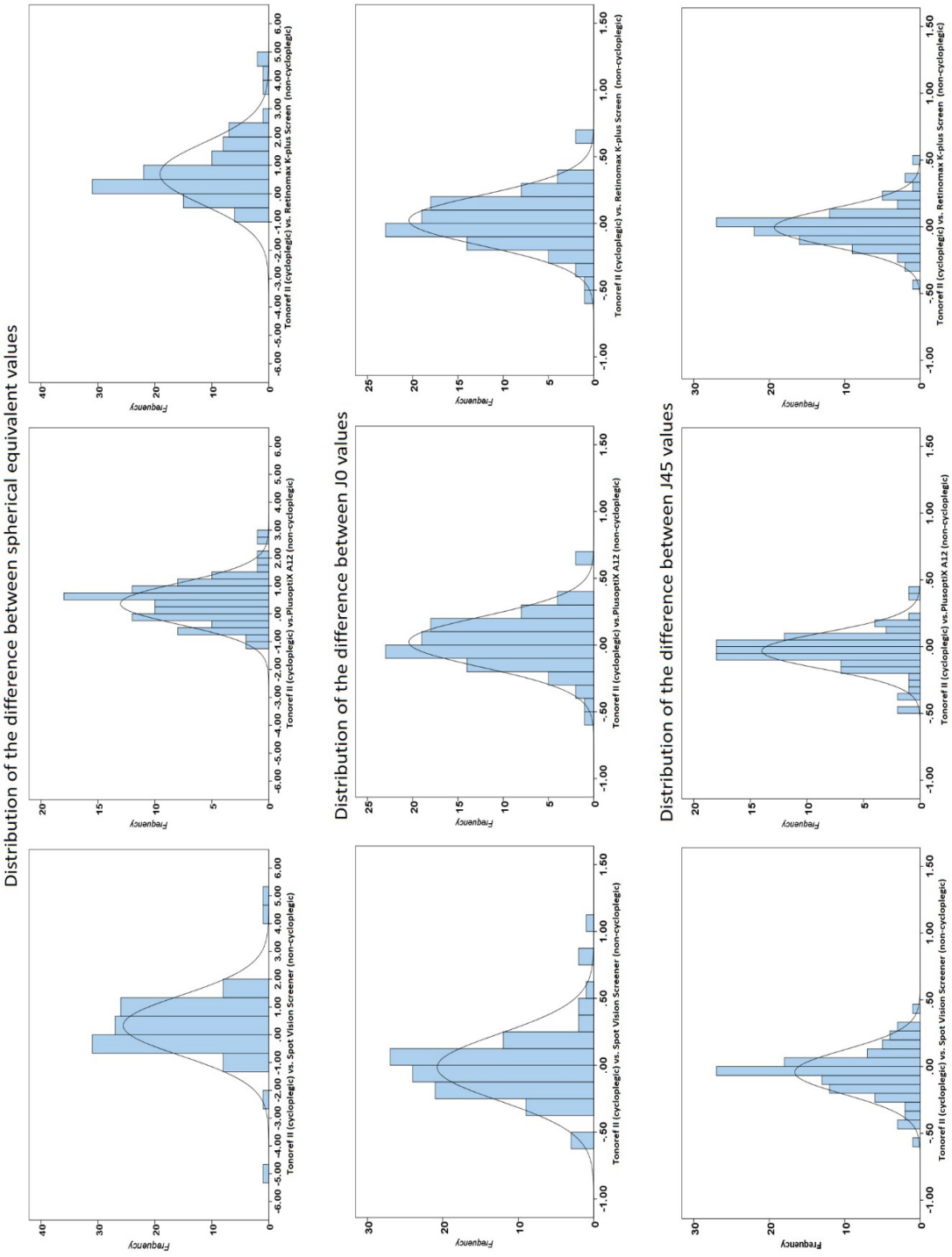

Figure 2. Frequency distribution of the differences in terms of spherical equivalent, J0 and J45 values between devices

Spot Vision Screener). The difference of J0 was similar between Spot Vision Screener, PlusoptiX A12, and Tonoref II. However, Retinomax K-plus Screen measured J0 significantly lower than the Tonoref II (p<0.001). On the other hand, the mean difference of J45 was similar between Retinomax K-plus Screen and the Tonoref II (p=0.7).

Table 3 summarizes the ICC between devices in terms of spherical power, cylindrical power, axis, SE, J0, and J45 values. The reliability of measurements between devices was variable. However, the best reliability results were obtained between Tonoref II and PlusoptiX A12. While excellent reliability was detected in spherical power, cylindrical power, SE and J0 between Tonoref II and PlusoptiX A12 [interclass correlation coefficient (ICC):0.930, 95% confidence interval (CI):0.801-0.909; ICC:0.921, 95% CI:0.882-0.947; ICC:0.927, 95% CI:0.891-0.951; and ICC:0.920, 95% CI:0.880-0.946, respectively], good reliability was determined in axis and J45 (ICC:0.752, 95% CI:0.685-0.852 and ICC:0.845, 95% CI:0.769-0.897, respectively).

Table 3. Interclass correlation coefficient (ICC), 95% confidence interval and reliability of measurements

Pairs-Parameters	ICC	95%Confidence Interval	*p* value	Reliability
Tonoref II (cycloplegic) vs. Spot Vision Screener (non-cycloplegic)				
Spherical power (D)	0.865	0.801-0.909	<0.001	Good
Cylindrical power (D)	0.955	0.934-0.970	<0.001	Excellent
Axis (Degrees)	0.421	0.129-0.616	0.004	Low
SE (D)	0.832	0.752-0.886	<0.001	Good
J0 (D)	0.917	0.877-0.944	<0.001	Excellent
J45 (D)	0.813	0.724-0.873	<0.001	Good
Tonoref II (cycloplegic) vs. PlusoptiX A12 (non-cycloplegic)				
Spherical power (D)	0.930	0.896-0.954	<0.001	Excellent
Cylindrical power (D)	0.921	0.882-0.947	<0.001	Excellent
Axis (Degrees)	0.752	0.685-0.852	<0.001	Good
SE (D)	0.927	0.891-0.951	<0.001	Excellent
J0 (D)	0.920	0.880-0.946	<0.001	Excellent
J45 (D)	0.845	0.769-0.897	<0.001	Good
Tonoref II (cycloplegic) vs. Retinomax K-plus Screen (non-cycloplegic)				
Spherical power (D)	0.879	0.821-0.918	<0.001	Good
Cylindrical power (D)	0.969	0.955-0.979	<0.001	Excellent
Axis (Degrees)	0.421	0.107-0.624	0.007	Low
SE (D)	0.858	0.790-0.904	<0.001	Good
J0 (D)	0.938	0.908-0.958	<0.001	Excellent
J45 (D)	0.879	0.822-0.918	<0.001	Good

ICC: Interclass Correlation, D: Diopter, SE: Spherical equivalent, J0: Jackson cross-cylinder power at axis 90° and 180°
J45:Jackson cross-cylinder power at axis 45° and 135°

Discussion

Previous studies showed that children with ADHD had a higher prevalence of refractive errors such as astigmatism, hypermetropia and amblyopia, Hence, vision screening is crucial for the early detection of refractive errors and prevent amblyopia in these children [3, 5, 14]. In this context, this is the first study that compares the noncycloplegic refraction measurement values obtained by Spot Vision Screener, PlusoptiX A12, and Retinomax K-plus Screen, and cycloplegic values by Tonoref II in children with ADHD. Although noncycloplegic measurements of hand-held devices underestimate or overestimate the values in terms of spherical and cylindrical powers, axis, SE, J0, and J45 compared to cycloplegic measurements of autorefractometer, PlusoptiX A12 had an excellent interclass correlation with Tonoref II regarding the spherical and cylindrical powers, SE, and J45.

Studies investigating the visual function and ocular abnormalities in children with ADHD reported a higher frequency of reduced visual acuity, subnormal stereoacuity, refractive errors, convergence abnormalities, small optic discs, heterophoria, and cognitive visual problems [14]. Also, a decreased accommodative response

has been demonstrated in these children [15]. Ocular abnormalities in ADHD were explained by various mechanisms consisting of neurological dysfunctions in cortical regions, inhibition impairment, decrease in the optic nerve's axonal volume, and balance control failure [3].

In children with ADHD, amblyopia, hypermetropia, astigmatism, and heterotropia were found 1.89, 1.82, 1.73, and 2.01 times more likely than without ones in a population based study [3]. Refractive errors may contribute to a decrease in concentration, leading to more evident symptoms of inattention and hyperactivity. Astigmatism and hyperopia affect both near and distance vision, unlike myopia, which affects only distance vision. Accordingly, astigmatism and hyperopia can affect an individual's attentional abilities more than myopia. In particular, uncorrected hyperopia and astigmatism, and amblyopia were found to be associated with reading impairment and thus academic performance [16, 17]. Therefore, detecting refractive errors is also important in the differential diagnosis of learning disabilities accompanying ADHD [18].

Amblyopia is a common, preventable cause of visual impairment in children and adults with a prevalence of 1.6-3.6% and one of the associated risk factors is uncorrected refractive errors. Higher prevalences of amblyopia in ADHD were mentioned in previous studies above. Furthermore, in a recent study, it was reported that the risk of developing ADHD is higher (1.81 fold) in children with amblyopia. The relationship between ADHD and amblyopia was speculated on sharing similar predisposing factors such as developmental delay, mental retardation, and respiratory allergic diseases [19]. Although the ARF rate may differ according to study groups, in our study group, the ARF rate at 24.5% was higher than the ARF rate which is 3.6% in the general population [20].

Photoscreeners and hand-held autorefractometers have been used for screening refractive errors, and several studies were conducted to compare devices in healthy school-aged children and also some specific patient populations, including autism and intellectual disability [7, 21, 22]. However, there was no study comparing non-cycloplegic measurements of photoscreeners and hand-held autorefractometers with cycloplegic measurements in children with ADHD in the literature.

Teberik et al. [7] evaluated the comparison of non-cycloplegic measurements of three different photoscreeners consist of Plusoptix A12, Spot Vision Screener, and Retinomax K-plus 3 with cycloplegic autorefractometer measurements in healthy school-aged children. They reported that Plusoptix A12 had compatible results with cycloplegic autorefractometer in all measurements, but the integration level was moderate. The accuracy of Plusoptix A12 was reported better in the myopic, astigmatic, and anisometropic eyes than hyperopic eyes due to underestimating hyperopic refractive errors [20]. Similarly, in our study, although PlusoptiX A12 underestimates hyperopia of 0.39 D, the device had an excellent interclass correlation with Tonoref II regarding the spherical and cylindrical powers, SE, and J45 in children with ADHD. The Plusoptix was found very useful compared with cycloplegic retinoscopy, especially in determining axis and cylinder power in various studies [23]. Ugurbas et al. [21] evaluated the validity of Plusoptix S04 as a vision screening device and reported that non-cycloplegic measurements of the device are accurate for children with intellectual disability. Furthermore, in a study by McCurry et al. [22], it was reported that vision screening with Plusoptix S08 reduced the full ophthalmologic examination requirement in one-third of the screening children with autism.

Yilmaz et al. [8] evaluated the comparison of Plusoptix A09 (non-cycloplegic) and Retinomax K-Plus-3 (cycloplegic) with retinoscopy (cycloplegic) in children. Both devices showed high agreement (ICC greater than 0.90 for all) with cycloplegic retinoscopy in terms of spherical power, cylindrical power, and SE. In our study, the Retinomax K-plus Screen acquired more myopic SE values compared other three devices, and the ICC showed good reliability (ICC between 0.75 and 0.90) in terms of spherical power, SE and J45. The difference between the two studies in terms of spherical values may have resulted from the inability of non-cycloplegic measurements to eliminate accommodation in our study, unlike Yilmaz et al. [8], [24]. Additionally, previous studies confirmed that non-cycloplegic measurements of

Retinomax devices were inaccurate. However, under cycloplegia, Retinomax had similar accuracy compared with retinoscopy and table-top autorefractometer in terms of spherical, cylindrical, and SE values [25]. Nevertheless, in another study, non-cycloplegic measurements of Retinomax demonstrated better results than photoscreening in terms of astigmatism and similar results in terms of hyperopia [26]. Similarly, in our study, the Retinomax K-plus Screen had excellent reliability (ICC greater than 0.90) in terms of cylindrical power and J0. Akil et compared the cycloplegic and non-cycloplegic refractive measurements of Retinomax K-plus 3 and table-top autorefractokeratometer with cycloplegic retinoscopy. They found a good agreement between Retinomax and autorefractokeratometer in J0 and J45, unaffected by cycloplegia [12].

The Spot Vision Screener has demonstrated good sensitivity and moderate specificity in detecting ARFs in children with developmental disabilities [6]. Teberik et al. [7] reported moderate integration levels between non-cycloplegic measurements of Spot and cycloplegic measurements of autorefractometer in terms of spherical, cylindrical, and SE values. In another study, Spot was found with high specificity to detect refractive errors; however, the sensitivity was found low for hyperopia [27]. In our study, we found excellent reliability in terms of cylindrical power and J0. However, there was a 0.33 D myopic shift of SE compared to cycloplegic measurements. Similarly, Qian et al. [28] indicated that Spot had a myopic shift of 0.17 D compared to cycloplegic retinoscopy. They also reported that J0 and J45 values had medium correlations with cycloplegic retinoscopy. Nevertheless, the Spot was found to strongly agree with cycloplegic retinoscopy for the refractive error and strabismus evaluation.

Small sample size, absence of cycloplegic measurements with hand-held autorefractometers, examination of both eyes of the children, and lack of comparison of the devices in terms of detecting ARFs are the limitations of this study. The cycloplegic retinoscopy has been used as the gold standard for refractive error measurements in most studies. However, in the present study cycloplegic autorefraction was utilized as a gold standard instead of retinoscopy. Given the controversy that this standardization might be debated, the validity of cycloplegic autorefraction for refractive error examination in children has been approved to be accurate and in sync with cycloplegic retinoscopy previously [9, 29, 30]. In addition, dynamic retinoscopy may be more beneficial in children with ADHD, since the accommodation response is reduced.

In conclusion, novel vision screening programs are widely used for detecting visual problems in children. In our study, all of the hand-held devices showed excellent reliability in terms of cylindrical power and J0 and good reliability for J45. This may guide for prescribing cylindrical power and axis; however, cycloplegic measurements may be necessary for prescribing accurate spherical values. Even though all devices had advantages or disadvantages, Plusoptix A12 showed excellent reliability for detecting refractive errors in children with ADHD. The elimination of the cycloplegia requirement, remote measurement, and short examination time in these children may increase patient compliance.

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Ethics committee approval

The Local Ethics Committee of Sivas Cumhuriyet University School of Medicine approved the study design and procedures (date: 03/12/2020 and number: 2020-12/01).

Authors' contributions to the article

Study Design: D.Y.Y., E.B., S.A.S.; Data Collection: E.B., D.Y.Y., D.D.;

Analysis: D.Y.Y.; Writing: D.Y.Y., E.B., C.K., S.A.S.;

Approval: D.Y.Y., E.B., C.K., S.A.S., D.D.

All authors have discussed the entire study and approved the final version.

Research Article

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Evaluation of clinical, laboratory and prognosis of patients in an influenza epidemic

Bir influenza epidemisinde hastaların klinik, laboratuvar ve prognozlarının değerlendirilmesi

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Abstract

Purpose: Influenza is an infectious respiratory disease that causes widespread epidemics and pandemics in the community. It is usually self-limiting but may cause significant morbidity and mortality due to complications. We aimed at evaluating the clinical, laboratory and prognosis of adult patients hospitalised with a pre-diagnosis of seasonal influenza.

Materials and methods: It is a descriptive study based on the data from 50 patients hospitalised with a preliminary diagnosis of seasonal influenza between 1 December 2015 and 31 January 2016.

Results: The average age of the patients was 55.44±17.17 years (20-89) and 64% of them were women. Of the cases, 36% were H1N1 positive, 30% were H3N2 positive and 34% had negative PCR results. The time from the onset of symptoms to hospital admission was 3.72±2.51 days (1-11). Comorbidities were present in 38 (76%) patients. Four patients had mortality.

Conclusion: Influenza can be self-limited according to the characteristics of the host, as well as cause mortality. In this study, it was found to cause severe illness and death in a healthy young adult. Therefore, it is important to avoid contact with infected patients, hand washing and vaccination to reduce influenza and its complications. In addition, if our preliminary diagnosis of influenza continues, further evaluations should be made even if the PCR is negative.

Key words: Influenza, epidemic, mortality, H1N1, vaccine.

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Öz

Amaç: İnfluenza, toplumda yaygın olarak görülen epidemi ve pandemilere yol açan bulaşıcı bir solunum yolu hastalığıdır. Genellikle kendi kendini sınırlayabilirken komplikasyonlar nedeniyle önemli morbidite ve mortaliteye neden olabilir. Mevsimsel grip ön tanısıyla yatırılarak takip edilen erişkin hastaların klinik, laboratuvar ve prognozlarının değerlendirilmesi amaçlandı**.**

Gereç ve yöntem: 1 Aralık 2015-31 Ocak 2016 tarihleri arasında mevsimsel influenza ön tanısı ile yatırılan 50 hastanın bilgilerine dayanan tanımlayıcı bir araştırmadır.

Bulgular: Hastaların ortalama yaşı 55,44±17,17 (20-89) ve %64'ü kadın cinsiyette idi. Olguların %36'sı H1N1 pozitif, %30'u H3N2 pozitif ve %34'ünün PCR sonucu ise negatif idi. Semptomların başlamasından itibaren hastaneye başvurana dek geçen süre 3,72±2,51 gündü (1-11). 38 (%76) hastanın komorbiditesi bulunmaktaydı. Dört hastada mortalite ile sonuçlandı.

Sonuç: İnfluenza, konağın özelliğine göre kendi kendi sınırlayabildiği gibi mortaliteye de neden olmaktadır. Bu çalışmada sağlıklı, genç bir erişkinde şiddetli hastalık ve ölüme neden olduğu görüldü. Bu nedenle influenzayı ve komplikasyonlarını azaltmak için enfekte hastalar ile temastan kaçınmak, el yıkama ve aşılama önemlidir. Ayrıca influenza ön tanımız devam ediyorsa PCR negatif olsa bile ileri değerlendirmeler yapılmalıdır.

Anahtar kelimeler: İnfluenza, epidemi, mortalite, H1N1, aşı.

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Introduction

Influenza is a common, highly contagious, acute viral respiratory disease worldwide [1]. Influenza virus is an RNA virus from the Orthomyxoviridae family and is classified in three groups as A, B and C. In recent years, influenza D has also been identified [2]. Influenza A and B are the most common types that cause disease in humans. Influenza C-related acute respiratory disease has been reported in children and is rare in adults [3].

Influenza virus is the causative agent that is prevalent in the community, whose clinic ranges from mild to severe and can cause death. High fever, dry cough, burning in the throat, malaise, generalised muscle and joint pain, headache and, to a lesser extent, nausea, vomiting and diarrhoea are some of the symptoms [4].

H1N1 virus has been identified as a new influenza strain that first emerged in Mexico in 2009 and carries the genes of human, swine and avian influenza viruses [5]. It was announced as the first pandemic of the 21st century by the World Health Organization on 11 June 2009 [6].

In this study, we aimed at evaluating the clinical, laboratory and prognosis of adult patients who were hospitalised with a pre-diagnosis of seasonal influenza in Kutahya Training and Research Hospital between 1 December 2015 and 31 January 2016.

Materials and methods

This is a descriptive study based on the data from patients admitted to the infectious diseases and pulmonology clinics with a preliminary diagnosis of seasonal influenza between 1 December 2015 and 31 January 2016. Nasopharyngeal swabs were taken on special transport medium and sent to the reference laboratory in special transport containers. Diagnosis of the samples was made by real time polymerase chain reaction (RT-PCR). The samples were analyzed by RT-PCR for respiratory viruses such as coronavirus, human bocavirus, parainfluenza type 1-2-3-4, respiratory syncytial virus A and B, rhinovirus, adenovirus, enterovirus, parechovirus, influenza A(H1N1), influenza A(H3N2), influenza B, human metapneumovirus. Those who did not show any of these viruses were considered negative.

The study included patients over 18 years of age. Based on the sample result, the patients were assigned into H1N1, H2N3 and negative groups and evaluated in terms of age, gender, duration of symptoms until admission to the hospital, clinical properties, laboratory tests at diagnosis and follow-up [complete blood count, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, C-Reactive Protein (CRP) etc.], radiologic imaging, treatments, and prognosis.

Information of the patients was retrieved from their medical records and hospital files by retrospective scanning.

The statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) 22.0 statistical package program. Descriptive statistics were expressed as number, percentage, mean, standard deviation, median and interquartile range. Kolmogorow Smirnov and Shapiro Wilk tests were used to evaluate whether the data met the normality hypothesis. Chi-square and Kruskall-Wallis tests were used for the data analysis (since the data did not meet the normality hypothesis). *P* values below 0.05 were considered statistically significant.

The study was approved by the Non-Interventional Clinical Research Ethics Committee of Kutahya Health Sciences University on 11/11/2021 with the decision numbered 2021/15-08.

Results

At the time of admission, patients complained of fever, cough, sputum, sore throat, headache, myalgia, and malaise. Fever, cough, myalgia, and malaise were the most common symptoms. The difference between genders was not statistically significant, although 32 (64%) of the cases were women and 18 (36%) were men ($p>0.05$). Of the cases, 36% were H1N1 positive, 30% were H3N2 positive and 34% had negative PCR results. The distribution of the samples by gender is given (Table 1). The age parameter met the normality hypothesis, whereas the blood leukocyte count, platelet, AST, ALT, LDH and CRP parameters did not. The average age was 55.44±17.17 years [minimum (min):20, maximum (max):89, median: 56.50]. Twenty-six percent of the patients received treatment in

intensive care, 24% in infectious diseases, 22% in pulmonology, 6% in internal medicine and 22% in other units. The time from the onset of symptoms to hospital admission was 3.72±2.51 days (min:1, max:11). An underlying disease was noted in 38 patients (76%), four (8%) had chronic renal failure (CRF), eight (16%) had diabetes mellitus (DM), and ten (20%) had hypertension (HT). Sixteen (32%) patients had chronic obstructive pulmonary disease (COPD), which was the most common comorbidity (Table 2).

Table 1. Nasopharyngeal sample results by gender

	Sample			
Gender	**H1N1 n(%)**	**H3N2 n(%)**	**Negative n(%)**	***p* value**
Female	12 (37.5%)	9 (28.1%)	11 (34.4%)	
Male	6 (33.3%)	6 (33.3%)	6 (33.3%)	>0.05

Table 2. Demographic and laboratory parameters of patients with prediagnosis of influenza

		Mean ± S.D	**Median (IQR**	**Min- max**
Age		55.44±17.17	56.5 (44-64.75)	20-89
Gender (n /%)	Female		32 (64%)	
	Male		18 (36%)	
Sample (n/%)	H1N1		18 (36%)	
	H3N2		15 (30%)	
	Negative		17 (34%)	
Time until hospital admission		3.72±2.51	3 (2-4)	1-11
Underlying disease (n/%)	COPD		16 (32%)	
	None		12 (24%)	
	HT		10 (20%)	
	DM		8 (16%)	
	CRF		4 (8%)	
Inpatient service (n/%)	Chest diseases		11 (22%)	
	Infectious diseases		12 (24%)	
	Internal medicine		3 (6%)	
	Other		11 (22%)	
	Intensive care		13 (26%)	
Blood Leukocyte count (mm3)		8894±4845.13	7350 (5750-11025)	3100-24000
Platelet (mm3)		225260±117713.04	198000 (140000-291000)	99000-657000
AST (IU/L)		68.62±220.89	30 (20-40)	8-1579
ALT (IU/L)		46.96±120.39	20 (15.75-31.25)	6-811
LDH (IU/L)		275.44±271.5	200 (147.25-280)	120-1482
CRP (mg/L)		98.12±77.57	86 (27.75-159)	5-319
Result (n/%)	Discharge		46 (92%)	
	Exitus		4 (8%)	

Abbreviations: S.D: standard deviation, IQR: Interquartile range, min: minimum, max: maximum AST: Aspartate aminotransferase ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, CRP: C-reactive protein, CRF: Chronic Renal Failure COPD: Chronic Obstructive Pulmonary Disease

The laboratory results of patients with a pre-diagnosis of influenza are summarized in Table 2.

Patients with elevated AST and ALT levels were tested for viral hepatitis and the results were negative. Patients with suspected influenza were isolated in the infectious diseases ward. They were trained on hand washing, use of masks, gloves and disinfectants. All suspected cases were treated with oseltamivir for five days and nonspecific antibiotic treatment was given to patients with suspected bacterial superinfection. All of the 50 patients we had inpatient follow-up and treatment had a pneumonia clinic. Pneumonic consolidation or ground glass findings were detected on lung imaging.

Forty-six patients (92%) recovered and were discharged, whereas two patients diagnosed with H1N1 (4%), one patient diagnosed with H3N2 (2%) and one patient with negative results (2%) died. There was no significant difference in the sample results (H1N1, H3N2 and other) between the discharged and deceased patients (p>0.05). One of the H1N1 positive patients who died was a 31-year-old man with no comorbidities and the other was an 89-year-old woman with CRF.

The younger patient was diagnosed with acute tonsillitis in an external center and was hospitalized with nonspecific antibiotic treatment approximately five days before his admission to our hospital. But, as fever persisted and his complaints of cough, shortness of breath and bloody sputum persisted, he was referred to our hospital. He was hospitalized with a pre-diagnosis of pneumonia due to influenza. Laboratory tests revealed WBC: 3300/mm^3, platelet: 116.000/mm^3, AST: 1579 IU/L, ALT: 811 IU/L, LDH: 1482 U/L, CRP: 105 mg/L (<5). Thoracic computed tomography showed bilateral diffuse pneumonic infiltration areas. The patient, who had previously used antibiotics, was started on piperacillin-tazobactam, levofloxacin, teicoplanin and oseltamivir. He was transferred to the intensive care unit on the second day of hospitalization due to low saturation and was followed up. The patient after being intubated one day after admission to the intensive care unit died on the fourth day of hospitalization due to respiratory arrest. Among the deceased patients, the 89-year-old patient also had a pneumonia clinic.

An 81-year-old H3N2 positive woman with CRF and a 54-year-old woman with a negative influenza result were also deceased. The 81-year-old patient complained of respiratory distress and had a pneumonia clinic. She was started on oseltamivir and piperacillin-tazobactam treatment. The patient died on the third day of hospitalization due to respiratory and cardiac arrest. The 54-year-old patient with underlying COPD who was hospitalized with a pre-diagnosis of influenza-associated pneumonia and whose PCR result was negative died on the 15th day of hospitalization. Posteroanterior chest x-ray showed infiltration areas in the right lower and mid lobes.

Discussion

A novel H1N1 virus emerged in 2009 [7]. Seasonal influenza cases are seen every year due to Influenza A (H1N1, H3N2 subtypes) and Influenza B, usually with one subtype being more dominant [8]. Some data have reported that severe influenza infection in prepubertal period is more common in men [9, 10]. In a study conducted in Australia, it was found that the proportion of women to men was low in children under 15 years of age and adults over 75 years of age, but higher between 20-65 years of age [11]. In a study by Saltoglu et al. [12] during the H1N1 pandemic in 2009, the number of women was found to be higher, and although it was similar in our study, it was not statistically significant.

The average age of patients followed up with a diagnosis of influenza was found to be 41.91 (18-82) years in a study of 34 cases and 33 (17-82) years in another study of 84 cases [12, 13]. In a systematic review and meta-analysis study, aging was found to be a risk factor for seasonal influenza [14]. In line with the literature, the oldest patient in our study was 89 years of age and 24% of our patients were over 65 years of age (median age 56.50 years). In addition to the patients who died at an advanced age, a 31-year-old male patient with no known comorbidities had H1N1 virus-associated pneumonia and died due to respiratory failure.

In Spain, 10% of nasopharyngeal samples from intensive care patients with severe respiratory failure had false negative results and it was concluded that a negative RT-PCR result does not exclude H1N1 [15]. In

critically ill patients with lower respiratory tract disease, there may be influenza viral clearance in the upper respiratory tract, so molecular testing of lower respiratory tract specimens is recommended to detect the virus [16]. When the virus reaches the lower respiratory tract, the probability of detecting the agent in nasopharyngeal specimens decreases. In line with this information, the patient with a negative influenza PCR result being admitted to the hospital five days after the onset of symptoms may have had a negative effect on the PCR result. In addition, a negative PCR result despite testing for other viruses causing respiratory tract infections other than influenza suggests that there may have been false negativity due to sample collection or transport errors.

In influenza, the virus starts to spread one day before the onset of symptoms and continues for up to seven days. This period may be prolonged in children, the elderly and immunocompromised patients. In the study of Yasar et al. [17] the time from the onset of symptoms to hospital admission was found to be 3 days. During the season of influenza between December 2015 and April 2016, the duration was found to be 2 days in 132 cases evaluated in the paediatric age group [18]. Similarly, the mean duration in this study was 3.72±2.51 days (min:1, max:11).

A study found that H1N1 positivity was 57%, H3N2 positivity was 2%, and Influenza B positivity was 40.4% in samples taken from hospitalized patients [19]. The CDC reported that 70.8% were influenza A and 29.2% were influenza B according to the United States of America (USA) influenza surveillance data from the 2015-2016 season. Subtyping was reported as 80.7% H1N1 and 19.3% H3N2 for influenza A; 68.5% B/Yamagata and 31.5% B/Victoria for influenza B [20]. In another study during the same influenza season, an analysis of the subtypes of 234 Influenza A cases revealed that the dominant type was H1N1 with a rate of 94.9% and the rate of H3N2 was 5.1% [21]. In the present study, the rates were close to each other with H1N1 positivity at 36% and H3N2 positivity at 30%; influenza PCR results were negative in 34% of the samples and influenza B was not detected.

The presence of comorbidities increases the risk of serious illness. The disease was more severe in patients with chronic lung disease, pregnancy, and obesity [22]. In New York, of the 47 cases who died due to H1N1, 79% were found to have underlying diseases and risk conditions [23]. In the present study, risk factors for mortality were not investigated, but the 31-year-old case with mortality had no comorbidities, which is controversial with this information. There are studies describing that elderly patients may have cross-reactive antibodies that protect against H1N1 due to exposure to influenza infection from an early age and a history of influenza vaccination [24, 25]. According to this study, this may have been due to the lack of sufficient protective antibodies in our patient who died at a young age or the presence of another factor that could not be identified. The other patients who died had underlying diseases such as CRF and COPD.

Empirical antiviral treatment should be considered in patients with a pre-diagnosis of seasonal influenza in the absence of comorbidities and in the younger age group, and treatment can be adjusted, if necessary, upon further evaluation. Patients with underlying diseases should be informed about influenza, vaccinated, and should be admitted to hospital as soon as possible when symptoms occur.

The limitations of this study are the small number of patients, as it was a single-center, retrospective study and did not include outpatients. We aimed to draw attention with the conclusion of our study, but multicenter studies with large patient coverage are warranted to generalize this conclusion.

In conclusion, other than the patients who died at an advanced age, mortality was also reported in a young patient without comorbidities. H1N1 can also cause severe illness and death in healthy young adults. As in our study, RT-PCR negativity in nasopharyngeal samples is not conclusive evidence to exclude influenza. False negativity should be kept in mind. If our strong pre-diagnosis of influenza continues, we should perform further examinations and evaluations. The best way to prevent influenza and influenza-related complications is to be vaccinated annually, morbidity and mortality can be reduced by vaccination. Avoiding contact with infected patients and hand washing are important in protection against influenza other than vaccination, training on this issue should be increased.

Conflict of interest: The authors declare that there is no conflict of interest.

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Ethics committee approval: The study was approved by the Non-Interventional Clinical Research Ethics Committee of Kütahya Health Sciences University (Date: 11/11/2021 and decision no: 2021/15-08)

Authors' contributions to the manuscript

The study conception was planned by T.T. and D.M.O. Patient data were provided by T.T., D.M.O., S.E.P., and A.B.U. edited the materials and methods section. T.T. and A.B.U. performed the analysis and interpretation of the study. Writing was done by T.T, D.M.O, S.E.P., A.B.U. reviewed and made corrections. All authors read, discussed, and approved the final version of the manuscript.

Research Article

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The role of coagulation mechanism in the development of acute thrombosed hemorrhoidal disease

Akut tromboze hemoroidal hastalığın gelişiminde pıhtılaşma mekanizmasının rolü

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Abstract

Purpose: Hemorrhoidal disease is a common benign anorectal disease. Acute thrombosis that occurs during the course of hemorrhoidal disease is a painful complication. Factors affecting its etiopathogenesis are not known definitively. The aim of this study, therefore, was to assess predisposition to coagulation during the development of the disease.

Material and methods: 30 patients with acute thrombosed hemorrhoidal disease and 30 other patients with hemorrhoidal disease but no thromboses were included in the study. Samples collected from these patients were analyzed with thromboelastography machines. The results were compared with patients' demographic data.

Results: No statistically significant difference was found between the groups as per age, sex, diarrhea, history of a similar attack, history of surgical treatment, spicy food consumption, fibrous food consumption, and regular exercise. The results of our study revealed that the alpha angle was smaller in patients with acute thrombosed hemorrhoidal disease.

Conclusion: Hypercoagulability does not occur in patients with acute thrombosed hemorrhoidal disease.

Key words: Hypercoagulability, thrombosed hemorrhoid, thromboelastography.

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Öz

Amaç: Hemoroidal hastalık sık görülen benign anorektal bölge hastalığıdır. Hemoroidal hastalığın seyri sırasında oluşan akut tromboz ağrılı bir komplikasyondur. Etyopatogenezinde etkili etmenler tam bilinmiyor. Çalışmamızdaki amacımız hastalığın gelişiminde pıhtılaşmaya yatkınlığı değerlendirmektir.

Gereç ve yöntem: Akut tromboze hemoroidal hastalığı olan 30 hasta ile hemoroidal hastalığı olan ancak tromboz gelişmeyen 30 hasta çalışmaya dahil edildi. Bu hastalardan alınan numuneler tromboelastografi cihazında incelendi. Demografik verileri ile kıyaslandı.

Bulgular: Gruplar arasında yaş, cinsiyet, ishal, benzer atak öyküsü, cerrahi tedavi öyküsü, baharatlı gıda tüketimi, lifli gıda tüketimi ve düzenli egzersiz açısından istatistiksel olarak anlamlı bir fark bulunmamıştır. Akut tromboze hemoroidal hastalığı olan hastalarda alfa açısının daha küçük olduğunu tespit ettik.

Sonuç: Akut tromboze hemoroidal hastalığı olan hastalarda hiperkoagülabilite durumu oluşmamaktadır. Ancak kanamaya yatkınlık gelişmektedir.

Anahtar kelimeler: Hiperkoagülabilite, tromboze hemoroid, tromboelastografi.

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Introduction

About 40% of individuals with hemorrhoidal disease are asymptomatic [1]. Hemorrhage, pain, pruritus or perianal wetness are usually seen in symptomatic patients. Some of the patients present with swelling and acute pain in the perianal site [2]. Such presentation can be observed both in internal and external hemorrhoidal disease. Acute thrombosis of the external hemorrhoidal cushion is referred to as acute thrombosed external hemorrhoidal disease, while thrombosis of the external hemorrhoid is also known as “acute perianal hematoma” [3]. The strangulation of prolapsed internal hemorrhoids and conditions of thrombosis, gangrene or ulceration are called acute thrombosed internal hemorrhoidal disease. Both conditions are defined as acute hemorrhoidal disease [3-6]. Thrombosis of external hemorrhoids often accompany the thrombosed protrusion of internal hemorrhoids from the anal canal. External or internal acute hemorrhoidal disease is an extremely painful clinical condition. It significantly affects patients’ daily lives.

Researchers have put forth many theories on the pathogenic mechanism of thrombosed hemorrhoidal disease. However, the factor that extensively explains the condition is yet to be known. The most important question within this framework of theories is about the reason why all patients with the same conditions do not have thrombosis. The aim of this study was, therefore, to identify other factors causing this condition, except those laid out in these theories, along with an assessment on whether patients were more prone to coagulation since whether coagulation factors have any effect on this condition was not known either.

Materials and methods

This prospective randomized study was conducted between 1 January 2019 and 1 January 2020. Local board of ethics approval was also obtained (Decision No: 2018/1602). The first 30 patients with acute thrombosed hemorrhoidal disease (ATHD) and the first 30 patients with hemorrhoid disease without thrombosis (HD) who applied to our clinic between these dates were included in the study. The exclusion criteria for the patients were ascertained to be the presence of a disorder affecting the coagulation system and history of medication. All the patients were evaluated by an experienced colorectal surgeon. Patients who did not exceed 72 hours from the onset of ATHD were included in the study.

The patients’ data on age, sex, pain, hemorrhaging, diarrhea, history of a similar attack, history of medical and surgical treatment, spicy food consumption, fibrous food consumption, and regular exercise routines, or lack thereof, were collected.

After we obtained written and oral consents from the patients, 1 cc citrated blood was drawn from each patient. These samples were then analyzed by thromboelastography (TEG) machines at the hematology laboratory within an average of 30 mins. TEG® analyzer (TEG 5000 Thromboelastograph® Hemostasis System, Haemonetics Corporation, Niles, USA) was used in the study (Figure 1). TEG analyzers were regularly calibrated and tested. Intrinsic pathways, extrinsic pathways, fibrinogen and hyperfibrinolysis values were analyzed in the samples. Screened parameters included prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), R time (reaction time, representing the period from the beginning of the measurement to the time when the distance between the two curves reach 1 mm), K time (signifying clot formation time and representing the period during which the clot reaches an amplitude of 20 mm), alpha angle (angle deg, i.e. the angle between the tangent line drawn from the curve separating from the horizontal axis and the horizontal axis and representing the speed at which the clot gains maximum force), maximum amplitude (MA, reflecting the maximum clot amplitude or maximum clot elasticity and is rather associated with the number of thrombocytes, thrombocyte functions and fibrinogen levels] and LY30 value [representing the depression in the amplitude of the clot at 30 minutes after the maximum amplitude point).

Within the scope of statistical analyses for the study, the Kolmogorov-Simirnov and Shapiro-Wilk tests were used to control the distribution of parameters. Student’s t-test was used for the comparison of independent groups. Non-categorical data, chi-square tests were used in cross tables. In the interpretation of statistical hypothesis tests, type 1 error was set at 0.05. The collected data were analyzed by the SPSS program.

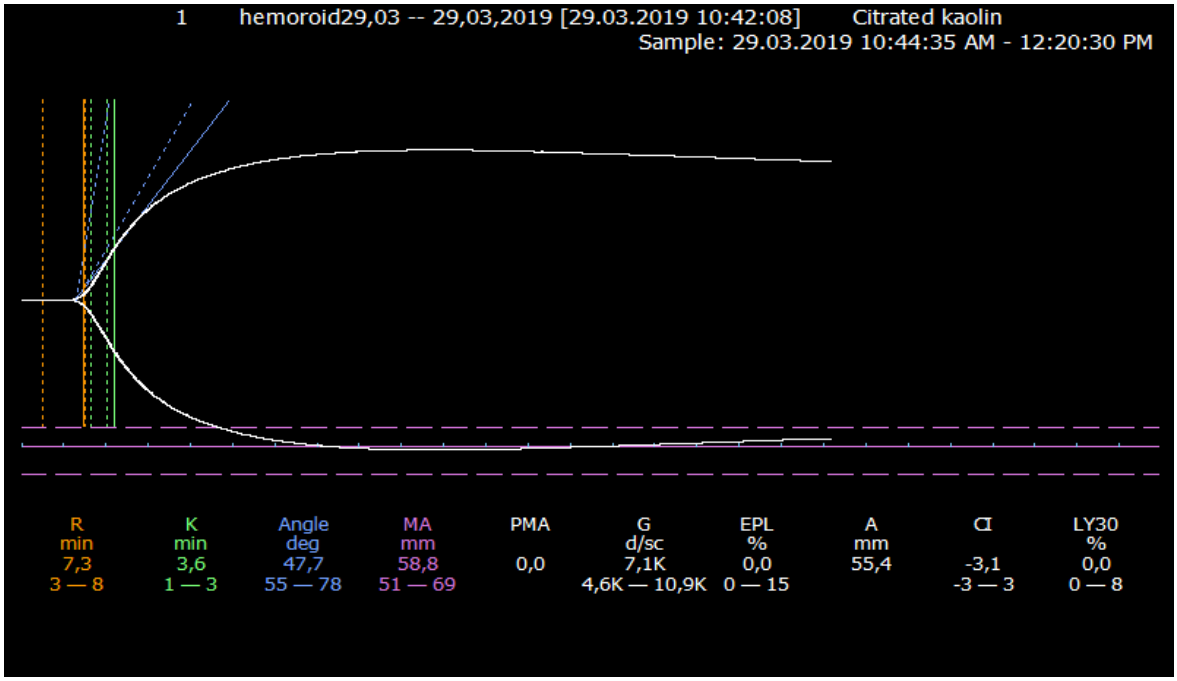

Figure 1. Thromboelastogram graphic of the patient with thrombosed hemorrhoids

Results

The study included a total of 60 patients. 30 of these patients had ATHD, while another 30 had HD. The mean ages of the patients with ATHD and HD were 35.10 (23-63) and 35.63 (20-65) respectively. No statistically significant difference was found between the groups as per age, sex, diarrhea, history of a similar attack, history of surgical treatment, spicy food consumption, fibrous food consumption, and regular exercise (Table 1). However, the results of our study revealed a statistically significant difference between patients with ATHD and HD in terms of pain, hemorrhaging and history of medical treatment. Pain and hemorrhaging rates were higher in patients with ATHD. The rate of history of medical treatment, on the other hand, was higher in patients with HD.

TEG analysis showed that the alpha angles were smaller in the ATHD group. This result indicated that patients with ATHD were more prone to hemorrhaging than those with HD. This result was statistically significant as well. No statistically significant difference was found in any of the other values (Table 2).

Discussion

Thrombosed hemorrhoidal disease is a common complication of hemorrhoidal disease. Acute thrombosed hemorrhoidal diseases can develop during the course of external and internal hemorrhoidal disease. Thrombosed external hemorrhoidal disease occurs in the inferior anal plexus structure beneath the squamous epithelium. Some of the patients have over-straining due to constipation. Yet this situation does not explain the physiopathology of all patients' clinical conditions [7]. External hemorrhoidal disease is seen more frequently in young individuals.

Thrombosis is usually formed due to over-exercise in this age group. It is suggested that over-exercise leads to the laceration of external veins by causing a sudden and extreme increase in intraluminal pressure [7, 8]. Another theory put forth to explain the physiopathology of acute thrombosis pertains to the impairment of connective tissue support that enables engorged hemorrhoidal veins to hold on to the anal canal [7]. Prolapsed varicose veins in this area are further damaged by the traumatic impact of straining or defecating. Such traumatic impact initially leads to stasis then to clotting in hemorrhoidal veins in time through the effect of triggering factors as well. Such alterations result in acute edema formation in the skin and mucosa of the anal verge [3, 7]. The results of our study, however, revealed that thrombosis was formed in some of the patients while it did not in some others although similar conditions were seen in all. These theories failed to fully explain the

Table 1. Demographic data of patients

		Groups				
		ATHD		HD		
		n	Percent (%)	n	Percent (%)	*p*
Sex	Male	24	80%	21	70%	0.37
	Female	6	20%	9	30%	
Pain	Yes	27	90%	10	33.33%	**<0.02**
	No	3	10%	20	66.67%	
Hemorrhage	Yes	20	66.67%	10	33.33%	**<0.01**
	No	10	33.33%	20	66.67%	
Constipation	Yes	21	70%	19	63.33%	0.58
	No	9	30%	11	36.67%	
Diarrhea	Yes	0	0%	1	3.33%	0.80
	No	30	100%	29	96.67%	
History of a similar attack	Yes	21	70%	23	76.67%	0.55
	No	9	30%	7	23.33%	
Medical treatment	Yes	8	26.67%	19	63.33%	**<0.01**
	No	22	33.33%	11	36.67%	
Surgical treatment	Yes	5	16.67%	24	80%	0.73
	No	25	83.33%	6	20%	
Spicy food consumption	Yes	12	40%	12	40%	0.29
	No	18	60%	18	60%	
Fibrous food consumption	Yes	12	40%	12	40%	0.30
	No	18	60%	18	60%	
Regular exercise routines	Yes	12	40%	7	23.33%	0.16
	No	18	60%	23	76.67%	

Table 2. TEG values of patients

	Groups				
	ATHD		HD		
	Average value	Min/Max	Average value	Min/Max	*p*
INR	1.12	0.9/1.38	1.10	0.9/1.3	0.5
PTsec	14.66	12/18	13.93	12/18	0.13
aPTT	83.86	62/110	84.26	65/110	0.91
R	7.82	6/9.9	7.98	6.7/9.9	0.61
K	3.9	2.3/5.5	3.45	1.5/5.4	0.06
Alpha value	43.21	34.5/58.5	54.71	38/70	**<0.01**
MA	52.73	44/62.5	52.86	44/61	0.93
CL	-4.53	-7/-1	-3.86	-6/-1	0.12

event that occurred in all patients. We believe that there are other factors affecting the etiology of the disease which we do not know as of now. We, therefore, investigated whether coagulation factors had an impact on thrombosis formation within the scope of our study. Further, studies in literature have reported no data on this subject which proves to be a dark spot that has not been studied before. Should this theory be accurate, one could have discussed the administration and efficacy of medication in treatment that altered the coagulation system.

Edema develops in the anal area due to a cause that increases anal tonus in prolapsed internal hemorrhoids [particularly grades 3 and 4]. Initially strangulation, then subsequent thrombosis occurs in the cushions through further increase in the anal canal pressure. Ulceration and necrosis may develop in advanced stages. Reduction of prolapsed internal hemorrhoids is challenging because of edema and increased anal sphincter pressure. Severe pain caused by edema and thrombosis increases internal anal sphincter spasms as a reflex. Sphincter spasm, in turn, leads to an increase in the severity of pain. Spasm and pain result in a vicious circle exacerbating each other [7, 9]. Edematous mucosa over thrombosed veins also causes edema in the skin and subcutaneous tissues. This edema increases in time. External hemorrhoidal cushions are usually involved in the event in these patients. The most important clinical complaint of patients is severe pain. Urinary retention may develop secondary to pain in some patients [4-7]. Such clinical presentation generally occurs after the onset of the event, in other words, it does not set off the event. We suggest that this theory or process is not a beginning but a result. The formation of clot in engorged veins is usually the beginning and booster of this clinical condition. The reason why venous clots do not form in all patients with hemorrhoidal disease, who happen to have the same clinical presentation, is not known. Patients with grade 3-4 hemorrhoidal disease suffer from this condition at certain times in their lives but the life styles of these patients are usually the same except for essential deviations. Constipation and over-straining trigger or facilitate this event but the reason why patients have problems at certain times is not known either.

The aim of this study was to reveal whether there was a hematologic condition that increased coagulation but our results did not reveal any difference between the groups except for the alpha value. The fact that the alpha value was smaller in patients with ATHD indicates that such patients are prone to hemorrhaging. We, however, were not able to identify the clinical significance of this result. The hypothesis we established at the planning stage of this study was that patients were prone to coagulation but our results did not support this hypothesis. We encountered the exact opposite of our hypothesis, in other words, patients were more prone to hemorrhaging.

Consequently, the results of our study revealed no hypercoagulability in the development of ATHD. Blood drawn from patients immediately before thrombosis may prove to be more significant but we do not know at which time. We believe that one of the possible ways to clarify this situation can be a comparison of the course of hemorrhoidal disease and its complications in patients on anticoagulants and those who are not.

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

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Ethics committee approval: Permission was obtained from Necmettin Erbakan University Non-Pharmaceutical and Medical Devices Ethics Committee Decision No: 2018/1602.

Authors' contributions to the article

S.A. and M.Ç. have constructed the main idea and hypothesis of the study. S.A., M.Ç., A.T., M.Ş. developed the theory and arranged/edited the material and method section. A.V., Ö.K. S.A. M.Ş. have done the evaluation of the data in the Results section. Discussion section of the article written by S.A., M.Ç., A.T.; Ö.K., M.Ş., A.V. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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The effect of dynamic contrast magnetic resonance imaging (DCE-MRI) in the diagnosis of breast cancer cases

Meme kanseri olgularının tanısında dinamik kontrastlı manyetik rezonans görüntüleme (DCE-MRI) yönteminin etkisi

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Abstract

Purpose: The aim of this study is to investigate the effect of dynamic contrast magnetic resonance imaging (DCE-MRI) in the diagnosis of breast cancer (BC) and to compare it with ultrasonography (USG).

Materials and methods: In our study, 78 patients who underwent preoperative DCE-MRI and USG in our diagnosis center in TRNC between 2009 and 2022 and were diagnosed with BC histopathologically were investigated retrospectively.Findings obtained according to the BI-RADS classification in both methods, detection of BC, detection of tumor foci (TF) in multiple tumors (multicentric and multifocal tumors) (MT), correct diagnosis rates (CDR)s in invasive lobular cancers (ILC) and invasive ductal cancers (IDC) were compared and the results were evaluated statistically.

Results: The mean age of the ILC and MT cases was found to be significantly lower than the IDC and unifocal tumor(UF) cases (p<0.05). CDRs in BC cases; 94.8% of DCE-MRI, 78.2% of USG (p<0.05), in the detection of TFs; 94.5% of DCE-MRI, 73.6% of USG (p<0.05), in detecting ILC cases; DCE-MRI 87.5%, USG 37.5% (p>0.05) in detecting IDC cases; it was determined as 95.7% in DCE-MRI and 80.2% in USG (p<0.05).

Conclusion: DCE-MRI is a more effective diagnostic method than USG in the diagnosis of BC cases and TFs in MT cases.

Key words: Magnetic resonance with dynamic contrast, breast cancer, multiple tumors, ultrasonography.

Akalin A, Acar HZ. The effect of dynamic contrast magnetic resonance imaging (DCE-MRI) in the diagnosis of breast cancer cases. Pam Med J 2023;16:222-228.

Öz

Amaç: Bu çalışmanın amacı meme kanseri (MK) tanısında; dinamik kontrastlı manyetik rezonans görüntülemenin (DCE-MRI) etkisini araştırmak ve ultrasonografi (USG) ile karşılaştırmaktır.

Gereç ve yöntem: Çalışmamızda 2009 ve 2022 yılları arasında KKTC deki tanı merkezimizde preoperative olarak DCE-MRI ve USG yapılan ve histopatolojik olarak MK tanısı konulan 78 olgu retrospektif olarak araştırılmıştır.

Olgularda her iki yöntemde Bİ-RADS sınıflamasına göre elde edilen bulgular; MK tespiti, multipl tümörlerde (multisentrik ve multifocal tümör) (MT) tümör odaklarının (TO) tespitinde, invaziv lobüler kanserlerde (İLK) ve invaziv duktal kanserlerde (İDK) doğru tanı oranları karşılaştırılmış, sonuçlar istatistiksel olarak değerlendirilmiştir.

Bulgular: İLK ve MT olgularının yaş ortalaması, İDK ve tek odaklı tümör (TOT) olgularına göre anlamlı ölçüde küçük bulunmuştur (p<0,05). DTO ları MK olgularında; DCE-MRI'ın %94,8, USG'nin %78,2 (p<0,05), TO larının tespitinde; DCE-MRI'ın %94,5, USG'nin %73,6 (p<0,05), İLK olgularını saptamada; DCE-MRI'ın %87,5, USG'nin %37,5 (p>0,05), İDK olgularını saptamada; DCE-MRI'ın %95,7, USG'nin%80,2 (p<0,05) olarak tespit edilmiştir.

Sonuç: DCE-MRI, MK olgularının ve MT olgularında TO'ların tanısında USG'ye göre daha etkili bir tanı yöntemidir.

Anahtar kelimeler: Dinamik kontrastlı manyetik rezonans, meme kanseri, multipl tümör, ultrasonografi.

Akalın A, Acar HZ. Meme kanseri olgularinin tanisinda dinamik kontrastli manyetik rezonans görüntüleme (DCE-MRI) yönteminin etkisi. Pam Tıp Derg 2023;16:222-228.

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Introduction

According to the global cancer statistics of 2020, BCs are the most common type of cancer in women in many countries around the world and the most common cause of death from cancer [1]. According to the data of The International Agency for Research on Cancer (IARC), there is a 66% increase in cancer-related death rates compared to 1960 [2]. Every year, 685,000 deaths are seen in the world due to BC [1].

Early diagnosis is of great importance to reduce mortality rates in cancer cases. Although studies with serum molecular markers have opened a new era in the early diagnosis of BC, as in many cancer types, the most widely used diagnostic tests are still USG and mammography (MG) [3]. Despite this, there are publications reporting that the absolute diagnosis rates of USG and MG in MTs are quite low, especially in BC types such as ILC [4, 5]. Some studies have reported that other advanced diagnostic tests such as DCE-MRI are more effective in these cases [6, 7]. The DCE-MRI imaging method is based on the quantitative measurement of enhancement. The number of studies investigating the effect of DCE-MRI in BCs, MTs and subtypes of BC and comparing it with conventional USG is not very large.

Therefore, in this study, we investigated the effect of DCE-MRI in the diagnosis of BC, MT cases and some BC subtypes (ILC and IDC) and compared it with USG.

Materials and methods

Study type and ethical approval

This study was a retrospective observational study and approval was obtained from The Girne American University Health Sciences Ethics Committee.

Patients and data collection

In our study, breast DCE-MRI and USG examinations of 359 patients who admitted to our diagnosis center in Cyprus between 2009 and 2022 were evaluated retrospectively.

Inclusion criteria of these cases in our study:

1.Cases in which DCE-MRI and USG were performed together preoperatively,

2.Cases biopsied after imaging tests,

3.Cases with malignant histopathological results as a result of biopsy.

Exclusion criteria of the cases in our study:

1.Cases who underwent secondary operation,

2.Recurrent cases,

3.Cases in which USG was not performed together with DCE-MRI,

4.Cases without biopsy,

5.Cases with benign histopathological results.

Cases were evaluated according to the American College of Radiology (ACR) Breast Imaging Reporting and Data System (BI-RADS) classification.

BI-RADS I, II, III cases, low probability of malignancy,

BI-RADS IV, V cases were considered as cases with a high probability of malignancy (correct diagnosis).

Imaging techniques

DCE-MRI images were obtained with a 1.5 Tesla MRI device (Signa HDx, 1.5 T, GE Healthcare) using a double-stranded breast coil. Precontrast T2 FSE axial, T2 FSE fatsat axial, T2 fatsat sagittal, diffusion sequences were obtained. Postcontrast images were obtained in the dynamic phase. Following the images taken without contrast, 0.1 mmol/kg IV contrast material (gadolinium) was injected at a rate of 3 ml per second in the dynamic examination and 6 consecutive multiphase images were taken in the same region. Added axial and sagittal vibrant sequences. After the examination, subtraction images and enhancement curves of early and late contrast sections were obtained in all patients. It was examined whether the lesions had contrast enhancement pattern, dynamic curve types, diffusion restriction. Nonmass enhancement lesions were investigated without background parenchymal enhancement. The time-intensity curve (TIC) was classified as persistent (type I), plateau (type II), and wash-out (rapid contrast loss) (type III).

USG examinations were performed using GE Logiq 730 pro, GE Logiq 9, GE Logiq S7 expert devices and 4-15MHz, 5-13MHz linear transducers. Both breasts and axilla were examined in different planes. PI and RI values were measured. Contour features, spicular extension, aspect ratio, echopattern, posterior acoustic shadowing and presence of calcification were investigated for benign-solid differentiation.

Statistical analysis

In our study, whether the data were suitable for normal distribution was examined using the Shapiro Wilk test. Descriptive statistics for continuous variables are given as (mean±standard deviation) in those that fit the normal distribution. Descriptive statistics for categorical variables are given as frequency and percentage (n (%)). In the independent group comparisons of continuous variables, the t-test was used for those showing conformity with the normal distribution. Pearson chi-square test and Yates Correction test were used to compare categorical variables between groups. Statistical analysis was made in IBM SPSS v.21 package program. The significance level was taken as α=0.05.

Results

All of our patients are women. Age range of our cases; 25-82, mean age; 48. The mean age of our ILC cases (n=6) was 37.6, our IDC cases (n=66); 48.5, the difference was statistically significant (p<0.05).MT (11.5%) was detected in 9 of our patients (n=78), and UF tumor was detected in 69 of them (88.4%). The mean age of our UF cases (n=69) was 49.1, the mean age of our MT cases (n=9) was 37.3, the difference was statistically significant (p<0.05).

The distribution of our BC cases according to the age variable is shown in (Table 1).

The histopathological distribution of TFs (n=91) in our cases (n=78) is as follows: IDC in 71 cases (78.0%), ILC in 16 cases (17.6%), mucinous in 2 cases (2.1%), tubular carcinoma in 1 case (1.1%), comedocarcinoma in 1 case (1.1%) (Table 2).

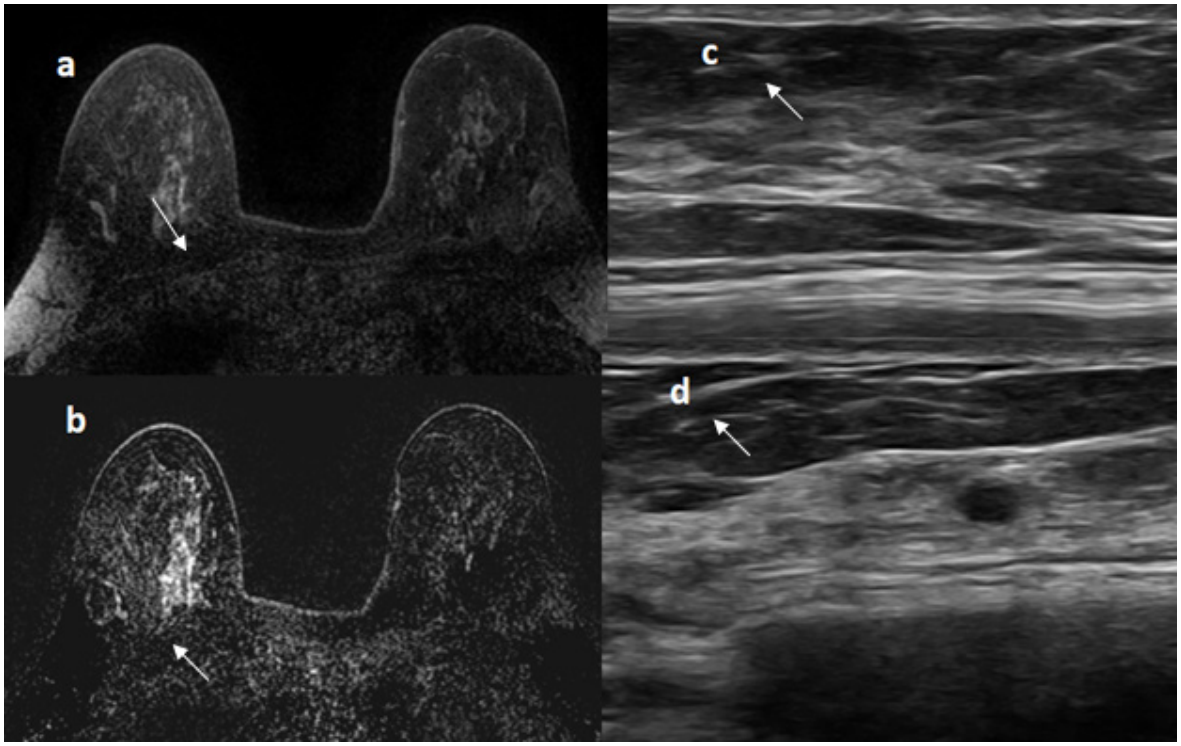
It was reported as BI-RADS III (n=13), BI-RADS IV (n=29) and BI-RADS V (n=29) in 71 foci with histopathological diagnosis of IDC. DCE-MRI results of the same cases were BI-RADS III (n=3), BI-RADS IV (n=19), BI-RADS V (n=49). It was reported as BI-RADS III (n=11), BI-RADS IV (n=3) and BI-RADS V (n=2) in 16 foci with histopathological diagnosis of ILC. DCE-MRI results of the same cases are BI-RADS III (n=2), BI-RAD 80.2%S IV (n=1), BI-RADS V (n=13) (Picture1, 2).

Table 1. Distribution of our breast cancer cases according to age variable

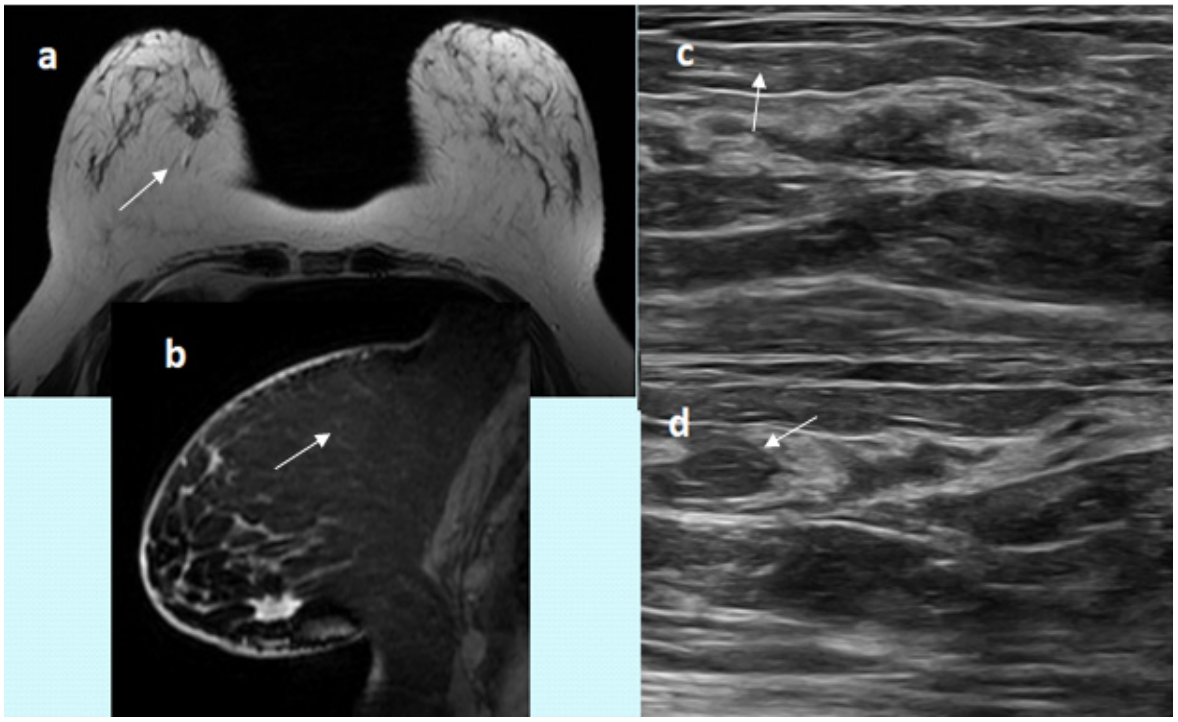
Breast cancer (n=78)		***p***
ILC (n=6) 37.6±3.0	IDC (n=66) 48.5±10.9	**<0.05**
Unifocal (n=69) 49.1±11.0	Multiple (n=9) 37.3±3.0	**<0.05**

Table 2. Histopathological findings in tumor foci (n=91) in our breast cancer cases (n=78)

Breast cancer	**Number of cases**	**%**
Invasive ductal carcinoma	71	78.0
Invasive lobular carcinoma	16	17.6
Mucinous carcinoma	2	2.1
Tubular carcinoma	2	1.1
Comedo carcinoma	1	1.1



Picture 1. DCE-MRI and USG images in an ILC case (DCE-MRG BI-RADS IV, USG BI-RADS II)
a and b- Segmental pathological contrast enhancement that does not form a mass in the right breast inner quadrant in postcontrast series in DCE-MRI
c and d- No fibrocystic changes and no solid mass were detected on USG



Picture 2. DCE-MRI and USG images in an ILC case (DCE-MRI BI-RADS V, USG BI-RADS IV)
a- Irregular contoured lesion in precontrast MRI examination
b- The same lesion showing significant contrast enhancement in the postcontrast image in DCE-MRI
c and d- No mass form on USG. Suspected hypoechoic field partially distributed in different sections

USG examination of 2 foci diagnosed with mucinous carcinoma detected BI-RADS IV (n=1) and BI-RADS V (n=1). DCE-MRI results of the same cases were BI-RADS IV (n=1), BI-RADS V (n=1). In 1 focus diagnosed with comedocarcinoma, it was BI-RADS IV in USG and BI-RADS V in DCE-MRI. In 1 focus diagnosed with tubular carcinoma, both modality results were BI-RADS V (Table 3).

CDRs (BI-RADS IV+V); 78.2% in BC (n=78) (61/78) on USG, 73.6% in TFs (n=91) (67/91), ILCs (n=16) (6/16) 37.5%, IDCs (n=71) (57/71) 80.2%, DCE-MRI in BC (n=78) (74/78) 94.8%, TFs (n=91) (86/91) 94.5%, ILCs (n=16) (14/16) 87.5%, IDCs (n=71) (68/71) 95.7% (Table 3).

Table 3. BI-RADS IV+V (Absolute diagnosis) findings in DCE-MRI and USG in breast cancer cases

	DCE-MRI (BIRADS IV+V)	USG (BIRADS IV+V)	*p*
Breast cancer (n=78)	74 (94.8%)	61 (78.2%)	**<0.05**
Tumor focus (n=91)	86 (94.5%)	67 (73.6%)	**<0.05**
Invasive lobular cancer (n=16)	14 (87.5%)	6 (37.5%)	1.00
Invasive ductal cancer (n=71)	68 (95.7%)	57 (80.2%)	**<0.05**

Discussion

Today, the most commonly used methods for BC screening and diagnosis are mammography (MG) and USG. In a national study conducted by the Japanese strategic anticancer randomized trial (J-START) organization in Japan, in the scans performed on 72,998 women with MG and USG between 2007 and 2011; it has been reported that MG is still the most important screening test in the diagnosis of BC, but the sensitivity decreases as the breast density increases, and its effectiveness increases more when USG is performed together [8]. In a study by Freer, it was reported that the sensitivity of MG decreased to 62-68% in women with dense breasts [9]. It has been reported that BC develops at a rate of 1/156 or 1/312 depending on the number and dose of MG in patients who underwent MG in BC scans [10]. Despite its advantages such as being cost-effective and portable, no radiation risk, and being able to be performed in a short time, USG in the diagnosis of BC is insufficient in the diagnosis of microcalcifications, MTs and ILCs [3, 4]. Therefore, more advanced diagnostic tests are needed. In a meta-analysis by Mann et al. [11], the sensitivity rates of DCE-MRI in BC were found to be between 81-100%. A meta-analysis of the effectiveness of USG in the diagnosis of BC by Sood et al. [12] found an overall sensitivity and specificity of 80.1%.

In our study, the ADR of USG in the diagnosis of BC was 78.1%, and DCE-MRI 94.8%, the difference was statistically significant ($p<0.05$) (Table 3).

In a study by Partridge et al. [13], it has been reported that diffusion-weighted imaging (DWI) and apparent diffusion coefficients (ADC) values in DCE-MRI, morphological criteria, and dynamic contrast enhancement curves of lesions are different in malignant lesions as reasons for the superiority of DCE-MRI method over USG in the diagnosis of BC cases who underwent DCE-MRI. Some other studies have also reported that DCE-MRI shows significant diffusion restriction and low ADC values in malignant lesions compared to benign ones [14, 15]. Zhang et al. [16], in the models they developed for the diagnosis of BC, demonstrated that ADC mean values and delayed enhancement are independent and significant factors in the diagnosis of BC [16]. In the study conducted by the same researchers, high AUC values such as 0.952 were obtained with DCE-MRI to differentiate malignant tumors in 188 cases.

ILC is the second most common (5-15%) malignancy of the breast [17]. ILC is often undetectable by MG because it is of equal or lower density to fibroglandular tissue, and microcalcifications are often undetectable in contrast to IDC or carcinoma in situ [18]. In addition, ILC cases that do not form a mass in USG and present only as structural distortion may be overlooked [19]. In a study by Wilson et al. [17], it was reported that the sensitivity was 93% with DCE-MRI, 57-81% with MG, and 68-98% with USG in ILCs. However, in the same study, it was found that false-positive diagnosis rates with USG in ILC cases could increase up to 29.9%.

In our study, the CDR of DCE-MRI (14/16) was 87.5% and USG (6/14) was 37.5% in ILC cases, and the difference was statistically insignificant (p>0.05) (Table 3). The reason why the difference is statistically insignificant may be the small number of our cases. In a meta-analysis conducted by Vera Badillo et al. [20] in 67,557 women, it was reported that 4-50% of BCs are multicentric or multifocal, ILC cases are more common, and their prognosis is worse. In a retrospective study conducted by Neri et al. [21] in 1158 BC women, it was reported that the mean age of MT cases was significantly lower than that of UF cases, and the prognosis was worse.

In our study, the mean age of MF cases was found to be significantly lower than that of UF and BC cases, and the mean age of ILC cases was significantly lower than that of IDC cases (Table 1). For these reasons, early diagnosis is more important in MT cases, especially at young ages, and more advanced diagnostic methods with high sensitivity and specificity are needed. In a study by Acar et al. [4], the sensitivity of DCE-MRI, which is one of the advanced diagnostic methods in MT cases, was found to be high, reaching 98.6%. In a study by Song et al. [7], it was reported that the sensitivity and specificity of DCE-MRI in MTs is high, tumor foci smaller than 1 cm can be detected more easily, and unnecessary biopsies can be prevented.

In our study, CDR was found to be 94.5% (86/91) in tumor foci with DCE-MRI, 73.6% (67/91) with USG, and the difference was found to be significant (p<0.05) (Table 3).

The limitation of our study is that it was not a prospective randomized study. The different aspect of our study is the limited number of studies in the literature comparing the effects of DCE-MRI and USD in BC cases.

In conclusion, DCE-MRI imaging method is more effective in detecting tumor foci than USG, and it is recommended to be applied together with USG, especially in risk group cases (genetics, young age, obesity, fibrocystic disease, bilateral mass).

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

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Authors' contributions to the article

Both authors equally contributed to the study conception and design. Both authors read and approved the final manuscript.

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Predictive value of serum vitamin B12 elevation in acute leukemia

Akut lösemilerde serum vitamin B12 yüksekliğinin prediktif değeri

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Abstract

Purpose: In this study; we sought to assess whether elevated vitamin B12 levels during the course of diagnosis might be a predictor of acute leukemias.

Materials and methods: The study was prepared by retrospectively evaluating the anamnesis and laboratory information of 95 patients diagnosed with acute leukemia (AML or ALL). Those who had any of the conditions clearly known to increase vitamin B12 levels by scanning their anamnesis and laboratory information were not included in the study.

Results: In total, it was observed that serum vitamin B12 level at the time of diagnosis was above the normal reference range (>771ng/L) in 36% of the patients. In the survival analysis performed to evaluate the effect of high serum vitamin B12 levels on prognosis, no statistically significant difference was found.

Conclusion: The data we obtained from this study shows that high serum vitamin B12 levels may have predictive value for acute leukemia.

Key words: Acute leukemia, cancer, cobalamin, vitamin B12.

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Öz

Amaç: Bu çalışmada; tanı anında bakılan yüksek vitamin B12 düzeylerinin akut lösemilerde prediktif bir faktör olup olmadığını belirlemeyi amaçladık.

Gereç ve yöntem: Çalışmamız akut lösemi (AML ve ya ALL) tanısı alan 95 hastanın anamnez ve laboratuvar bilgileri retrospektif olarak değerlendirilerek hazırlanmıştır. Anamnez ve laboratuvar bilgileri taranarak B12 vitamini düzeyini yükselttiği açıkça bilinen durumlardan herhangi birine sahip olanlar çalışmaya dahil edilmemiştir.

Bulgular: Toplamda hastaların %36'sında tanı anındaki serum vitamin B12 düzeyinin normal referans aralığının (>771ng/L) üzerinde olduğu görüldü. Yüksek serum vitamin B12 düzeyinin prognoza etkisini değerlendirmek için yapılan sağ kalım analizinde istatistiksel olarak anlamlı bir fark gözlenmedi.

Sonuç: Bu çalışmadan elde ettiğimiz veriler; yüksek serum vitamin B12 düzeylerinin akut lösemi için öngörü değeri olabileceğini göstermektedir.

Anahtar kelimeler: Akut lösemi, kanser, kobalamin, vitamin B12.

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Introduction

Acute leukemias are important due to their rapid progression and mortality, although they are relatively rare malignancies, considering their share in all malignancies 1% for acute myeloid leukemia (AML), 0.3% for acute lymphoblastic leukemia (ALL)] [1, 2]. Despite all the improvements achieved, 5-year survival rates are 28.7% for AML and 68.8% for ALL [1, 2]. Therefore, advances in the management of these diseases are very important.

Vitamin B12 (cobalamin, Cbl) is an essential vitamin for humans [3]. A cobalamin level measured from peripheral blood above the reference values is called hypercobalaminemia [4]. Hypercobalaminemia has been reported to be iatrogenic as well as been associated to some diseases such as liver diseases, alcoholism, kidney diseases, autoimmune diseases and infections [3-5]. Although varying degrees of vitamin B12 levels have been reported in different studies in solid neoplasms and hematological malignancies, the frequency of hypercobalaminemia at the time of diagnosis and the underlying pathogenesis are not clearly clarified in neoplastic patients [6-8]. It has been reported that the increase in circulating cobalamin binding protein haptocorrin may be associated with high plasma cobalamin levels. Although the physiological function of this protein, which can originate from many tissues, is not fully known, it has been shown to increase in some cancer types [6, 7]. Therefore, it has been stated that haptocorrin may be a cause of serum vitamin B12 elevation detected in malignancies [4, 6, 7].

Among hematological malignancies, high serum cobalamin levels can be detected due to the haptocorrin-like protein transcobalamin-III (TC-III), which is caused by increased leukocytes in myeloproliferative diseases, especially in chronic myeloid leukemia (CML) [9, 10]. There is not enough data in the literature regarding hypercobalaminemia in acute leukemia. Therefore, the purpose of this study was to determine the frequency of high levels of vitamin B12 in acute leukemias at the diagnosis time, to evaluate whether increased vitamin B12 levels could be a predictive factor for this disease and to reveal its relationship with prognosis in the patients. In this way, we aimed to contribute to the literature in terms of early diagnosis and management of acute leukemia.

Materials and methods

Participants, procedures and demographics

This study was prepared by retrospectively evaluating the anamnesis and laboratory information of 95 patients diagnosed with acute leukemia (AML or ALL) at Hospital between 24.10.2006-08.04.2019. Patients whose diagnosis was made at an external center and those whose blood vitamin B12 level was not measured at the time of diagnosis were not included in the study. In addition, by screening the anamnesis and laboratory information, those who had any of the conditions known to increase the levels of vitamin B12 at the diagnosis time were also excluded from the study. At this point, the following are determined as exclusion criteria: reduced glomerular filtration rate (tGFR CKD-EPI <45 ml/min/1.73 m^2), ALT (Alanine Aminotransferase) and / or AST (Aspartate Aminotransferase) >100IU/L, history of chronic hepatic failure, hepatitis (HAV, HBV, and HCV) infection, history of rheumatological disease, history of autoimmune disease, clinical or laboratory findings of infectious disease, intake of vitamin B12 supplements in oral or injection form, excessive alcohol consumption (for women more than seven drinks, for men more than fourteen drinks which contain about 14 grams of pure alcohol per week), excessive meat consumption (more than 3 servings per week) [11, 12]. All individuals who did not meet these criteria and were diagnosed with acute leukemia between the ages of 18-90 were included in the investigation. Patients were classified as male or female based on their gender. They were grouped as Aegean, Mediterranean and other according to the regions they live in. They were classified as low or normal (≤10 K/uL) and higher than normal (>10 K/uL) according to the absolute leukocyte count and they were grouped as low or normal (≤771 ng/L) and higher than normal (>771 ng/L) according to B12 level. Normal serum vitamin B12 reference values in

Pamukkale University Hospital Laboratory were 211-911 pg/mL. Our research was carried out in accordance with the Helsinki Declaration 2008 principles. This retrospective study was approved by the Pamukkale University Non-Invasive Clinical Research Ethical Committee.

Statistical analysis

The data was analyzed utilizing SPSS 22.0 [IBM SPSS Statistics 22 software (Armonk, NY: IBM Corp.)] package program. Continuous variables were represented as mean ± standard deviation (S.D.), while categorical variables were represented as frequencies and percentages. In order to examine the relationships between categorical variables, the Pearson chi-square test was used. The log-rank test was used to compare survival curves calculated by the Kaplan-Meier method. Statistical significance was defined as $p<0.05$.

Results

Eighty-one (85%) of the patients included in the study had AML and 14 (15%) had ALL. There was a relatively balanced distribution in terms of gender, with 41 (43%) female and 54 (57%) male patients. The average age of the patients at the diagnosis was 48±18.1. At the beginning of the work; while 21 (22%) of the examined patients passed away, 74 (78%) were alive.

In total, it was observed that 32 (34%) of the patients had absolute leukocyte count above normal (>10 K/uL) at the time of diagnosis, 63 (66%) were lower than normal or in the normal reference range (≤10 K/uL). There was no statistical significant difference between the groups with low or normal / high leukocyte count in terms of high vitamin B12 level [x2 (1, N=95) 0.06, (p=0.8)]. In other words, vitamin B12 elevation in the patients studied was not associated with leukocytosis.

In total, it was observed that the vitamin B12 level at the time of diagnosis was above the normal reference range (>771 ng/L) in 34 (36%) of the patients. These data were crucial in terms of showing that high vitamin B12 levels can be an important finding for the early diagnosis of acute leukemias. This rate was determined as 37% (30/81) among patients with AML diagnosis and 29% (4/14) among patients with ALL diagnosis, and this difference was not statistically significant [x2 (1, N=95) 0.3, (p=0.54)]. No significant difference was observed between the groups in terms of high B12 level according to gender and living area. [respectively; x2 (1, N=95) 0.02, (p=0.88). x2 (2, N=95) 1.8, (p=0.54)].

Survival analyzes were also performed to evaluate the effect of elevated serum vitamin B12 levels on prognosis in patients with acute leukemia. However, there was no statistically significant difference in terms of survival between the groups with and without vitamin B12 levels above normal ((log-rank test, p=0.641). There was also no significant difference between the 1-year survival, 2-year survival, 3-year survival, and 5-year survival subgroups (Table 1). In terms of overall survival rates, no statistically significant difference was found between the groups (Figure 1). This data was important in that it showed that the high vitamin B12 level at the time of diagnosis did not affect the survival rate in patients with acute leukemia, and therefore it was not directly related to the prognosis.

Mean survival time for low/normal and high B12 levels was 116.9 (100.9-133.0) and 105.0 (78.5-131.5) mo, respectively.

Table 1. Vitamin B12 level elevation ratios and p values by groups

	B12≤771 ng/L		B12>771 ng/L		Chi-square value	*p* value
	N	**%**	**N**	**%**		
Diagnosis						
AML	51	63	30	37	0.372	**0.542***
ALL	10	71.4	4	28.6		
Gender						
Woman	26	63.4	15	36.6	0.02	**0.888***
Male	35	64.8	19	35.2		
Region of residence						
Aegean	58	64.4	32	35.6		
Mediterranean	3	75	1	25	1.874	**0.546****
Other	0	0	1	100		
Absolute leukocyte count						
>10 K/uL	20	62.5	12	37.5	0.061	**0.804***
≤10 K/uL	41	65.1	22	34.9		
1 year survival						
Yes	56	64.4	31	35.6	0.11	**1.00****
No	5	62.5	3	37.5		
2 years survival						
Yes	40	63.5	23	36.5	0.154	**0.695***
No	11	68.8	5	31.2		
3 years survival						
Yes	36	69.2	16	30.8	0.004	**0.948***
No	13	68.4	6	31.6		
5 years survival						
Yes	18	64.3	10	35.7	0.003	**0.959***
No	13	65	7	35		

*: Pearson Chi-square test, **: Fisher's Exact test

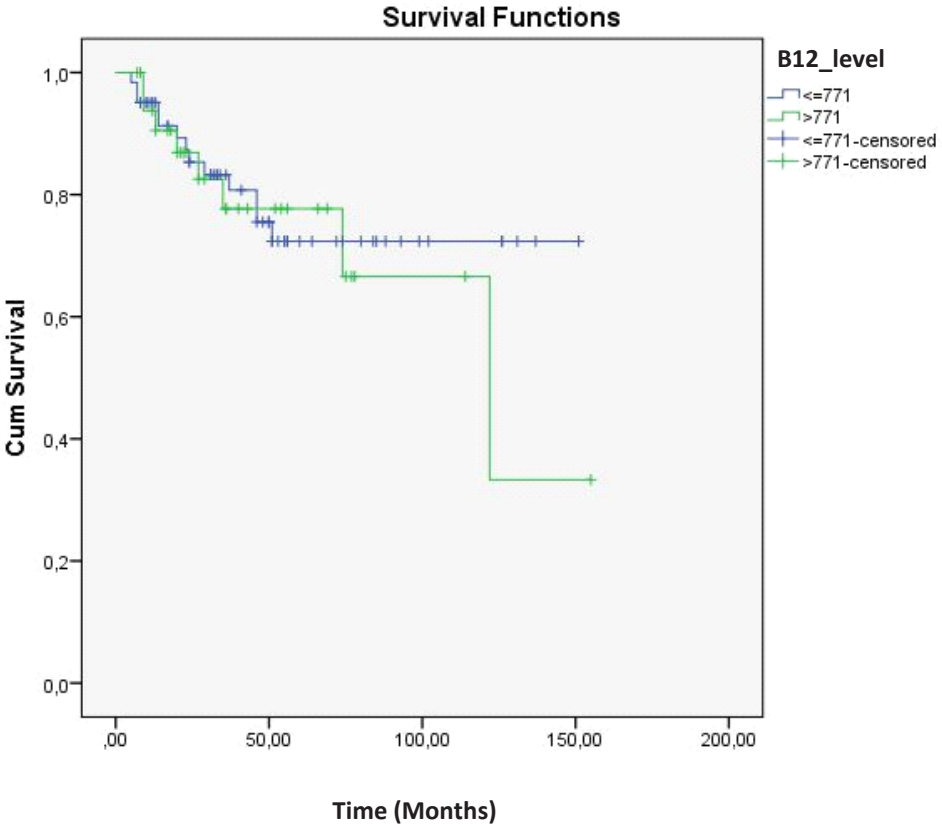

Figure 1. Survival curve according to serum B12 levels of patients with acute leukemia

Discussion

A high-level serum vitamin B12 is an important clinical finding as much as low-level serum vitamin B12 [3]. The relation of some clinical conditions (liver diseases, alcoholism, kidney diseases, autoimmune diseases and infections) with serum vitamin B12 level is well known [3, 4]. In the study; Those with high vitamin B12 levels had a significantly higher risk of malignancy during the 1-year follow-up. In addition, it has been observed that this risk is especially higher for hematological malignancies [13]. In a Latvian cohort study, the odds ratios for total and myeloid leukemia (acute, chronic, and unspecified) in patients with elevated B12 were 6.0 (95% CI 4.7-7.6; p<0.0001) and 19.2 (95% CI 13.1-28.0; p<0.0001), when compared to the control group. Thus, it was concluded that increased serum vitamin B12 levels may be a potential marker for oncohematological disorders [14]. Similarly, in a study conducted in Denmark; approximately 40,000 people who did not use vitamin B12 injections and had no known cancer history were examined for 2 years and malignancy was detected in 6.7% of those with high serum vitamin B12 levels in the first 6 months (mo) of the follow-up, whereas malignancy was detected in 2.6% of those with normal vitamin B12 levels [15]. However, there is not enough data regarding the predictive value of serum vitamin B12 level elevation in acute leukemia. Therefore, this issue was discussed in this study and as a result, serum vitamin B12 level of 36% of the patients with acute leukemia was found above normal reference values at the time of diagnosis. This indicates that high serum vitamin B12 levels may be predictive in terms of acute leukemia. At this point, performing complete blood count and peripheral blood smear tests in patients with high vitamin B12 levels in hospital admissions may be beneficial in terms of early diagnosis.

Although there are various studies in the literature regarding the relation of high vitamin B12 level with prognosis and mortality, there is not enough data on this issue in patients with acute leukemia. In a cohort study published in the journal Cancer Epidemiology, data on approximately 25,000 cancer patients were examined, and it was discovered that patients with high vitamin B12 levels had significantly lower 1-year survival rates than patients with normal levels [(1-year survival,%) Cbl: 200-600 pmol/L: 69.3%; 601-800 pmol/L: 49.6%; >800 pmol/L: 35.8%; comparison cohort: 72.6%] [16]. In another study published in 2018, the data of 523 metastatic cancer patients were evaluated retrospectively. The median survival time in the high B12 group (>911 pg/mL) was 1.8 months and 5.1 months in the normal B12 group (211-911 pg/mL) (p<0.001). According to multivariate analysis, serum vitamin B12 level was a distinct prognostic factor for overall survival (p<0.001) [17]. In another study evaluating 90 patients with hepatocellular carcinoma, it was found that survival times were lower in patients with high B12 levels. HCC patients with the highest serum B12 levels (>1,500 ng/l) had significantly lower survival (mean survival of 13.5 mo) than those in the second and lowest levels group [18]. In another study published in 2017 with the participation of 190 patients over 65 years of age in the geriatric ward, it was observed that hematological disorders were 5.7 times higher in those with high vitamin B12 levels compared to normal ones (OR=5.7; p=0.001) [19].

In our study, no significant difference was observed in terms of survival between acute leukemia patients with and without high vitamin B12 levels at the time of diagnosis, thus it was concluded that high vitamin B12 levels in these patients did not have prognostic value. The study's main limitation is the relatively small sample size. Furthermore, due to the study's retrospective design, selection bias may exist. The strengths of this study include the good quality of the data. Furthermore, we were able to exclude persons who had been given cobalamin supplement and most of those who had any of the conditions known to increase vitamin B12 levels.

In conclusion, although the data we obtained from this study indicate that high serum vitamin B12 levels may have predictive value for acute leukemia, but do not have a prognostic value, more studies are needed to reach a clear conclusion.

Conflict of interest: The authors declare no conflict of interests for this study.

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Informed consent: Patients alive at the time of data collection provided informed consent.

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Authors' contributions to the article

A.U. and B.U.K. constructed the main idea and hypothesis of the study. A.U., B.U.K., S.H., G.A.C. and N.G.; they developed the theory and arranged/edited the material and method section. A.B., B.U.K., S.H., G.A.C. and N.G. have done the evaluation of the data in the Results section. Discussion section of the article written by A.U. and B.U.K. who also reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version. It should be written after the references.

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Evaluation of tuberculosis frequency in children using biological agents

Biyolojik ajan kullanan çocuklarda tüberküloz sıklığının değerlendirilmesi

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Abstract

Purpose: Anti-TNF drugs increase the risk of tuberculosis. In this study we aimed to investigate the incidence of tuberculosis in patients using anti-TNF drugs.

Materials and methods: One hundred and fifteen pediatric cases which were received anti-TNF drugs were included in the study. The clinical and epidemiological characteristics of the cases were analyzed retrospectively.

Results: One hundred and fifteen cases using anti-TNF drugs were included in the study. The diagnoses of the cases were as follows; Juvenile Rheumatoid Arthritis 76 (66%), Ulcerative Colitis 11 (9.6%), Crohn's 7 (6%), Ankylosing Spondylitis 6 (5.2%), FMF 5 (4.3%), Psoriasis 4 (3.5%). The distribution of the agents used by the patients was; etanercept 74 (64.3%), infliximab 17 (14.8%), adalimumab 17 (14.8%), anakinra 5 (4.3%), and canakinumab 2 (1.7%). It was learned that all cases had BCG vaccinations when they were two months old, confirmed by the vaccination cards and the ministry of health's vaccination follow-up system. TST was performed in all of the cases and TST response was measured as <5mm in 89 (77.4%), 5-9 mm in 11 (8.7%), 10-14 mm in 8 (7.4%), >15 mm in 7 (5.6%) cases. Isoniazid (INH) prophylaxis was started for nine months in 17 cases with the diagnosis of latent tuberculosis. Active tuberculosis was not detected in any of the cases.

Conclusion: All patients receiving anti-TNF need to be evaluated for tuberculosis. Although it is not detected at the beginning of the treatment, regular tuberculosis screening should be continued during the treatment with contact history, symptoms, physical examination, chest X-ray, and TST/IGRA in light of current guidelines.

Key words: Tuberculosis, pediatric, anti-TNF.

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Öz

Amaç: Anti-TNF ilaçlar tüberküloz enfeksiyon riskini arttırırlar. Bu çalışmayla Anti-TNF ilaç kullanan hastalarda, tüberküloz gelişme sıklığını araştırmayı amaçladık.

Gereç ve yöntem: Çalışmaya anti-TNF ilaç kullanan 115 çocuk hasta dahil edildi. Olguların klinik ve epidemiyolojik özellikleri retrospektif olarak değerlendirildi.

Bulgular: Anti-TNF ilaç kullanan 115 olgu çalışmaya dahil edildi. Olguların tanıları; Juvenil Romatoid Artrit 76 (%66), Ülseratif Kolit 11 (%9,6), Crohn's 7 (%6), Ankilozan Spondilit 6 (%5,2), FMF 5 (%4,3), Psoriasis 4 (%3,5) şeklindeydi. Hastaların kullandığı ajanların dağılımı ise; etanersept 74 (%64,3), infliximab 17 (%14,8), adalimumab 17 (%14,8), anakinra 5 (%4,3) ve kanakinumab 2 (%1,7) şeklindeydi. Tüm vakaların iki aylıkken BCG aşısı olduğu öğrenildi, aşı kartları ve sağlık bakanlığının aşı takip sisteminden teyit edildi. Tüm olgulara TDT yapıldı ve TDT yanıtı <5mm 89 (%77,4), 5-9 mm 11 (%8,7), 10-14 mm 8 (%7,4), >15 mm 7 (%5,6) olarak ölçüldü. Latent tüberküloz tanısı alan 17 olguya 9 ay izoniazid (INH) profilaksisi başlandı. Olguların hiçbirinde aktif tüberküloz saptanmadı.

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Sonuç: Anti-TNF alan tüm hastaların tüberküloz açısından değerlendirilmesi gerekmektedir. Tedavi başlangıcında saptanmasa da tedavi süresince güncel kılavuzlar ışığında temas öyküsü, semptomlar, fizik muayene, akciğer grafisi ve TDT/IGRA ile düzenli tüberküloz taramasına devam edilmelidir.

Anahtar kelimeler: Tüberküloz, çocuk, anti-TNF.

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Introduction

Tumor Necrosis Factor-alpha (TNF-alpha) is a proinflammatory cytokine that plays an essential role in the pathogenesis of many inflammatory diseases. TNF-alpha increases the release of many cytokines and chemokines, causing the migration and proliferation of lymphocytes to the inflammation area. In this way, granuloma formation occurs, and even if the bacilli cannot be destroyed, they are imprisoned in this structure, preventing their proliferation and spread [1, 2]. Many studies have shown the importance of TNF-alpha in controlling Mycobacterium species, *Aspergillus fumigatus, Histoplasma capsulatum, Coccidioides species, Toxoplasma gondii, Cryptococcus neoformans, Candida albicans* and viral pathogens [3, 4]. TNF-alpha plays an essential role in the pathogenesis of inflammation in many diseases. Therefore, in recent years, TNF-alpha inhibitors used in the treatment of autoimmune and inflammatory conditions such as Juvenile Idiopathic Arthritis (J.I.A.), Rheumatoid Arthritis (R.A.), Psoriasis, Psoriatic Arthritis, Crohn's Disease, Ankylosing Spondylitis (AS), Familial Mediterranean Fever (FMF), Autoimmune Uveitis, etc. The most commonly used anti-TNF-alpha agents clinically today are infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol [5-8]. Anti-TNF agents are effective in autoimmune diseases by suppressing inflammation. Still, also they increase the risk of granulomatous infections such as *Histoplasma capsulatum*, Nocardia, and especially tuberculosis by preventing granuloma formation, chemotaxis of neutrophils and macrophages, and cytokine release [3, 4].

Tuberculosis is a global public health problem that affects one-third of the world's population and is the second most common cause of death from an infectious disease. Although the primary area of the disease is predominantly the lungs, other organs and systems may also be affected. It has been shown that the use of anti-TNF drugs increases the risk of tuberculosis 11-40 times. Based on this, guidelines on the use of anti-TNF recommend screening for latent tuberculosis infection (LTBI) before anti-TNF therapy and isoniazid (INH) prophylaxis for positive cases [9-11]. During the screening, patients should be evaluated with their medical history, physical examination findings, chest radiographs, and TST or interferon-gamma release assay (IGRA). Furthermore, TNF-alpha inhibitors are contraindicated as soon as active T.B. infection is detected, and anti-TNF therapy should be discontinued immediately if tuberculosis develops during treatment [12]. In this study, we aimed to investigate the risk of tuberculosis development and their follow-up and treatment protocols in pediatric patients using anti-TNF drugs.

Material and method

One hundred and fifteen pediatric cases were followed up in our University Faculty of Medicine, Department of Pediatrics, between January 2011 and December 2021. They received anti-TNF, and biological agent treatment was included in the study. The clinical and epidemiological characteristics of the cases included in the study, the primary disease, the anti-TNF and immunosuppressant agents used and their duration, physical examination and laboratory findings, and TST and IGRA results were analyzed retrospectively. The study was approved by University Medical Ethics Committee (decision no: 32-1542). All cases were followed up for tuberculosis with clinical and physical examination every three months, chest X-ray every six months, and TST test

once a year. The Mantoux method was used for TST. For this purpose, five units of P.P.D. were injected intradermally into the forearm ulnar surface, and the induration's transverse diameter was measured 48-72 hours later. The TST result was evaluated according to the current guideline recommendations of the time it was performed. Induration of 5 mm or more was considered positive in those not vaccinated with BCG, and induration of 10 mm or more in those immunized with BCG. In patients who did not react, the test was repeated ten days later, and the booster effect was evaluated. Results 6 mm larger than the first test or greater than 10 mm were considered positive. IGRA test was performed in cases with negative TST Isoniazid (INH) was used for nine months for prophylactic treatment in patients diagnosed with LTBI.

Statistical analysis

The IBM SPSS Statistics 18 package program was used to analyze the data in the study. Central and prevalence measures such as number, percentage, minimum, maximum values, mean, median, and standard deviation were used to create descriptive statistics, and Pearson, Chi-square, and McNemar tests were used to determine the difference between categorical variables. $p \leq 0.05$ was considered statistically significant.

Results

One hundred fifteen cases using anti-TNF drugs were included in the study. The mean age of the cases was 13 (2-18) years. Of the patients, 66 (57%) were female, and 49 (43%) were male. Of the cases, 76 (66%) had Juvenile Rheumatoid Arthritis, 11 (9.6%) Ulcerative Colitis, 7 (6%) Crohn's, 6 (5.2%) Ankylosing Spondylitis, 5 (4.3%) FMF and 4 of them were followed up due to Psoriasis (3.5%) (Table 1). Etanercept in 74 (64.3%) cases, infliximab in 17 (14.8%) cases, adalimumab in 17 (14.8%) cases, anakinra in 5 (4.3%) cases, and canakinumab was used in 2 (1.7%) cases. In patients with anti-TNF drugs, 66 (57.4%) were using methotrexate, 11 (9.6%) were using systemic steroids, 4 (3.5%) were using salazopyrin, 2 (1.7%) were using cyclophosphamide (Table 1). The patients had the chronic disease for an average of 6.5 years and had been using anti-TNF drugs for an average of 4 years. All the cases declared that they had BCG. vaccination, but six patients did not have a BCG scar. P.P.D. was performed in all of the patients, and P.P.D. response was measured as <5mm in 89 (77.4%), 5-9 mm in 11 (8.7%), 10-14 mm in 8 (7.4%), >15 mm in 7 (5.6%) cases. The IGRA test was performed on ten patients and was positive in one. Isoniazid (INH) prophylaxis was started for nine months in 17 cases with the diagnosis of latent tuberculosis (Table 1, 2). Of the 17 cases in which prophylaxis was initiated, 4 had a cough. Therefore, they were examined for active tuberculosis with Acid-Fast Stain (A.F.S.) in sputum, tuberculosis culture, tuberculosis PCR, and 2 with computed tomography. Active tuberculosis was not detected in any of the cases (Table 2). Of the 17 patients who received INH prophylaxis, nine were using etanercept, five were using infliximab, and three were using adalimumab. There was no statistically significant difference between the use of INH prophylaxis and the type and duration of the Anti-TNF agent (p:0.32) (Table 3).

Table 1. Clinical and epidemiological characteristics of the cases

	n:115 (%)		**n:115 (%)**
Age (year)	13 (2-18)		
Gender		**Chest X-Ray**	
Girl	66 (57)	Normal	113 (98.2)
Boy	49 (43)	Pathological	2 (1.8)
		TST	
JIA	76 (66.1)	0-4 mm	89 (77.3)
U. Colitis	11 (9.6)	9-10 mm	11 (9.5)
Crohn's	7 (6.1)	10-14 mm	8 (6.9)
AS	6 (5.2)	>15 mm	7 (6.1)
FMF	5 (4.3)	**Quantiferon**	
Psoriasis	4 (3.5)	Negative	9 (7.8)
Behcet	1 (0.9)	Positive	1 (0.9)
PAN	1 (0.9)		
Sarcoidosis	1 (0.9)		
Posterior Scleritis	1 (0.9)		
Etanercept	74 (64.3)	Methotrexate	66 (57)
Infliximab	17 (14.8)	Steroid	11 (9.6)
Adalimumab	17 (14.8)	Salozopyrin	4 (3.5)
Anakinra	5 (4.3)	Cylophosphamide	2 (1.7)
Canakinumab	2 (1,7)		
		ARB	
Disease Duration (month)	77 (4-204)	Negative	4 (3.5)
		Positive	0 (0)
Anti-TNF Duration (month)	45 (2-137)	Latent Tuberculosis	17 (14.8)
INH Prophylaxis	17 (14.8)	Active Tuberculosis	0 (0)

JIA: Juvenile Idiopathic Arthritis; AS: Ankylosing spondylitis; FMF: Familial Mediterranean Fever; PAN: Polyarteritis Nodosa
INH: Isoniazid; PPD: Pürified Protein Derivation; ARB: A*cid resistant* bacilli

Table 2. Characteristics of latent tuberculosis cases

	Age	Gender	Anti-TNF	Anti-TNF Duration (month)	Disease	Disease Time (year)	DMARD	BCG scar	PPD (mm)	IGST	Symptom	Chest X-ray	ARB	INH (month)
Patient-1	17	B	Etanercept	24	JIA	8	MTX	+	12	-	+	N	Negative	9
Patient-2	16	G	Adalimumab	60	JIA	11	MTX	+	16	-	-	N	-	9
Patient-3	18	G	Etanercept	48	JIA	10	MTX	+	12	-	-	N	-	9
Patient-4	16	B	Etanercept	72	JIA	14	MTX	+	20	-	+	P	Negative	9
Patient-5	17	B	Etanercept	120	JIA	12	MTX	+	14	-	-	N	-	9
Patient-6	12	G	Etanercept	54	JIA	8	MTX	+	18	Negative	-	N	-	9
Patient-7	16	G	Infliximab	12	U.Colitis	10	-	+	18	Negative	-	N	-	9
Patient-8	9	G	Etanercept	60	JIA	6	-	+	12	-	-	N	-	9
Patient-9	11	B	Adalimumab	36	JIA	6	-	+	10	-	+	N	Negative	9
Patient-10	17	B	Etanercept	60	JIA	9	MTX	+	13	-	-	N	-	9
Patient-11	17	G	Adalimumab	53	Uveitis	5	Steroit	-	19	-	-	N	-	9
Patient-12	17	B	Infliximab	46	Crohn's	4	MTX	+	11	Negative	-	N	-	9
Patient-13	17	B	Infliksimab	50	U.Colitis	4	-	-	5	-	-	N	-	9
Patient-14	4	B	Etanercept	12	JIA	2	MTX	+	10	-	+	N	Negative	9
Patient-15	16	B	Infliximab	38	Crohn's	5	MTX	+	18	-	-	N	-	9
Patient-16	17	G	Infliximab	15	Crohn's	6	Steroit	+	1	Positive	-	N	-	9
Patient-17	13	G	Etanercept	60	JIA	8	MTX	+	18	-	-	N	-	9

G: Girl, B: Boy, JIA: Juvenile Idiopathic Arthritis, AS: Ankylosing spondylitis, FMF: Familial Mediterranean Fever, PAN: Polyarteritis Nodosa, MTX: Methotrexate

Table 3. Comparison of cases who received and did not receive INH prophylaxis

	INH Prophylaxis (-) 98 (%)	**INH Prophylaxis (+) 17**	***P***
Age(year)	13 (2-20)	14 (4-18)	0.08
Gender			0.2
Girl	58 (59)	8 (47)	
Boy	40 (12)	9 (53)	
Disease			0.25
JIA	65 (66.3)	11 (64.7)	
U.Colitis	9 (9.2)	2 (11.8)	
Crohn's	4 (4.1)	3 (17.6)	
AS	6 (6.1)	-	
FMF	5 (5.1)	-	
Psoriasis	4 (4.1)	-	
Sarcoidosis	1 (1)	-	
Behcet	1 (1)	-	
PAN	1 (1)	-	
Posterior Scleritis	-	1 (5.9)	
Diease Duration(year)	6 (0.5-12)	9 (1.5-17)	0.5
Anti-TNF			0.3
Etanercept	65 (66.3)	9 (52.9)	
Infliximab	12 (12.2)	5 (29.4)	
Adalimumab	14 (14.3)	3 (17.6)	
Anakinra	5 (5.1)	-	
Canakinumab	2 (2)	-	
Anti-TNF Duration(month)	44 (2-120)	55 (12-1221	0.8
DMART			0.7
Mtx	55 (56)	11 (64.7)	
Steroid	9 (9.2)	2 (11.8)	
Salozopyrin	4 (41)	-	
Cylophosphamide	2 (2)	-	
PPD			0.001
0-4 mm	88 (89)	1 (5.8)	
5-9 mm	6 (6.1)	1 (5.8)	
10-14 mm	-	8 (47)	
>15 mm	-	7 (41.4)	
Quatiferon			0.02
Negative	6 (6.1)	3 (17.6)	
Positive	0 (0)	1 (5.9)	
ARB			0.001
Negative	-	4 (23.5)	
Positive	-	-	

JIA: Juvenile Idiopathic Arthritis, AS: Ankylosing spondylitis, FMF: Familial Mediterranean Fever PAN: Polyarteritis Nodosa INH: Isoniazid, PPD: Pürified Protein Derivativ, ARB: *Acid resistant* bacilli, MTX: Methotrexate

Discussion

With the clinical use of anti-TNF agents, significant progress has been made in treating many autoinflammatory diseases, especially rheumatologic diseases. However, it has been reported that the widespread use of anti-TNF drugs increases the risk of mycobacterial infections, especially tuberculosis (T.B.), and bacterial, viral, and fungal infections. Especially in countries with a high prevalence of tuberculosis, reactivation of latent tuberculosis infection poses an essential problem for anti-TNF therapy. The World Health Organization (WHO) report reported that the incidence of T.B. in Türkiye was 16/100.000. In patients using anti-TNF therapy, the risk of tuberculosis is 10-20 times higher than in the average population. Currently, a Guideline for Tuberculosis in Patients Using Anti-TNF Therapy was published by the Public Health Agency of the Ministry of Health, Türkiye, in 2016 [9]. Therefore, all patients scheduled for anti-TNF therapy should be screened for tuberculosis before treatment. Our study found LTBI in 17 (14.8%) of 115 cases. Similarly, in a survey conducted by Kilic et al. [13] with 144 children receiving anti-TNF in our country, they reported the rate of LTBI as 4.8% (13) (Table 1, 2). Girit et al. [14] reported the rate of LTBI before treatment as 28.1% in their study with 57 cases. There are different recommendations in different guidelines regarding the method of screening for LTBI in patients receiving anti-TNF therapy and what should be the cut-off value taken, especially for TST. While the cut-off value for TST was ≥5 mm in the American Thoracic Society guideline published in 2017, the cut-off value was recommended as ≥10 mm in the consensus report of The Tuberculosis Network European Trials Group [15, 16]. In our country, the Rheumatism Research and Education Association (RAED) recommended the TST cut-off value as 5 mm for adults and children [17]. In the Tuberculosis Guidelines for Patients Using Anti-TNF Therapy, which the Ministry of Health recently updated, Public Health Agency of Türkiye, the cut-off value is recommended as ≥10 mm for pediatric patients with BCG vaccine and ≥5 mm for those who have not been vaccinated [9]. It has been stated that concomitant rheumatic and autoimmune diseases and other concurrent immunosuppressive drugs may affect the results of TST and IGRA used to detect tuberculosis development and LTBI [9]. In our study, all cases were screened primarily with TST. The cut-off value was ≥10 mm for those vaccinated with BCG and ≥5 mm for those not vaccinated. TST value was ≥10 mm in 15 of 17 patients with LTBI diagnosis, and INH prophylaxis was started, while TST was ≥5 mm in 1 patient (patient 13), and TST was 1 mm in 1 patient (patient 16). Since the TST=5 mm case had no BCG scar and the TST=1 mm case had a positive IGRA test, which was studied simultaneously, INH prophylaxis was given to 2 patients for nine months.

Different rates have been reported in studies conducted in many other countries and centers regarding the risk of developing active tuberculosis in patients receiving anti-TNF therapy. Kilic et al. [13] reported that tuberculosis developed in 1 (0.69%) of 144 pediatric patients receiving anti-TNF therapy. Similarly, the rate of tuberculosis development was reported as 0.85% by Cagatay et al. [18] and 1.5% by Hanta et al. [19]. Contrary to these studies, Girit et al. [14] and Kurt et al. [20] reported that tuberculosis did not develop in any of the patients receiving anti-TNF. Different results have been reported in studies investigating the protection of prophylaxis for tuberculosis. Borekci et al. [12] showed no significant difference between the groups that received and did not receive INH prophylaxis regarding the development of active tuberculosis. Kaptan et al. [21] reported that active tuberculosis developed in 7 of 389 patients receiving anti-TNF therapy, and all patients received INH prophylaxis. In a multicenter study conducted by Noguera Julian et al. [22], they reported that out of 19 cases who developed tuberculosis, 15 were previously screened for LTBI, and one case was under INH prophylaxis. In our study, although 17 cases received INH prophylaxis for LTBI, none of our patients developed active tuberculosis. The absence of a case of T.B. in our study was attributed to the fact that all cases were screened appropriately for LTBI, and the administration of INH prophylaxis with patients' compliance in necessary cases reduced the risk of T.B.

Previous studies have shown that the risk of tuberculosis development differs depending on the primary disease and the type and duration of use of the Anti-TNF agent. In a

study evaluating the incidence of T.B. in 10.000 patients who received anti-TNF therapy in the U.K., it was shown that T.B. development was higher on adalimumab (144/100.000) and infliximab (136/100.000) treatments compared to etanercept (39/100.000) [23]. Active T.B. can be seen in an average of 13.6 months after etanercept treatment and 5.5 months and 18.5 months after infliximab and adalimumab treatment, respectively [23]. This is also due to the effect of infliximab on the elimination of granulysin-expressing CD45RA+ subgroups of effector memory CD8+ T cells, which are involved in the intracellular killing of M. tuberculosis [24, 25]. In another study evaluating chronic disease and TST response, the lowest response was observed in R.A. patients, while the highest response was observed in Ankylosing spondylitis (AS) patients [26]. Our study did not have any patients who developed active tuberculosis. Acid-Fast-Stain (A.F.S.), tuberculosis culture, and radiological findings were normal in the active tuberculosis screening performed in 4 patients who received INH prophylaxis for LTBI and had suspicious symptoms for tuberculosis.

The main limitations of our study are the limited number of cases, the limited information availability on patient follow-up due to the retrospective nature of the study, and the lack of Quantiferon test for most of the cases.

As a result, all patients planning to receive anti-TNF therapy should be screened for tuberculosis. Although it is not detected at the beginning of the treatment, regular tuberculosis screening should be continued during the treatment with contact history, symptoms, physical examination, chest X-ray, and TST/IGRA in light of current guidelines.

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Authors' contributions to the article

All authors took part in the planning and design of the study. Y.K. and M.C.K. participated in data collection and statistical analysis. Y.K., M.C.K., Ö.K., and E.Ç.D. drafted the manuscript. All authors read and approved the final manuscript.

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The relationship between sexual behavior and well-being during the COVID-19 pandemic: an online survey study

COVID-19 pandemisinde cinsel davranış ve iyi oluş arasındaki ilişki: çevrimiçi bir anket çalışması

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Abstract

Purpose: In addition to its psychologically and socially negative effects on society, the COVID-19 pandemic has affected the sexual behavior of individuals. This study investigated the relationship between changes in sexual drive and well-being during the COVID-19 pandemic period.

Materials and methods: The data of this cross-sectional study were collected using a web-based survey between May 12 and August 12, 2021, with the voluntary participation of 231 individuals. The survey form included questions on sociodemographic and health-related characteristics, employment status, sex life before and during the COVID-19 pandemic period and status of having a partner, and COVID-19 diagnostic status, in addition to the Short Warwick-Edinburgh Mental Well-Being Scale.

Results: A reduction in sexual drive in the pandemic period was reported by 71 (30.7%) participants. The group whose sexual drive decreased had significant differences in terms of age, marital status, the status of having children, and Warwick-Edinburgh scale scores compared to the groups whose sexual drive increased or remained unchanged (respectively, p=0.009, p=0.039, p=0.041, and p<0.001). According to the logistic regression model, for every one-year increase in age corresponded to 8% (95% CI: 3-12%) increase in the risk of reduced sexual drive (p=0.001), and a 1-unit increase in Warwick-Edinburgh scale scores corresponded to a 16% (95% CI: 10-22%) decrease (p<0.001).

Conclusion: The COVID-19 pandemic affected the sexual drives and behavior of the participants. Increased age and lower mental well-being levels were associated with lower sexual drive. Programs aimed at improving mental well-being will have a beneficial effect on sexual behavior.

Key words: Sexual behavior, COVID-19 pandemic, mental health, sexual activity.

Sofuoglu Z, Baysan C, Gulmez H, Palanbek Yavas S, Ayvat P. The relationship between sexual behavior and well-being during the COVID-19 pandemic: an online survey study. Pam Med J 2023;16:248-256.

Öz

Amaç: COVID-19 pandemisi toplum üzerindeki psikolojik ve sosyal açıdan olumsuz etkilerinin yanı sıra bireylerin cinsel davranışlarını da etkilemiştir. Bu çalışma, COVID-19 pandemi döneminde cinsel dürtü değişiklikler ile iyilik hali arasındaki ilişkiyi araştırmayı amaçlamıştır.

Gereç ve yöntem: Kesitsel tipteki bu çalışmanın verileri, 231 kişinin gönüllü katılımıyla 12 Mayıs-12 Ağustos 2021 tarihleri arasında web tabanlı bir anket formu kullanılarak toplanmıştır. Anket formunda Kısa Warwick-Edinburgh Mental iyilik ölçeğine ek olarak sosyodemografik ve sağlıkla ilgili özellikler, istihdam durumu, COVID-19 pandemi öncesi ve pandemi dönemindeki cinsel yaşam ve eş sahibi olma durumu ve COVID-19 tanı durumu ile ilgili sorular yer almıştır.

Bulgular: Katılımcıların 71'i (%30,7) tarafından pandemi döneminde cinsel istekte azalma olduğu bildirilmiştir. Cinsel dürtü azalan grup, cinsel dürtüsü artan veya değişmeyen gruplara göre yaş, medeni durum, çocuk sahibi olma durumu ve Warwick-Edinburgh ölçeği puanları açısından anlamlı farklılık gösterdi (sırasıyla, p=0,009, p=0,039, p=0,041 ve p<0,001). Lojistik regresyon modeline göre, yaştaki her 1 yıllık artış, cinsel dürtü azalma riski %8 (%95 GA: %3-12) artışı sağlarken (p=0,001) Warwick-Edinburgh ölçeği puanlarındaki 1 birimlik artış %16'lık (%95 GA: %10-22) azalmaya neden oluyordu (p<0,001).

Sonuç: COVID-19 pandemisi katılımcıların cinsel dürtülerini ve davranışlarını etkilemiştir. Artan yaş ve düşük zihinsel refah seviyeleri, daha düşük cinsel dürtü ile ilişkilendirildi. Zihinsel sağlığı iyileştirmeyi amaçlayan programlar, cinsel davranış üzerinde faydalı bir etkiye sahip olacaktır.

Anahtar kelimeler: Cinsel davranış, COVID-19 pandemisi, ruh sağlığı, cinsel aktivite.

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Introduction

After the identification of more than 118,000 cases in 114 countries by 11.03.2020, the novel coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) [1]. The COVID-19 pandemic has had negative psychological and social effects on societies. Its psychological effects have included not only fear of infection but also increased levels of anxiety and depression symptoms. Social distancing measures have reduced interpersonal communication and interactions and had negative effects on empathetic processes. These issues also have negative effects on the psychological well-being of individuals [2].

WHO defines sexual health not only as of the absence of a disease, dysfunction, or disability, but also a complete state of emotional, physical, mental, and social well-being related to sexuality [3]. In addition to reducing physical contact between partners from kissing to sexual intercourse due to fears of infection, COVID-19 leads to arguments and differences of opinion between partners who have to spend 24 h of the day together and negatively affects their sex lives [4]. The negative psychological effects of depression and anxiety symptoms developing due to the pandemic reduce sexual interest and intercourse frequency between partners [5, 6]. While some publications have shown lower frequencies of sexual intercourse in the COVID-19 pandemic period [7, 8], others have shown higher frequencies [9, 10].

Different results have been obtained about changes in sexual intercourse frequency in the COVID-19 pandemic period in the literature. In this study, it was aimed to reveal the effects of the COVID-19 pandemic on the sexual drives of individuals and investigate the relationships of these results to mental well-being and other factors.

Materials and methods

Population

The sample of the study consisted of individuals over the age of 18 living in Türkiye. The inclusion criteria were determined as being over 18 years old, being male or female, and being sexually active heterosexual or homosexual individuals. Individuals who did not agree to participate in the study or did not completely fill out the survey form were excluded. Before they started to fill out the survey, all participants consented to participate in the study and provided permission for their data to be used. The sample size required for the study was calculated using the OpenEpi [11] program and the formula: $n=[DEFF*Np(1-p)]/[(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$. The minimum required sample size for the study was calculated as 164 participants based on prevalence (reduced sexual drive) of 46.45% (P) [7], in an 80% confidence level, with a 5% error margin, and by taking the design effect as one.

Data collection

The data were collected between May 12 and August 12, 2021 on the Survey Monkey platform. Messages including the invitation to participate in the study and information about the study were sent to the phones of members of professional associations through electronic messaging platforms (WhatsApp). These individuals also shared the link to the survey with other individuals. Participation in the study was voluntary, and before starting to collect data, the participants were provided with an informed consent form to confirm. No monetary or non-monetary incentives were offered to the participants for their participation. The online survey form was open-access, and IP addresses were checked to identify potential duplicate responses. There were 4 duplicate IP addresses. As it was determined that data of different individuals were entered from these addresses, these data were not removed from the dataset. As the settings on the system where the survey was implemented (Survey Monkey) were made to ensure that uncompleted or incompletely filled survey forms would not be transferred to the database, no participants were excluded after the data were collected.

Survey form

The survey form consisted of questions on the sociodemographic and health-related characteristics of the participants (12 items),

questions on their employment status (3 items), questions on their sex lives before and in the pandemic period and their status of relationship with their partner (4 items), questions on their history of COVID-19 infection (2 items), and the Short Warwick-Edinburgh Mental Well-Being Scale (7 items).

The Short Warwick-Edinburgh Mental Well-Being Scale was tested for validity and reliability in Turkish by Demirtas and Baytemir [12] in 2019. It is a 5-point Likert-type scale (1= None of the Time, 5= All of the Time) consisting of 7 items in the form of positive statements. Higher scores indicate higher levels of mental well-being. The Cronbach's alpha coefficient of the scale was determined as 0.86, while its goodness-of-fit indices were found as χ^2/df: 1.58, RMSEA: 0.065, CFI:0.99, and NFI: 0.97. Permission to use the scale in our study was obtained from Demirtas and Baytemir on 19.04.2021.

Statistical analysis

In the reporting of the descriptive statistics, the continuous data are presented as mean, standard deviation, median, minimum, and maximum values, and the categorical data are presented as frequencies and percentages. Chi-squared test was conducted to investigate the relationships between the categorical variables. Binary logistic regression analysis was carried out for the multivariate analysis of factors associated with reduced sexual drive. Variables that had values of $p<0.200$ in the univariate analyses were included in the logistic regression model, and the ideal model was obtained using the backward method. For all statistical analyses, the level of statistical significance was taken as $p<0.05$. All data were analyzed using the Statistical Package for the Social Sciences (SPSS, Version 23.0).

Approval for conducting the study was obtained from the Noninterventional Studies Ethics Committee of Buca Seyfi Demirsoy Research and Training Hospital (Decision Date: 28.04.2021, Decision Number: 2021/4-41). Data were collected in accordance with the declaration of Helsinki.

Results

Among the 231 individuals who participated in the study, 135 (58.4%) were women, and 96 (41.6%) were men. The mean age of the participants was 45.0±9.2 (min: 22, max: 71) years, and their mean BMI was 25.4±4.0 (min:17.1, max: 37.6) kg/m^2. It was found that 175 (75.8%) of the participants were married or marriage lite. The sociodemographic characteristics of the participants are summarized in Table 1.

While 181 (78.4%) of the participants were working at any job, 23 (10.0%) were retired, 16 (6.9%) were homemakers, 6 (2.6%) were unemployed, and 5 (2.2%) were students. The ratio of the participants who worked 8 hours or longer per day was 124 (53.7%). It was found that 110 (47.6%) of the participants had a monthly income of 9,000 TL or higher, 102 (44.2%) had a monthly income of 3,000-8,999 TL, and 19 (8.2%) had a monthly income of 2,999 TL or lower.

It was determined that 46 (19.9%) of the participants had at least one chronic disease. When they were asked about their COVID-19 infection history, 29 (12.6%) of the participants stated that they had been diagnosed with COVID-19, and among those who had been diagnosed, 2 received treatment in intensive care, whereas one received treatment as an inpatient. The rates of the participants who were smokers and those who consumed alcohol were respectively 75 (32.5%) and 116 (50.2%).

Most participants shared the same home with their partners (n:168 (72.7%)). The information about the participants regarding the time they spent with their partners and changes in their sexual drive in the pandemic period is presented in Table 2. The mean Short Warwick-Edinburgh Mental Well-Being Scale score of the participants was found as 26.3±4.7.

It was determined that the participants whose sexual drive levels decreased during the pandemic period had a significantly higher mean age than the participants whose sexual drive levels increased or remained unchanged ($p=0.009$). The degree of decrease in the sexual drive levels of the participants who were married/marriage lite was significantly higher than the degree of decrease among others ($p=0.039$). The participants who had children also experienced a significantly higher degree of decrease in their sexual drive levels compared to others ($p=0.041$). The participants whose

Table 1. Sociodemographic characteristics of the participants

Variables	n	%
Marital Status		
Unmarried	28	12.1
Married/Marriage lite	175	75.8
Separated/Divorced/Widowed	26	11.2
Does not want to specify	2	0.9
Educational Status		
Primary school	3	1.3
Secondary school or high school	22	9.5
University	107	46.3
Master/Doctorate	99	42.9
Duration of Relationship		
None	23	10.0
5 years or shorter	35	15.1
6 years or longer	173	74.9
Has Children		
Yes	179	77.5
1 child	93	40.3
2 children	76	32.9
3 or more children	10	4.3
No	52	22.5
Living		
With partner/children	177	76.6
With parents	18	7.8
Alone	30	13.0
With roommate(s)	6	2.6

Table 2. Living and sexual drive statues of the participants in the pandemic period

Variables	n	(%)
Shares the same home with partner		
No	63	27.3
Yes	168	72.7
Shares the same home partner in the pandemic		
Yes	168	72.7
No	50	21.7
Sometimes	13	5.6
Time spent in the same environment with partner		
0 h	21	9.1
1–6 hours	74	32.0
7–12 hours	56	24.2
13–18 hours	32	13.9
19–24 hours	48	20.8
Sexual drive in the pandemic		
Decreased	71	30.7
Unchanged	130	56.3
Increased	30	13.0

sexual drive levels decreased in the pandemic period had a significantly lower mean Warwick-Edinburgh score than those whose sexual drive increased or remained unchanged (p<0.001) (Table 3).

According to the logistic regression model of the factors affecting the sexual drive reduction status of the participants, a 1-year increase in age corresponded to a 1.08 (95% CI: 1.03-1.12) increase in the risk of reduced sexual drive (p=0.001), and a 1-unit increase in Warwick-Edinburgh scale scores corresponded to a 16% (95% CI: 10-22%) decrease (p<0.001) (Table 4).

Table 3. Relationship between the sociodemographic and some other characteristics of the participants and changes in their sexual drive during the pandemic period

Variables	Unchanged and Increased (n: 160)	Decreased (n: 71)	*p*
Age, Mean±SD	44.0±9.5	47.4±8.0	**0.009**[*]
Sex, n (%)			
Female	89 (65.9)	46 (34.1)	0.192[†]
Male	71 (74.0)	25 (26.0)	
BMI, Mean±SD	25.3±4.0	25.7±4.0	0.431[*]
Marital Status, n (%)			
Married/Marriage lite	115 (65.7)	60 (34.3)	**0.039**[†]
Unmarried/No Marriage lite	45 (80.4)	11 (19.6)	
Has Children, n (%)			
No	42 (80.8)	10 (19.2)	**0.041**[†]
Yes	118 (65.9)	61 (34.1)	
Relationship Duration, n (%)			
None	20 (87.0)	3 (13.0)	0.151[†]
Shorter than 5 years	24 (68.6)	11 (31.4)	
6 years or longer	116 (67.1)	57 (32.9)	
Living Status, n (%)			
Living with partner/children	116 (65.5)	61 (34.5)	0.080[†]
Living with parents/roommate(s)	19 (79.2)	5 (20.8)	
Living alone	25 (83.3)	5 (16.7)	
Educational Status, n (%)			
High school or lower	20 (80.0)	5 (20.0)	0.218[†]
University or higher	140 (68.0)	66 (32.0)	
Monthly Income, n (%)			
0-2,999 TL	13 (68.4)	6 (31.6)	0.993[†]
3,000-8,999 TL	71 (69.6)	31 (30.4)	
9,000 TL or higher	76 (69.1)	34 (30.9)	
Working hours per day, n (%)			
7 h or less	73 (68.2)	34 (31.8)	0.750[†]
8 h or more	87 (70.2)	37 (29.8)	
Has a chronic disease, n (%)			
No	131 (70.8)	54 (29.2)	0.307[†]
Yes	29 (63.0)	17 (37.0)	

Table 3. Relationship between the sociodemographic and some other characteristics of the participants and changes in their sexual drive during the pandemic period (continued)

Variables	Unchanged and Increased (n: 160)	Decreased (n: 71)	*p*
Smoker, n (%)			
No	107 (68.6)	49 (31.4)	0.749†
Yes	53 (70.7)	22 (29.3)	
Consumes alcohol, n (%)			
No	80 (69.6)	35 (30.4)	0.921†
Yes	80 (69.0)	36 (31.0)	
Stays in the same home with partner in the pandemic, n (%)			
Yes	112 (66.7)	56 (33.3)	0.139†
No	36 (72.0)	14 (28.0)	
Sometimes	12 (92.3)	1 (7.7)	
Shares the same home with partner, n (%)			
No	48 (76.2)	15 (23.8)	0.162†
Yes	112(66.7)	56 (33.3)	
Time spent in the same environment with partner per day, n (%)			
12 h or less	105 (69.5)	46 (30.5)	0.902†
13–24 hours	55 (68.8)	25 (31.3)	
Has been diagnosed with COVID-19, n (%)			
No	137 (67.8)	65 (32.2)	0.210†
Yes	23 (79.3)	6 (20.7)	
Warwick Edinburgh Skor, Mean±SD	27.2±4.7	24.3±4.0	**<0.001***

* Student T test, † Chi-squared test

Table 4. Logistic regression model of the factors affecting the sexual drive reduction status of the participants in the pandemic period

	B	S.E.	Wald	*p*	OR	95% C.I. for OR	
						Lower	Upper
Age	0.073	0.02	13.01	0.001‡	1.08	1.03	1.12
No marriage/marriage lite (ref.: marriage/marriage lite)	-1.393	0.789	3.115	0.078‡	0.25	0.05	1.17
Shares home with partner (ref.: yes)							
No	1.001	0.769	1.691	0.193‡	2.72	0.60	12.26
Sometimes	-2.159	1.149	3.531	0.060‡	0.12	0.01	1.10
Warwick-Edinburgh Score	-0.175	0.037	21.777	<0.001‡	0.84	0.78	0.90
Constant	0.492	1.108	0.198	0.657	1.64		

Nagelkerke R^2=0.238, ‡: Binary logistic regression analysis

Discussion

The COVID-19 pandemic, which has been going since the end of 2019, has led to social and economic difficulties in many countries including Türkiye [13]. In Türkiye, the government has occasionally issued complete and partial closures (workplaces, schools, movie theaters, and other social activities), and "social distancing" has been going on in daily life [14]. This situation has led to essential changes in the lives of individuals and affected their quality of life and well-being negatively [15, 16]. Previous studies have determined that the social measures and restrictions caused by the COVID-19 pandemic [17], experiencing financial concerns [7], and increased depression, anxiety and negative mood levels [6, 18] have a transformative effect on the sexual behavior of individuals.

While more than half of the participants in our study (56.3%) reported that their sexual drive levels did not change, 30.7% stated that their sexual drive decreased. The reason for this result may be the fact that our study was conducted in a relatively late period of the pandemic, where the period of uncertainty (e.g., lack of a vaccine, lack of specific treatments) had been put behind, and we were closer to a normal life. Additionally, the high education and income levels of the participants may have made it easier for them to manage stressful situations experienced in the pandemic period (e.g., financial difficulties, infodemic) and prevented these issues from being reflected in their sex lives. The results of studies conducted on sex life throughout the COVID-19 pandemic period have shown differences, including reports of decreases and increases [19]. In most studies conducted in different societies in different countries, reductions have been observed in "sexual function," "sexual desire," "sexual intercourse," and "sexual satisfaction" levels [20-22]. Feng et al. [8] reported that the frequency of sexual intercourse decreased by 43.3% in the period of restrictions, while sexual desire, satisfaction, and quality of sex remained unchanged in most participants. As reasons for lowered levels of sexual behavior, fear of infection, fear of disease-related death, concerns created by news stories about deaths and disease that are constantly on the global agenda, job termination and financial unpredictability, and intense exposure to external stressors such as social isolation and loneliness may have influenced the romantic relationships of individuals and their interest in sex [4]. Mumm et al. [17] reported that sexual intercourse frequency and sexual satisfaction during intercourse increased in Germany, Micelli et al. [23] reported increased levels of desire to become parents among Italian individuals, especially women, and Yuksel et al. [9] observed similarly that sexual intercourse frequency and sexual desire increased. Among studies conducted in Türkiye, Karagoz et al. [24] reported that sexual intercourse frequency decreased, but sexual function levels increased in both sexes among those who spent more time together, Baran et al. [25] found that the number of sexual intercourse per week among their participants, which was 2.34±1.35 in the pre-pandemic period dropped to 1.54±1.45 in the pandemic period, and Kaya et al. [26] determined lower frequencies of sexual intercourse and reduced sexual satisfaction among women who were diagnosed with COVID-19. The varying effects of the COVID-19 pandemic on each country (e.g., treatment opportunities, spread of the virus, mortality and morbidity rates), differences in quarantine practices in countries, and differences in terms of sex life in different societies may explain the different results reported in these studies [17]. Perspectives on sexuality in different cultures show great variations depending on history regarding views on sexuality, the importance of sexuality, and sexual orientation [27]. In our study, it was found that as the ages of the participants increased, their sexual drive decreased. Similarly, Karsiyakali et al. [28] identified lower sexual desire levels among individuals at a higher mean age. Aging brings about physiological, behavioral, and psychosocial changes, and all these changes affect sexual function [29]. COVID-19 progresses more severely and deadlier in the elderly population, while social restriction measures in Türkiye were stricter for older individuals in Türkiye, and these reasons may have increased the pressure on older individuals and led them to experience a loss of sexual drive [30].

Mental well-being, which represents the positive aspect of mental health, is closely

related to the physical and social well-being of individuals, and individuals with a positive state of well-being usually possess the resources and skills that are required for coping with problematic situations [12]. Studies have reported that the catastrophic environment experienced throughout the COVID-19 pandemic period has reduced the mental well-being of individuals [15, 16, 23]. Cito et al. [7] observed a decrease in the frequency of sexual intercourse in the quarantine period, while they also identified a positive correlation between well-being scores and sexual intercourse frequency. Likewise, in our study, the participants whose sexual drives decreased had lower scores in the Short Warwick-Edinburgh Mental Well-Being Scale.

Strengths and limitations

Our study is one of the first studies that have presented the relationship between mental well-being and sexual drive during the COVID-19 pandemic in Türkiye. Unfortunately, our sample size does not represent the entire Türkiye. It was a limitation that we used questions that we prepared by reviewing the literature because no specific, validated survey was available regarding the effects of quarantine on sexuality. The fact that the participants of this study included only those who had access to online platforms was another limitation.

To sum the stressful situations created by the COVID-19 pandemic has inevitably affected humanity as a whole. It has not been easy for everyone to accept and manage the necessary changes it has brought, and in this period, the sexual drives of individuals toward their partners and their sexual behavior have been affected. In this study, it was determined that older age, being married/marriage lite, and having children were related to reduced sexual drive. Low mental well-being scale scores were also associated with reduced sexual drive. Sex is a part of people's lives. By considering the psychological pressure brought about by the COVID-19 pandemic, researchers should design effective programs to improve the sexual behavior of people.

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

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Authors' contributions to the article

Z.S. have constructed/constructed the main idea and hypothesis of the study. Z.S., H.G., C.B., S.P.Y. developed the theory and arranged/edited the material and method section. Z.S., H.G, P.A. collected data. Z.S., C.B., S.P.Y. have done the evaluation of the data in the Results section. All authors conducted a literature review. Discussion section of the article written by all authors reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Is there a superiority of the stone volume measured in 3-D non-contrast tomography to the stone area in predicting the stone-freeness of the retrograde intrarenal surgery success?

Retrograd intrarenal cerrahinin taşsızlığını öngörmede üç boyutlu kontrastsız bilgisayarlı tomografi ile ölçülen taş volümünün taş alanına bir üstünlüğü var mıdır?

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Abstract

Purpose: To examine the predictive effect of preoperative stone volume (SV) against stone area (SA) on stone-free status (SF) following retrograde intrarenal surgery (RIRS).

Materials and methods: We retrospectively examined the medical records of 68 RIRS patients with renal calculi who were eligible. Patients having Non-Contrast Tomography (NCCT) before and subsequent to RIRS were included, however staghorn stones and inability to access were omitted. SF status was determined by the absence of visible stones on the NCCT three months after RIRS. Using a software reconstruction tool using 3-D NCCT, a radiologist determined stone load characteristics, such as SA and SV. Using a logistic regression model, the assessment of potential SF status determinants was conducted.

Results: Age, stone density, quantity and position of stones, usage of access sheath, failed prior SWL, and procedures were not substantially linked with non-SF status, however gender (p=0.014), SA (p=0.001), and SV (p=0.002) were strongly associated with non-SF status. The association between SV and SA was strong (r=0.866, p<0.001). A pairwise assessment of the ROC curves for SV and SA revealed no statistically significant difference in their specificities (p=0.274). Nevertheless, the multivariate analysis showed that SA was the sole independent predictor of SF status (p=0.001).

Conclusions: Both SA and SV were strongly suggestive of SF status after the RIRS. However, SA was only identified as an independent predictor of SF status after RIRS and as a sufficient predictor of SF status after RIRS.

Key words: Stone burden, 3-D measurement, lazer litotripsi, success treatment.

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Öz

Amaç: Retrograd intrarenal cerrahi (RIRC) sonrasında preoperatif taş volümü (TV) ve taş alanı (TA) ölçümlerinin taşsızlık durumu (TD) üzerine prediktif etkisini değerlendirmek.

Gereç ve yöntem: Böbrek taşları için RIRC yapılan, çalışma kriterlerine uygun 68 hastanın medikal kayıtlarını retrospektif olarak değerlendirdik. Operasyon öncesi ve sonrası kontrastsız bilgisayarlı tomografileri (KBT) olan hastalar çalışmaya dahil edilirken, sataghorn taşı olan ve taşa ulaşılamayan hastalar çalışma dışı bırakıldı. TD durumu RIRC operasyonu sonrasındaki 3. aydaki KBT'de görülebilir taş olmaması olarak tanımlandı. Bir radyolog 3 boyutlu KBT üzerindeki bir yazılım programı kullanarak TA ve TV gibi taş yükü karakteristiklerini tespit etti. Lojistik regresyon analizleri kullanılarak potansiyel TD tanımlayıcılarının değerlendirilmesi yapıldı.

Bulgular: TD ile taşın dansisitesi, lokalizasyonu ve sayısı, erişim kılıfı kullanımı, önceden geçirilmiş başarısız SWL ve operasyonlar ilişkili bulunmazken, cinsiyet, TA ve TV güçlü şekilde ilişkili bulundu (p=0,014), (p<0,001), ve (p=0,002), sırasıyla. TA ve TV'nin pairwise ROC eğri analizleri kıyaslandığında özgünlükleri açısından TD belirlemede aralarında istatistiki anlamlı bir fark olmadığı görüldü (p=0,274). Bununla birlikte, multivariate analizler SA'nın TD'yi belirlemede tek bağımsız prediktör olduğunu ortaya koydu (p=0,001).

Sonuç: Hem SA hem de SV RIRC operasyonu sonrasında TD'yi tahmin etmede fikir vericidir. Bununla birlikte SA, RIRC operasyonu sonrasında TD'yi bilmede tek bağımsız prediktördür ve tek başına yeterlidir.

Anahtar kelimeler: Taş yükü, 3 boyutlu ölçüm, lazer litotripsi, tedavi başarısı.

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Kaynak Y, Kılıçarslan H, Okeer E, Coşkun B. Retrograd intrarenal cerrahinin taşsızlığını öngörmede üç boyutlu kontrastsız bilgisayarlı tomografi ile ölçülen taş volümünün taş alanına bir üstünlüğü var mıdır? Pam Tıp Derg 2023;16:258-264.

Introduction

Pretreatment stone load is an independent determinant in patients receiving retrograde intrarenal surgery (RIRS) stone-free rates (SFRs) [1-4]. On plain X-ray, IVU, or non-contrast-enhanced CT (NCCT), the maximum stone length or stone surface area has historically been measured [5, 6].

European Association of Urology (EAU) suggests using maximum stone diameters to forecast stone load [7]. Due to the fact that kidney stones are irregular three-dimensional objects, two-dimensional measures may not be sufficient to accurately forecast stone load [8, 9]. EAU advises using a scalene ellipsoid formula to assess stone volume (SV). Recent research shown, however, that the scalene ellipsoid formula cannot offer an exact volume for all stones, since the typical shape of renal stones varies with diameter [10]. Using contemporary CT scanning tools, it is possible to precisely quantify the volume of a stone using 3D reconstruction [11]. Few studies have focused on this topic to yet, therefore it has not yet been conclusively determined [12, 13].

In the current research, we wanted to assess the prediction value of SV and stone area (SA) on SF status following RIRS using a specialized CT software reconstruction tool.

Materials and methods

Patients

We analyzed the medical records of 387 patients who had RIRS for renal calculi between October 2009 and January 2014 and recruited 68 individuals who met the inclusion criteria. Before and after RIRS, each patient was obliged to have a documented radiographic evaluation of the urinary tract by NCCT. Exclusion criteria included staghorn stones, inability to reach the site of the stones, and pediatric patients (less than 18 years of age). The demographic information, positioning of the access sheath, past SWLs and operations on the same side, as well as the quantity and location of the stones, were documented. A post-operative month 3 (POM 3) NCCT was used to classify patients as SF or non-SF. SF status was defined as the absence of any visible stone on the NCCT at POM 3.

Technique

Each patient had surgery under either general or spinal anesthesia. The standard lithotomy posture for patients was a modified combination Trendelenburg (head inclined about 20 degrees). During the procedures, a Storz Flex-XTM 2 (Karl Storz, Tuebingen, Germany, 7.5 F) flexible ureterorenoscope was used. Under endoscopic observation, the rigid ureterorenoscope was first introduced into the bladder. Through a working channel, a polytetrafluoroethylene-coated, 0.0035-inch guide wire was introduced into the ureter. Under the help of a guide wire, the rigid ureterorenoscope was inserted into the ureter. The second guide wire (sensitive, 0.0035 inch) was put into the other working channel of the ureterorenoscope after the ureterorenoscope was in the ureter. After removing the rigid ureterorenoscope, the access sheath was placed over the PTFE-coated guide wire. If it was difficult to insert the access sheath, the flexible ureterorenoscope was inserted without the access sheath. The visual picture was linked with the fluorescence image in order to enter the correct calyces. For lithotripsy, a 270-micron laser fiber was used. The holmium laser was calibrated at a rate of 10-25 Hz and an energy level of 0.5-0.8 joules. Stones were fractured until they were tiny enough (2 mm) to pass through the urinary system without difficulty. We implanted a 4.7 F ureteral double-j stent at the end of the procedures and withdrew it two weeks following RIRS.

Clinical and imaging evaluations

An expert radiologist reexamined the NCCTs to determine the number and location of kidney stones in the collecting system, as well as the SV, SA, and mean HU.

Statistical examination

The data were analyzed using SPSS (SPSS, Chicago, IL) and MedCalc (version 12.7.7) software. The Shapiro-Wilk test was performed to determine if variables were normally distributed.

The normally distributed variables are reported as mean standard deviation and compared using the Student's ttest. The non-normally distributed variables were given as median (minimum-maximum) and compared using Mann-Whitney U. ROC regression analysis was performed to evaluate the predictive abilities of age, mean HU, SV, and SA. The value of the threshold was established using the Youden Index. J. ROC curves for SV and SA were compared. Additionally, continuous data were presented as mean standard deviation (SD). To compare categorical variables provided as "n" or percentage (%), the Chi-Square Test and Fisher's Exact Test were used. Using the Spearman's rho test, the correlation between SA and SV was determined. We utilized a logistic regression model to undertake univariate and multivariate evaluation. All relevant factors were accounted for in the multivariate model (backward stepwise logistic regression method). All statistical tests were deemed significant if $p<0.05$. Chi-square tests and multivariate analysis using the Backward Stepwise Logistic Regression Method were used to identify determinants of stone-free status.

The quantity of stones was separated into two categories: solitary stones and stones with 2 sides. Lower pole, mid-pole+upper pole+renal pelvis, and multifocal stones are the three classifications of stone placement.

Results

Characteristics of patients and operative results

The average age was 51.73±13.70 (range: 18.0-80) years. There were 36 male patients (52.9%) and 32 female patients (47.1%). Lower pole, middle pole, upper pole, renal pelvis, and multifocal stone patients numbered 23, 10, 3, 17, and 15, respectively. The average total stone area and volume was 1.15 (min-max=0.28-5.89) cm^2 and 1.27 (min-max=0.32-9.35) cm^3, respectively. The average Hounsfield Unit value was 276.10±111.16 HU. The number of patients free of kidney stones was 54 (79%). The following metrics indicated significant differences between the SF and non-SF groups in favour of woman ($p=0.014$). Both SA and SV were associated with SF ($p<0.001$) and SV ($p=0.002$), respectively. Stone density, location and quantity of stones, age, past unsuccessful SWL, previous failed surgeries, and the use of an access sheath did not vary between the SF and non-SF groups. The background and treatment results are compared in Table 1 according to the stone status at POM3.

Correlation between two stone burden parameters

The Spearman's correlation test revealed that SA and SV are highly correlated (0.866 Spearman's rho) ($p<0.001$). This association was statistically significant (Figure 1).

Establishment of cutoff points

Area under the ROC curve (AUC) values for the SA and SV were 0.810 and 0.765, respectively (Figure 2).

Both stone load indicators were strongly predictive of SF at POM 3 (for SA $p<0.001$ and for SV $p=0.001$).

The ROC curves suggested that the SA and SV cutoff thresholds were 1.84 cm^2 and 1.650 cm^3, respectively. For SA, sensitivity was 85.19 (95% confidence interval: 72.9-93.4) and specificity was 71.43 (95% confidence interval: 41.9-91.6). For SV, the sensitivity was 75.93 (95% confidence interval: 62.4-86.5) and the specificity was 71.43 (95% confidence interval: 41.4-91.6). A pairwise assessment of the ROC curves for SA and SV revealed no statistically significant difference in their specificities ($p=0.274$). The AUC value for age was 0.689% ($p=0.027$), while the age cutoff value was 38 years. It had a sensitivity of 90.74 (95% confidence interval [CI]:79.7-96.9) and a specificity of 42.86 (95% CI:17.7-71.1). Also suggestive of SF status at POM 3 was not age.

Multivariate analysis of SF status predictors

Our univariate analysis revealed that the SA ($p<0.001$), SV ($p=0.002$), and gender ($p=0.014$) were significantly linked with non-SF at POM3.

In our backward stepwise logistic regression model, we kept the age and SA parameters. However, the SA was only an independent predictor of SF status ($p=0.001$) OR=12.68 (95% CI=2.933-54.825). The findings of univariate and multivariate analyses are summarized in Table 2.

Table 1. Comparison of patient and stone data between cases with stone-free and non-stone-free at postoperative month 3 after RIRS

	SF at POM3 (n=54)	Non – SF at POM 3 (n=14)	*p*
Age (years)	51.66±14.04	52.00±12.79	0.936[a]
≤38	9 (81.8%)	2 (18.2%)	1.000[b]
>38	45 (78.9%)	12 (21.1%)	
Gender			
Male	24 (66.7%)	12 (33.3%)	0.014[b]
Female	30 (93.8%)	2 (6.3%)	
Number of stones			
1	38 (86.4%)	6 (13.6%)	0.054[b]
≥2	14 (63.6%)	8 (36.4%)	
Renal stone location			
Lower pole	17 (73.9%)	6 (26.1%)	0.121[b]
Mid-pole, Upper pole, Renal pelvis	27 (90.0%)	3 (10.0%)	
Multiple location	10 (66.7%)	5 (33.3%)	
Stone Burden (Cumulative)			
Area (cm^2)	0.97 (0.28-4.76)	2.12 (0.78-5.89)	<0.001[c]
≤1.84 cm^2	46 (92%)	4 (8%)	<0.001[b]
>1.84 cm^2	8 (44.4%)	10 (55.6)	
Volume (cm^3)	1.150 (0.032-8.090)	2.895 (0.720-9.350)	0.002[c]
≤1.650 cm^3	41 (91.1%)	4 (8.9%)	0.001[b]
>1.650 cm^3	13 (56.5%)	10 (43.5%)	
Stone Density (Mean Hounsfield Unit)	272.83±113.32	288.71±105.43	0.637[a]
Use of access sheath			0.523[b]
Need	35 (76.1%)	11 (23.9%)	
No need	19 (86.4%)	3 (13.6%)	
Failed previous SWL			0.584[b]
Yes	24 (75%)	8 (25%)	
No	30 (83.3%)	6 (16.7%)	
Failed previous operations			0.758[b]
Yes	17 (77.3%)	5 (22.7%)	
No	37 (80.4%)	9 (19.6%)	

[a] Independent Samples test, [b] Chi-square test [c] Mann-Whitney U test
SF=Stone-free; POM=Postoperative month; SWL=Shock-wave lithotripsy

Table 2. Univariate and multivariate analysis of stone status after RIRS

Parameter	Category	Non-SF rate	Univariate *p*	Multivariate *p*	OR	95% CI
Age (years)	>38	21.1%	1.000	-	-	-
	≤38	18.2%				
Gender	Female*	6.3%	0.014	0.063	_	_
	Male	33.3%				
Number of stones	1	13.6%	0.054	-	_	_
	≥2	36.4%				
Area (cm^2) (cumulative)	≤1.84 *	8%	<0.001	0.001	12.68	2.93-54.83
	>1.84	55.6%				
Volume (cm^3) (cumulative)	≤1.650 *	8.9%	0.003	_	_	_
	>1.650	43.5%				

Model significancy; *p*<0.001 (Omnibus Tests of Model Coefficients) for Hosmer and Lemeshow Test *p*=0.326 *reference value

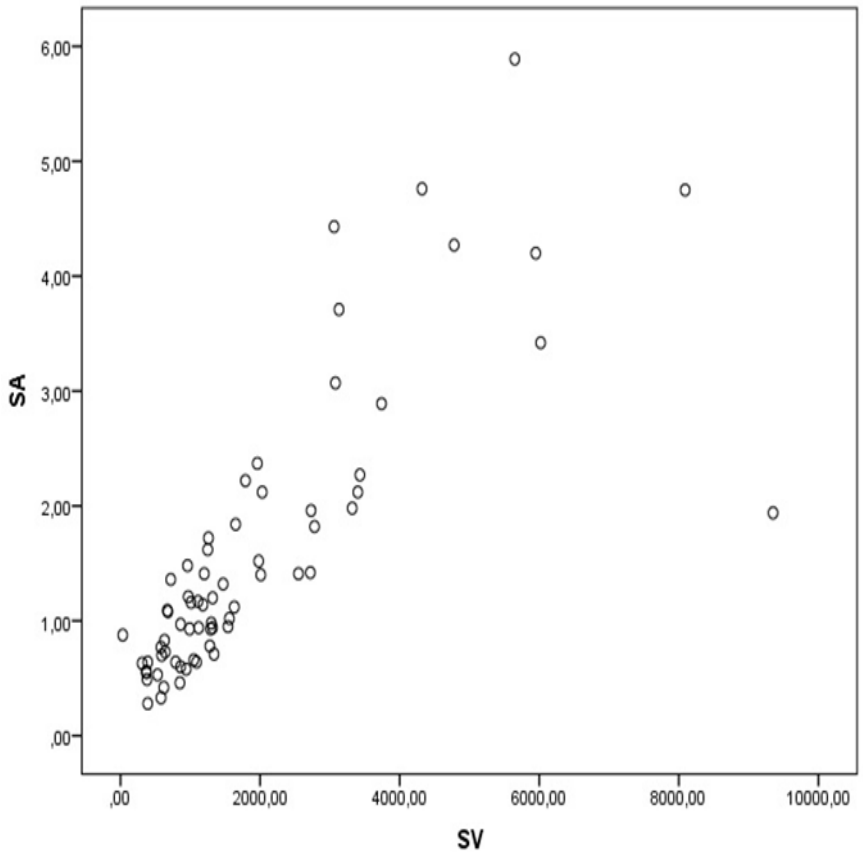

Figure 1. Scatter diagram for stone volume and stone area

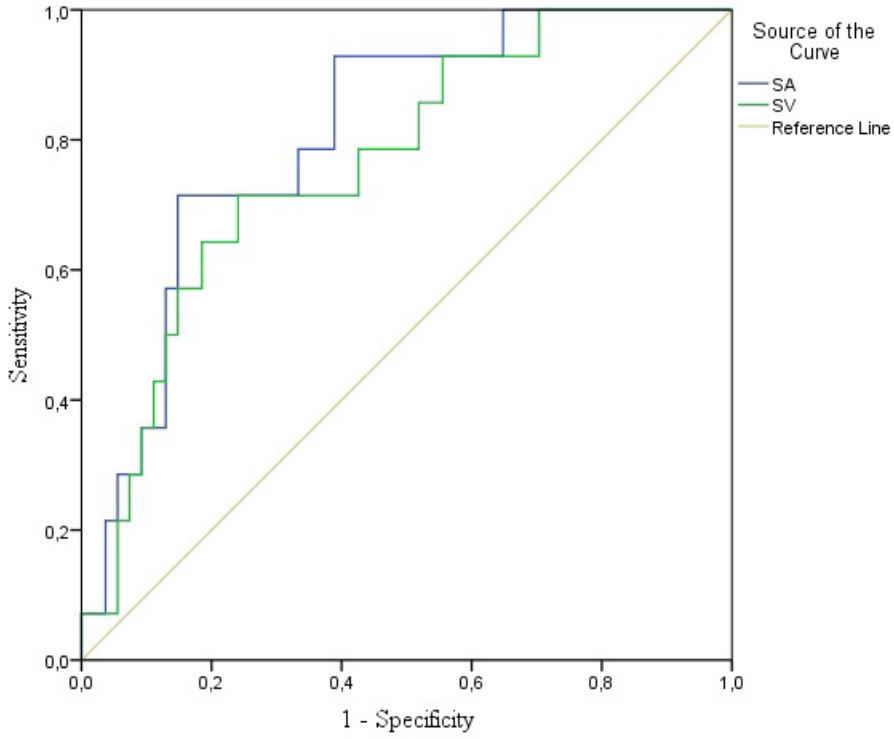

Figure 2. ROC curves of Stone area and stone volume for predicting stone-free stat

Discussion

In this work, we examined the importance and value of SV in predicting SF status after RIRS. The SA was computed by multiplying the longest dimension of the stone in axial plan by its perpendicular dimension in 3D NCCT. On 3D-NCCT, the SV was assessed using software intended to quantify tissue density within a certain range inside a defined area of interest. As a result, we hypothesized that the SV may be superior than the SA in predicting SF status for the reasons stated above, and that there may be high correlations between these stone load metrics. Our Spearmen test demonstrated high connections between SA and SV, as anticipated. In univariate analysis, SV was identified as a clinical predictor of SF status, although suggesting a lower value of predictability for SF status than SA. In addition, a pairwise assessment of the ROC curves of SA and SV revealed that their specificities for predicting stone status following RIRS were not significantly different. However, the SA outperformed the SV in predicting non-SF status after RIRS. Our multivariate backward logistic regression analysis demonstrated, however, that only SA is an independent predictor of SF status after RIRS. SV as determined by NCCT, is the most accurate predictor of SF status after extracorporeal shock wave lithotripsy (ESWL) [8, 9]. In addition, the SV was revealed to be an independent predictor of SF status following RIRS [1, 4, 13]. Thus, our findings seemed to vary from those of previously published research. However, SA has also been demonstrated to be prognostic of SF status following RIRS and linked well with SV [1, 4]. In these experiments, SA was estimated using KUB films and SV using NCCT. They discovered a significant relationship between SA and SV. Based on what we discovered and what others have indicated, SA as assessed by KUB films may be sufficient to determine SF status after RIRS.

Our ROC analysis determined that the SA cutoff value is 1.84 cm^2. This cutoff value predicted SF status with great sensitivity and specificity. According to a recent research, this figure for the predicted stone area is 1.25 cm^2 [1]. According to a previous research by the same authors, the traced stone area is 1.50 cm^2 [4]. We determined the SV threshold to be 1.650 cm^3. Previous research has indicated values between 0.84 cm^3 and 1.120 cm^3 [1, 4]. These thresholds were not comparable to ours. In addition, we can report that the imaging modalities, assessment methodology, and characterization of SF status vary amongst investigations.

In our investigation, we discovered that the SF rate after RIRS was somewhat greater in solitary stones than in non-solitary stones (p=0.054), but was not a predictor of SF status on its own. This data revealed that stone load had a far greater effect on SF status after RIRS than stone count. Our result was consistent with earlier findings [1, 4].

After RIRS, we determined that the location of the stone was not statistically significant for SF status. EAU standards identify lower pole stone as an adverse criterion for SF status [14]. We eliminated patients whose stones could not be accessible in order to prevent our findings from being corrupted by theirs, since we were interested in the effect of SV on the SF status of RIRS. According to us, this may have been the reason why we were unable to determine its effect on SF status after RIRS.

This research has significant limitations. Due to the retrospective nature of the study, confounding variables and measurement bias cannot be addressed to the same extent as in prospective randomized research. Another limitation was that this research only included findings from a single institution. Reviewing the relevant literature, we can state that published data regarding the imaging modalities, measuring methodology, and characterization of SF status are heterogeneous. Thus, further homogenous research from many sites are required.

In conclusions, SA was a predictor independent of SF status after RIRS. SV was strongly suggestive of SF status after RIRS, but it was not a predictor of SF status on its own. These predictors of SF status have cutoff values of 1.84 cm^2 for SA and 1.650 cm^3 for SV. These cutoff points may aid doctors in predicting the state of SF after RIRS. With the exception of stone load measures, the quantity of stones was not predictive of SF status. Based on prior publications and our results, it is possible that the SA evaluated by KUB films is sufficient to predict SF status in clinical practice.

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

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Ethics committee approval: The study was conducted without ethics commission approval because it was conducted before the years of 2020.

Authors' contributions to the article

H.K. have constructed/constructed the main idea and hypothesis of the study. Y.K., E.O. and B.C. developed the theory and arranged/edited the material and method section. B.C. and Y.K. have done the evaluation of the data in the Results section. Discussion section of the article written by Y.K. and H.K who also reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Brain death and effect of diagnosis on organ donation: a 10-year analysis

Beyin ölümü ve tanının organ bağışına etkisi: 10 yıllık bir analiz

Aslı Mete Yıldız, İlker Kiraz, İlknur Hatice Akbudak, Burak Eral, Simay Karaduman

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Abstract

Purpose: Brain death can be defined as the irreversible loss of brain functions. It is evident that a delay occurred in the diagnosis of brain death will result in the loss of many cadaveric organs and thus, the number of patients waiting for organ transplantation will increase. Despite there being many studies in the literature about the diagnosis and difficulties of brain death, family interviews, and organ donation, the studies regarding the effects of the regulation change in Türkiye on organ donation are limited. The present study includes 10-year retrospective data and it has been conducted to offer an insight to the scientists about the diagnosis of brain death and organ donation.

Material and method: The study was conducted by using data obtained from the examination of the retrospective files of patients diagnosed with brain death between 2011 and 2021 at Pamukkale University, School of Medicine after obtaining the approval of the ethics committee.

Results: After the study was initiated, the files of 71 patients diagnosed with brain death between 2011 and 2021, were accessed. Due to the missing information in the files of 4 patients, these patients were excluded from the study. Of 67 patients with registered brain death, 36 were male (53.7%) and 31 were female (46.2%). The age average was 49.07. When the treatment units of these patients were evaluated, 50 patients (74.6%) diagnosed with brain death were treated at the neurosurgery intensive care unit, 7 (10.4%) at the neurology intensive care unit, 6 (9%) at the anesthesia intensive care unit, and 4 (6%) at the cardiovascular surgery intensive care unit.

Conclusions: The study concluded that the importance of the diagnosis duration of brain death and the number of specialists who diagnosed brain death in the previous years may have affected this process. It is clear that the formal process, which changed after 2014, showed an acceleration in diagnosis. The importance of the interviews with family members besides the brain death diagnosis has emerged as a result of the study.

Key words: Brain death, organ donation, transplantation.

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Öz

Amaç: Beyin ölümü, beyin fonksiyonlarının geri dönüşümsüz kaybı olarak tanımlanabilir. Beyin ölümü tanısında yaşanacak bir gecikmenin birçok kadavra organının kaybına yol açacağı ve dolayısıyla organ nakli bekleyen hasta sayısının artacağı açıktır. Literatürde beyin ölümü tanı ve güçlükleri, aile görüşmeleri ve organ bağışı ile ilgili çok sayıda çalışma olmasına rağmen, Türkiye'deki mevzuat değişikliğinin organ bağışına etkilerine ilişkin çalışmalar sınırlıdır. Bu çalışma 10 yıllık retrospektif verileri içermekte olup, beyin ölümü tanısı ve organ bağışı konusunda bilim insanlarına ışık tutmak amacıyla yapılmıştır.

Gereç ve yöntem: Çalışma, etik kurul onayı alındıktan sonra Pamukkale Üniversitesi Tıp Fakültesi'nde 2011-2021 yılları arasında beyin ölümü tanısı alan hastaların retrospektif dosyalarının incelenmesi sonucu elde edilen veriler kullanılarak yapıldı.

Bulgular: Çalışma başlatıldıktan sonra 2011-2021 yılları arasında beyin ölümü tanısı alan 71 hastanın dosyalarına ulaşıldı. 4 hastanın dosyasında eksik bilgi olması nedeniyle bu hastalar çalışma dışı bırakıldı. Kayıtlı beyin ölümü olan 67 hastanın 36'sı erkek (%53,7), 31'i kadındı (%46,2). Yaş ortalaması 49.07 idi. Bu hastaların tedavi üniteleri değerlendirildiğinde beyin ölümü tanılı 50 (%74,6) hasta beyin cerrahisi yoğun bakım ünitesinde, 7 (%10,4) hasta nöroloji yoğun bakım ünitesinde, 6 (%9) hasta anestezi yoğun bakım ünitesinde ve 4 (%6) hasta da kalp damar cerrahisi yoğun bakım ünitesinde idi.

Sonuç: Çalışma, beyin ölümü tanı süresinin öneminin ve önceki yıllarda beyin ölümü tanısı koyan uzman sayısının bu süreci etkilemiş olabileceği sonucuna varmıştır. 2014 yılından sonra resmi prosedür değişiminin teşhiste hızlanma gösterdiği açıktır. Çalışma sonucunda aile bireyleri ile yapılan görüşmelerin önemi de ortaya çıkmıştır.

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Anahtar kelimeler: Beyin ölümü, donör organ, transplantasyon.

Mete Yıldız A, Kiraz İ, Akbudak İH, Eral B, Karaduman S. Beyin ölümü ve tanının organ bağışına etkisi: 10 yıllık bir analiz. Pam Tıp Derg 2023;16:266-272.

Introduction

Brain death can be defined as the irreversible loss of brain functions [1]. It was primarily declared by the identification of clinical and pathological findings by French Mollaret and Goullon in 1959 [2]. Following the exclusion of reversible conditions mimicking this clinical status, brain death is a situation that includes the irreversible loss of all the brain activities, volitional acts, response to the painful stimulus, consciousness, lower brain stem functions, and spontaneous ventilation. There are differences between severe brain damage and brain death [3]. In order to detect such differences, the situation causing irreversible brain damage should be confirmed via neurological imaging methods. Pre-requisites include the exclusion of drug exposure that may cause coma, exclusion of serious metabolic disorders that are present in the patient, and close assessment of the absence of hypothermia that may cause this situation [4].

Brain death is an accepted medical and legal death criterion in many countries around the world [5]. Among the European countries, Finland is the first country that has accepted brain death as a manner of death since 1971. In Türkiye, the diagnosis of brain death has started to be made via No. 2238 Law on organ and tissue procurement, storage, vaccination, and transplantation, which has been in effect since 1979 [6]. Diagnosis of brain death is made by clinical assessment, apnea test, and radiological imaging triad in Türkiye. While the regulation on organ and tissue transplantation services dated 01.02.2012 and numbered 28191 stated that there was a requirement for the opinion of 4 specialist physicians for the diagnosis of brain death, the Ministry of Health amended this regulation in 2014 and entered the requirement of 2 specialist physicians for the diagnosis of brain death into effect [7, 8].

Although the criteria for the diagnosis of brain death vary in different countries around the world, the invariable criteria of brain death are coma, loss of brain stem reflexes, and positivity of the apnea test [3, 4]. In case an apnea test cannot be conducted or no definitive diagnosis can be made, auxiliary imaging methods are applied. These methods are transcranial doppler, electroencephalography, cerebral tissue perfusion scintigraphy, and cerebral angiographic computerized tomography [4, 6]. After all the assessments, a diagnosis of brain death was made by 4 specialists in Türkiye before 2014 and since then, it has been made by 2 specialist physicians (anesthesiology and reanimation specialist or intensive care specialist and neurosurgery or neurology specialist) [6].

Since patients with brain death are the hope for multi-organ transplantation for the patients waiting for organ transplantation for a long time, careful diagnosis of brain death will also be a source of hope for such patients [9]. The stage of diagnosing brain death is a very critical process and this process should progress rapidly. In Türkiye, time spent on both diagnosis and documentation, and also the difficulties in family interviews regarding organ donation, are troublesome processes for the patients waiting for organ transplantation. In recent years, there has been an increase in organ transplantation from cadavers together with the importance given to donor care in intensive care units [10].

It is evident that a delay occurred in the diagnosis of brain death will result in the loss of many cadaveric organs and thus, the number of patients waiting for organ transplantation will increase. Despite there being many studies in the literature about the diagnosis and difficulties of brain death, family interviews, and organ donation, the studies regarding the effects of the regulation change in Türkiye on organ donation are limited. The present study includes 10-year retrospective data and it has been conducted to offer an insight to the scientists about the diagnosis of brain death and organ donation.

Material and method

The study was conducted by using data obtained from the examination of the retrospective files of patients diagnosed with brain death between 2011 and 2021 at Pamukkale University, School of Medicine after

obtaining the approval of the ethics committee (permission for the study was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee. Patients, whose files could not be accessed or whose data were missing, were excluded from the study. In the light of this information, the following data (as of 2011) of the patients diagnosed with brain death was investigated in the study: Age, gender, department establishing the diagnosis, clinical diagnosis before brain death, apnea test applications, type of imaging methods applied at the time of diagnosis, the status of donating their organs, the degree of affinity of the interviewees, and if lack of donation, its reason. In addition, information on whether the patient was a judicial case and whether received vasopressor agents in intensive care units as well as the diagnosis dates of brain death were evaluated. After these evaluations, the units and numbers of the physicians making the diagnosis, who were responsible for the determination of brain death, were analyzed. As a result, times of cardiac death after the diagnosis of brain death were examined.

Statistical analysis

The behaviors of quantitative variables were specified by using centralization and variance measures. In order to show the behavioral differences of the group means, an Anova T-test was used; where normality and uniformity assumptions are met. Non-parametric methods, such as Kruskal-Wallis H Test (number of groups >2) and Mann Whitney U Test (number of groups=2), were used where those were not met. For all cases, statistical significance was specified as p=0.05. Statistical analyses were provided by IBM SPSS (Statistical Package for Social Sciences for Windows, Version 21.0, Armonk, NY, IBM Corp.) package program.

Results

After the study was initiated, the files of 71 patients diagnosed with brain death between 2011 and 2021, were accessed. Due to the missing information in the files of 4 patients, these patients were excluded from the study. Of 67 patients with registered brain death, 36 were male (53.7%) and 31 were female (46.2%). The average age was 49.07. When the treatment units of these patients were evaluated, 50 patients (74.6%) diagnosed with brain death were treated at the neurosurgery intensive care unit, 7 (10.4%) at the neurology intensive care unit, 6 (9%) at the anesthesia intensive care unit, and 4 (6%) at the cardiovascular surgery intensive care unit.

Primary reasons for the hospitalization of the patients were assessed. The frequently seen reasons for hospitalization included: Subarachnoid hemorrhage (25, 37.3%), subdural hematoma (7, 10.4%), intracerebral hematoma (6, 9%), hypoxic encephalopathy (5, 7.5%), and cerebral aneurysms (4, 6%). Other reasons for hospitalization were found to be different diseases such as acute myocardial infarction, anaphylactic shock, disseminated intravascular hemorrhage, ischemic cerebrovascular cases, and intracranial mass (Table 1).

When the methods used for diagnosing brain death were examined, the number of patients with apnea tests was 54 (80.6%) and without apnea tests was 13 (19.4%). When auxiliary imaging methods used for diagnosis were observed, it was found that 53 patients (79.1%) were diagnosed by cerebral angiography computerized tomography, 9 (13.4%) by transcranial doppler, 3 (4.5%) by electro-encephalopathy (EEG), and 2 (4%) by brain Digital Subtraction Angiography (DSA). Of the patients, 14 (20.9%) were in judicial cases according to the assessment of the judicial statuses of the patients.

When the use of vasopressor agents in the intensive care follow-up of the patients was evaluated, only 16 patients (23.9%) did not need vasopressor drugs, while dopamine, dobutamine, and adrenaline were administered in various doses and combinations to other patients. In the evaluation of the specialties of the physicians determining brain death, 14 patients (20.9%) were diagnosed with brain death by 4 specialist physicians (anesthesiology, cardiology, neurology, and neurosurgery specialists). The number of brain deaths determined by the anesthesia and neurosurgery specialists was 46 (68.7%).

Documents of 4 patients could not be accessed and the brain death of the other 3 patients (4.5%) was diagnosed by anesthesiologists and neurologists. When organ donation of patients with brain death was evaluated, the organs of 48 patients (71.6%) were not donated by their families. While the cornea and both kidneys of 7 patients (10.5%) were donated; the heart, cornea, liver, and kidneys of 3 patients (4.5%) were donated. Only

the kidneys of 2 patients were donated, while the number of patients donating liver and both kidneys were 3 (4.5%). Other donations were 1 liver (1.5%), 1 liver and cornea (1.5%), and 1 liver, cornea, and both kidneys (1.5%). In one patient (1.5%), organs were donated, however, the organs were not used as a donor. After the evaluations, when the periods between the diagnosis of brain death in the patients and the timing of the cardiac death were examined, the mean time elapsed was 4.24±21.52 days (Table 2).

Table 1. Diagnosis of Brain death (Primary reasons for the hospitalization of the patients)

Diagnosis	Group n(%)
Acute MI	1 (1.5%)
Anaphylactic Shock	1 (1.5%)
Arteriovenous Malformation / Hemorrhage	4 (6%)
Dissemine Intravasculer Coaugulophaty	1 (1.5%)
Hypoxic Encephalophaty	5 (7.5%)
Intacranial Mass	1 (1.5%)
Intracerebral Hematoma	6 (9%)
Iscemic Cerebrovasculer Disesase	1 (1.5%)
Cardiac Arrest	1 (1.5%)
Perforatig Trauma	1 (1.5%)
Subarachnoid Hemorrhage	25 (37.3%)
Subarachnoid Hemorrhage/ Arteriovenous malformation	2 (3%)
Subarachnoid Hemorrhage /Hematoma	1 (1.5%)
Cerebellar Mass Herniation	1 (1.5%)
Cervical Fracture	1 (1.5%)
Subdural Hematoma	7 (10.4%)
Cerebrovasculer Disesase	3 (4.5%)
Cerebrovasculer Disesase (Hemorragic)	1 (1.5%)
Intraventricular Hemorrage	3 (4.5%)

Table 2. Mean age of patients and length of hospital stay during the brain death assessment process

	Mean±SD	**Medlan (Min-Max)**
Age	49.07±18.02	50 (9-87)
Day difference between brain death and cardiac death	4.24±21.52	2 (-71-85)
Day difference between hospitalization and brain death	6.22 ±14.09	3 (-20-92)
Day difference between hospitalization and cardiac death	10.76±15.0	7 (1-87)

In the examination of the family interviews conducted during the organ donation process, organ donation interviews with the relatives of the patients were most frequently made with the spouses of the patients (27, 40.3%). The second-highest rate in the family interviews was found to be done with the sons of the patients (13, 19.4%). Other interviews included father (8, 11.9%), both father and mother (3, 4.5%), elder brother (1, 1.5%), elder sister (1, 1.5%), sibling (2, 3%), daughters and sons (1, 1.5%), daughter-in-law and son-in-law (1, 1.5%), sister-in-law and brother-in-law (1, 1.5%) and uncle (1, 1.5%). When the reasons for not donating organs were examined, the most frequent answer of the families was the opposition to organ donation (28, 41.8%). Of the families who did not donate organs, 19 (28.4%) left this question unanswered; while 8 families (11.9%) did not want to accept that their patient had died. Table 3 shows the reasons for rejecting organ donation besides other reasons.

Table 3. Reason for rejecting organ donation

Reason for rejecting organ donation	Group n (%)
Empty	19 (28.4%)
Family rejects organ donation	28 (41.8%)
Brain death is not declared	3 (4.5%)
Patient Didn't Want To Have Organs Donated Before He/She Dies	5 (7.5%)
Inability to accept the death of the patient	8 (11.9%)
They Swore Not to Donate Organs/The Patient Was Alcoholic	1 (1.5%)
Left MCA flow was observed	1 (1.5%)
Conscientious discomfort	2 (3.0%)

Discussion

Establishing the diagnosis of brain death and following up with these patients as potential donors for organ transplantation have become a glimmer of hope for organ transplant patients in many centers in Europe and Türkiye. Statistics of brain death diagnosis have been shared by many countries in the literature. In a study conducted in England, the rate of diagnosis of brain death for potential organ transplantation was determined as 99%, while the rate of brain death diagnosis was only by using neurological criteria, which was 86%. In the same study, the rate of family interviews was 91% [11].

In the study of Karakoc et al. [12], 113 brain death diagnoses were made in Eskisehir, Türkiye in a 4-year examination and 25.7% of them resulted in organ donation. In another study, when 9-year retrospective brain death cases were analyzed, it was observed that 118 patients were diagnosed with brain death [6], and the time for the brain death diagnosis became easier after 2014.

The present study made a 10-year retrospective examination of brain death diagnoses in a university hospital, and similarly, it was found that the rate of brain death diagnosis and organ donation increased after 2014. This can be explained by the fact that the diagnosis was a more difficult process due to the legislation in the past, the awareness of organ donation increased, and the importance of communication in family meetings. However, it can also be observed that increasing the donation curve sometimes decreases in some situations. As an example, in a study conducted in Korea, despite the continued increase in Türkiye and the world until 2016, there was a sharp decrease in 2017. The reasons for this were the maltreatment of the donor after the removal of organs, the elimination of compensation given to family members, the cancellation of the life-sustaining treatment protocol provided to family members, and the regulation changes in the working hours and conditions of the doctors [13].

These assessments show that brain death diagnosis, legal, and social interviews conducted with the family members are sensitive situations. They also remind us of the importance of donor care at the stage of organ procurement.

In a relevant study, new ways of communication between the family members and hospital staff were highlighted and the fact that the main theme was empathy stood out [14]. In a study conducted in Türkiye regarding this issue, the reasons for not donating organs in the interviews of brain death declaration and organ donation in pediatric patients were found to be the thought of the deterioration of body integrity and that they would suffer if their organs were procured [15]. This study is partially correlated with the current study about the rejection of organ donation. The reason for that was the family's disapproval of organ transplantation and not accepting that their patients died. These two studies bring the lack of knowledge and education about organ donation forth. In addition, the need for donor care and rapid organ procurement in case of organ donation is a need that should not be forgotten. Unfortunately, the time elapsed between brain death and cardiac death is not very long.

A study conducted suggests that donor care should not be performed before interviewing the families [16]. In another study, it was stated that

the diagnosis of brain death was made 3 days on average after hospitalization [17], while a Turkish study reported that the diagnosis period before 2014 was 4.8 days and the period after was 2.3 days [6]. In the assessments conducted in the current study, this period was found to be 6.2 days on average (Table 1). Any time elapsed to make a diagnosis means a delay in potential organ transplantation and it should be aimed to accelerate this process.

To that end, auxiliary imaging methods are frequently used both in Türkiye and throughout the world. In a study examining the brain death criteria [1], the brain death criteria of 80 different countries were compared and it was observed that auxiliary tests are compulsory in 40% of these countries. The conditions, when auxiliary tests are required, have been explained by the Ministry of Health in Türkiye [8]. In the present study, auxiliary imaging methods were applied to all the patients diagnosed with brain death. The reason was the difficulties in performing the apnea tests because most of our patients received vasopressor drug support. Evaluation of all these practices was conducted in a study including 492 hospitals in the USA in which the hospital procedures regarding brain death were examined. When the protocols of the hospitals included in the study were examined, it was found that the diagnosis of brain death should be made by specialist physicians, auxiliary imaging methods should be applied when necessary, and the conditions before diagnosis should be determined correctly (such as hypothermia and exclusion of drug exposures mimicking brain death...). Since this diagnosis should be 100%, the USA published a guideline in 2010 related to this issue and tried to standardize the diagnosis of brain death [18]. In Türkiye, the necessary legislation has been updated by the Ministry of Health, and these practices are carried out in a standard way at hospitals [8].

Limitations of the study

The present study was conducted by gathering retrospective data for 10 years and some of the data were affected by the change in the number of physicians making the diagnosis and the change in the physicians during this period. When this situation is evaluated and individual differences in physicians are considered, the diagnosis process of brain death may have been affected during the study.

In conclusion, the study concluded that the importance of the diagnosis duration of brain death and the number of specialists who diagnosed brain death in the previous years may have affected this process. It is clear that the formal process, which changed after 2014, showed an acceleration in diagnosis. The importance of the interviews with family members besides the brain death diagnosis has emerged as a result of the study. The reality of both increasing the education regarding this issue and showing empathy and sensitivity in organ donation have emerged. Further studies regarding this issue are required both in Türkiye and in the world. Enlightenment together with sensitivity to this issue is required.

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

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Authors' contributions to the article

A.M.Y. constructed the main idea and hypothesis of the study. A.M.Y. and İ.K. developed the theory and arranged/edited the material and method section. A.M.Y., İ.K., B.E. and İ.H.A. have done the evaluation of the data in the Results section. Discussion section of the article written by A.M.Y., İ.K., İ.H.A., B.E. and S.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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The relation of vitamin D receptor gene polymorphisms with risk of obesity, metabolic syndrome, hepatosteatosis in Turkish children

Vitamin D reseptör gen polimorfizmlerinin Türk çocuklarında obezite, metabolik sendrom ve hepatosteatoz ile ilişkisi

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Abstract

Purpose: The aim of this study was to determine the relation of vitamin D receptor gene (VDR) polymorphisms of TaqI (rs731236), ApaI (rs7975232), BsmI (rs1544410), FokI (rs10735810) with the risk of obesity, metabolic syndrome and hepatosteatosis in children.

Materials and methods: 130 obese and 130 healthy children of age range between 10-16 years were included in this study. Anthropometric measurements, biochemical evaluations and abdominal USG of all children were done. Obese and healty children were analyzed for the most common polymorphisms of the VDR gene by restriction fragment length polymorphism's technique. The diagnosis of metabolic syndrome was made using the International Diabetes Federation criteria.

Results: Genotypic distribution of BsmI, FokI, and TaqI polymorphism were found statistically different between obese patients and control group, but genotypic distribution of all studied polymorphisms were not found statistically different in obese patients with metabolic syndrome or hepatosteotosis.

Conclusion: BsmI polymorphism (rs1544410) was found to have a significant positive effect on the development of obesity, metabolic syndrome and hepatosteatosis. Children who carry risk factors for childhood obesity could be screened before the development of obesity and associated metabolic complications using the BsmI polymorphism of the VDR gene.

Key words: Obesity, polymorphism, Vitamin D, metabolic syndrome.

Ozhan B, Bilgihan E, Cetin GO, Agladioglu K. The relation of vitamin D receptor gene polymorphisms with risk of obesity, metabolic syndrome, hepatosteatosis in Turkish children. Pam Med J 2023;16:274-280.

Öz

Amaç: Bu çalışmanın amacı, TaqI (rs731236), ApaI (rs7975232), BsmI (rs1544410), FokI (rs10735810) gibi Vitamin D reseptör (VDR) polimorfizmlerinin çocuklarda obezite, metabolik sendrom ve hepatosteatoz riski ile ilişkisini belirlemektir.

Gereç ve yöntem: Çalışmaya yaşları 10-16 arasında değişen 130 obez ve 130 sağlıklı çocuk dahil edildi. Tüm çocukların antropometrik ölçümleri, biyokimyasal değerlendirmeleri ve batın USG'leri yapıldı. Obez ve sağlıklı çocuklar, VDR geninin en yaygın polimorfizmlerine restriksiyon fragment uzunluğu polimorfizm yöntemi kullanılarak analiz edildi. Metabolik sendrom tanısı Uluslararası Diyabet Federasyonu kriterleri kullanılarak konuldu.

Bulgular: BsmI, FokI ve TaqI polimorfizminin genotipik dağılımı, obez hastalar ve kontrol grubu arasında istatistiksel olarak farklı bulundu, ancak çalışılan tüm polimorfizmlerin genotipik dağılımı, metabolik sendromlu veya hepatosteotozu olan obez hastalarda istatistiksel olarak farklı bulunmadı.

Sonuç: VDR geni BsmI polimorfizminin (rs1544410) obezite, metabolik sendrom ve hepatosteatoz gelişimine katkısının olduğu bulundu. Obezite gelişim riski taşıyan çocuklarda, obezite ve komplikasyonlar oluşmadan önce tespit etmek için BsmI polimorfizmi tarama amaçlı kullanılabilir.

Anahtar kelimeler: Obezite, polimorfizm, D vitamini, metabolik sendrom.

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Introduction

Obesity is the most common metabolic disorder worldwide and a potential risk factor for many life-threatening preventable diseases. Environmental factors and genetic background may contribute to the development of obesity through an increase in adipocyte number and size. Adipose tissue (AT) is an active endocrine organ which not only takes part in the storage of energy and the regulation of thermogenesis but also known to synthesize and secrete a large variety of anti-inflammatory and pro-inflammatory molecules. These are adipokines, chemokines, and cytokines that enable communication with autocrine, paracrine or endocrine signaling pathways [1].

Vitamin D is a unique fat-soluble vitamin synthesized from 7-dehydrocholesterol by direct contact with sunlight on human skin playing an important role in calcium balance and bone metabolism as well as regulating cell proliferation and differentiation in extra skeletal tissues [2]. Paracrine and autocrine effects of vitamin D occur as a result of binding of active vit D to vitamin D receptor (VDR) that is a member of the nuclear receptor-family in the cell nucleus. VDR mediating the biological functions of vitamin D is found in varying concentrations in nearly all nucleated cells of our body shows that active vitamin D has a direct or indirect effect on the regulation of a large number of genes [3]. The genomic effect of vitamin D through VDR receptors is thought to be effective in the pathogenesis of health and diseases such as cancer, metabolic syndrome, obesity, diabetes, skin diseases, immunity, inflammation, and organ transplantation-related complications [4]. In addition to these genomic effects of Vit D, it is also known to have epigenetic effects by activating signaling pathways, DNA methylation and histone acetylation [5].

Though the association between vit D and obesity is known, whether obesity is a consequence of vit D deficiency or vice versa is not yet known. Nevertheless, the expression of VDR in white and brown adipose tissue and the existence on 3T3-L1 adipocytes was reported. This shows that vit D may be effective in the development of inflammation and insulin resistance, which have a role in the development of obesity-related diseases [6].

The aim of study was to determine the relation of VDR polymorphisms *of TaqI (rs731236), ApaI (rs7975232), BsmI (rs1544410), FokI (rs10735810)* with obesity, metabolic syndrome and hepatostetosis in children.

Materials and methods

Study Population

A total of 130 obese and 130 healthy control children aged 10-16 years, who were referred to pediatric endocrinology outpatient clinic of the university hospital between January 2015 and June 2015, were evaluated. Exclusion criteria were secondary obesity due to the presence of genetic and/or endocrinological disease or the use of any medication. Tanner criteria were used for pubertal staging. Weight was measured with a balance beam scale with an accuracy of 0.001 kg, and height was measured with a manual height board with an accuracy of 0.1 cm. Body mass index (BMI) was calculated according to the formula body weight (kg) / height squared (m^2). Obesity was defined as BMI ≥ 95th percentile for age and sex according to published standards [7]. The diagnosis of metabolic syndrome was made using the International Diabetes Federation criteria (IDF) [8].

This study was approved by Pamukkale University Non-Interventional Clinical Research Ethics Committee, and the parents of all children approved the written informed consent at the beginning of the study.

Biochemical assessments

After the overnight fasting, venous blood samples were drawn to assess glucose, insulin, alanine transaminase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C). In all subjects, thyroid function tests were assessed to exclude hypothyroidism. Serum glucose and insulin were used for the calculation of *homeostasis model assessment* (HOMA-IR)=[fasting insulin (mIU/mL)×fasting glucose (mg/dL)/22.5] (mg/dL=mmol/L×18.182).

Imaging of steatosis

Brightness mode ultrasound were performed by using SiemensSonoline G 50 / Italy with 3.5 MHz convex transducers. Degree of hepatic echogenicity scaled by visualization of the

intrahepatic vessels and diaphragm was used to show the presence or absence of steatosis.

Genotyping

DNA samples were obtained from blood leukocytes using a genomic DNA kit according to the manufacturer's instructions (QIAamp DNA Accessory Set, Micro and Min Kits-QIAGEN), and stored at –20 °C until further analysis. VDR *TaqI (rs731236), ApaI (rs7975232), BsmI (rs1544410), FokI (rs10735810)*, genotyping was conducted by restriction fragment length polymorphism's technique. Four different fragments, which consisted of recognized VDR variants, were produced by fragmenting samples of DNA with the application of restriction enzymes. The produced DNA products were subjected to agarose gel electrophoresis and allele identification and genotyping were done.

Statistical analysis

SPSS (Statistical Packard for Social Sciences) for Windows statistical software version 18.0 was used for all calculations. Data distribution was analyzed using the Kolmogorov-Smirnov test. Anthropometric, biochemical parameters were compared by Student t test. Chi-square test was used to assess categorical variables. A multiple regression model was used to predict the relationship of obesity, MetS, and hepatosteatosis with VDR BsmI polymorphism. In all analyses, a p-value ≤0.05 was considered significant.

Results

A total of 260 children, 130 of whom were obese and 130 of whom were healthy, participated in the study. The mean age of 70 (53.8%) girls and 60 (46.2%) boys (46.2%) of obese children participating in the study was calculated as 13.0±2.25 years. There were 69 (53.1%) girls and 61 (46.9%) boys (46.9%) in the control group with a mean age of 12.9±1.9 years. Of the obese children, 14 (10.8%) were in the prepubertal period, 116 (89.2%) were in the pubertal period, 8 (6.2%) of the children in the control group were in the prepubertal period and 122 (93.8%) were in the pubertal period. There was no difference between the two groups by means of age, gender, and puberty. In the comparison of anthropometric and biochemical findings of obese and control group children, no differences were found between height, blood glucose level and AST level, but all the other parameters were found to be statistically different between the two groups (Table 1, 2). Liver USG could not be performed in four of the obese children participated in the study because they did not come to their appointment. Fatty liver disease was diagnosed by B-Mod USG in 78 (61.90%) obese children and metabolic syndrome was diagnosed in 20 (15.38%) obese children according to IDF criteria. BMI and waist circumference measurement (30.72±4.63 kg/m^2 / 100.12±12.33 cm) in obese children with hepatic steatosis were found to be statistically significantly higher than those without hepatic steatosis (29.01±4.22 kg/m^2 / 94.64±12.61 cm) respectively (p=0.032, p=0.017).

Table 1. Anthropometric characteristics of children

	Obese children (n:130)	Healty control (n:130)	*p*
Sex (F/M) (n, %)	70/60 (53.8/46.2)	69/61 (53.1/46.9)	0.901
Age (years)	13.08±2.25	12.93±1.91	0.514
Weight (kg)	76.27±19.59	46.85±9.04	<0.001*
Weight SDS	2.47±1.03	-0.42±0.78	<0.001*
Height (cm)	158.23±12.70	155.94±11.18	0.222
Height SDS	0.24±1.16	-0.09±0.87	0.026*
BMI (kg/m^2)	30.04±4.48	18.93±2.13	<0.001*
Bmi SDS	2.41±0.62	-0.44±0.79	<0.001*
Waist circumference (cm)	98.03±12.58	69.86±6.97	<0.001*
Systolic blood pressure (SBP) (mmHg)	118.42±12.26	107.73±9.46	<0.001*
Diastolic blood pressure (DBP) (mmHg)	75.96±9.04	68.35±6.97	<0.001*
Prepubertal/pubertal) (n, %)	14/116 (10.8/89.2)	8/122 (6.2/93.8)	0.181

F/M; female/male, SDS; standart deviation score, BMI; body mass index, * *p* values <0.05 are statistically significant

Table 2. Metabolic variables of obese patients and control group

	Obese children (n:130)	Healthy conrol (n:130)	*p*
Glucose (mg/dl)	90.97±7.05	90.63±8.76	0.966
Insulin (uIU/mL)	20.87±10.48	9.05±2.78	<0.001*
HOMA-IR	4.72±2.51	2.02±0.63	<0.001*
AST (IU/L)	20.00±5.11	19.35±5.45	0.340
ALT (IU/L)	19.83±11.12	13.21±4.97	<0.001*
Trigliserid (mg/dl)	105.50±50.29	85.03±41.36	<0.001*
Total Kolesterol (mg/dl)	157.18±29.83	139.06±27.69	0.002*
LDL (mg/dl)	87.58±25.16	70.86±18.30	<0.001*
HDL (mg/dl)	48.55±11.19	56.90±12.93	<0.001*

HDL: high density lipoprotein, LDL: low density lipoprotein, ALT;alanine transaminase, AST;aspartate aminotransferase, HOMA-IR: homeostasis model assessment for insulin resistance, *p* values <0.05 are statistically significan

Genotypic distribution of all single nucleotide polymorphisms (SNP) is presented in Table 3. Genotypic distribution of BsmI polymorphism, FokI polymorphism, TaqI polymorphism were found statistically different between obese patients and control group. But genotypic distribution of all studied SNPs were not found statistically different in obese patients with metabolic syndrome or hepatosteotosis (respectively $p>0.286$, $p>0.341$, $p>0.563$, $p>0.401$).

All studied SNPs were evaluated by using the Hardy-Weinberg equilibrium. Only BsmI polymorphism is within the equilibrium ($p\geq0.05$). In contrast, FokI polymorphism, TaqI polymorphism, and ApaI polymorphism) showed a deviation from the Hardy-Weinberg equilibrium, so we didn't use them for further analysis in the study. BsmI polymorphism of VDR showed that the heterozygous 'Bb' genotype is predominant in the distribution of obese patients (79.2%), and the 'bb' genotype is predominant in the control group (92.3%). The frequency of allele 'B' and 'b' was 0.311 and 0.689 respectively in the study groups for BsmI polymorphism.

Regression analysis showed BsmI polymorphism was found to have significant positive effect on the development of obesity, metabolic syndrome and hepatosteotosis (Table 4).

Table 3. Vitamin D receptor gene BsmI, ApaI, FokI, TaqI, polymorphisms in children with obesity and the control group

Genotypes	Obese children (n:130)	Healthy children (n:130)	*p*
BsmI			
BB	18 (13.8%)	10 (7.7%)	<0.001*
Bb	103 (79.2%)	0 (0%)	
bb	9 (7%)	120 (92.3%)	
FokI			
FF	94 (72.3%)	60 (46.1%)	<0.001*
Ff	34 (26.1%)	64 (49.2%)	
ff	2 (1.6%)	6 (4.7%)	
ApaI			
AA	43 (33.0%)	48 (36.9%)	0.087
Aa	64 (49.2%)	71 (54.6%)	
aa	23 (17.8%)	11 (8.5%)	
TaqI			
TT	43 (33.0%)	1 (0.7%)	<0.001*
Tt	65 (50.0%)	114 (87.7%)	
tt	22 (17.0%)	15 (11.6%)	

p values <0.05 are statistically significant

Table 4. Association of VDR gene polymorphism with obesity, hepatosteotosis, metabolic syndrome in Turkish children at regression analysis

	Unstandardized Coefficients		Standardized Coefficients	R Square		
Model	**B**	**Std. Error**	**Beta**		**95.0%CI for B**	***p***
Bsm1[a]	0.495	0.048	0.539	0.29	0.400-0.590	0.000
Bsm1[b]	0.137	0.032	0.258	0.06	0.074-0.200	0.000
Bsm1[c]	0.854	0.032	0.854	0.72	0.79-0.918	0.000

a. Dependent Variable: Hepatosteotosis
b. Dependent Variable: Metabolic syndrome
c. Dependent Variable: Obesity

The 'BB' genotype showed a 36.92 times increased risk of obesity (p=0.000, (OR=36.92 (95%CI 5.48-248.77)), a 27.82 times increased risk of MS (p=0.003, (OR=27.82 (95%CI 3.1-249.23)), and a 6.10 times increased risk of hepatostetosis (p=0.002; (OR=6.1 (95%CI 1.93-19.25)). The 'Bb' genotype showed a 20.13 times increased risk of MS (p=0.003, (OR=20.13 (95%CI 2.60-155.89)) and 32.36 times increased risk of hepatostetosis (p=0.002; (OR=6.1 (95%CI 1.93-19.25)) in comparison to individuals who are homozygous for ''bb'' genotype.

Discussion

In this study, we investigated the relationship of vitamin D receptor ApaI, BsmI, TaqI and FokI gene polymorphisms with childhood obesity, metabolic syndrome and fatty liver disease, and only BsmI gene polymorphism was found to be related with obesity, fatty liver and metabolic syndrome. Those with 'BB' and 'Bb' genotypes of VDR BsmI were found to be significantly associated with an increased risk of having obesity, MS, and hepatosteatosis compared to the 'bb' genotype. To our knowledge, this is the first study to report that VDR polymorphisms are associated with obesity, hepatostetosis, and MS in Turkish children. The adipose tissue (AT) is known as an active endocrine organ playing an important role in metabolic, hormonal and immunological processes. AT contains a lot of cell types such as adipocytes, immune cells, endothelial cells, smooth muscle cells and fibroblasts. The degree of obesity determines secretory activity of adipose tissue by changing number of cell types, phenotype and distribution. A hypoxic environment, adipose tissue fibrosis, and macrophage infiltration of AT caused by obesity may trigger an inflammatory response and the production of inflammatory mediators [9]. This immune dysregulation in adipose tissue eventually results in chronic low-grade systemic inflammation that is accused of the pathogenesis of obesity related-disease processes [10]. Obesity induced adipose tissue remodeling explains how an inflammatory response is initiated, nevertheless most of the inflammatory triggers are still unknown. It was shown that obesity was associated with low serum 25 (OH) D levels as well as related to VDR in many cross-sectional previous studies [11-14]. Vit D controls the inflammatory response through the VDR located on adipocytes and macrophages in adipose tissue even though evidence to support a causal link is still inadequate and the contribution to the pathophysiological mechanism is not very clear [15, 16].

The present study showed that 'BB' and 'Bb' genotypes of BsmI gene polymorphism were associated with obesity, metabolic syndrome, hepatosteatosis. Of the obese patients, 72% of patients with obesity, 29% with hepatosteatosis, and 6% with metabolic syndrome could be explained by having the BsmI polymorphism alone.

Minor allele carriers of the SNP BsmI (rs1544410) were at higher risk of an increased BMI, and BsmI gene polymorphism was more frequent in obese individuals of Arabian people showed by the previous studies [17, 18]. Contrary to the study of Sharife et al. [17], individuals carrying the major allele (G) of the Bsm I gene were at risk of obesity in a study conducted in North China [19]. Wang et al. [20] genotyped Cdx2, BsmI, TaqI, ApaI, and FokI in the VDR gene of 106 overweight/obese and 86 healthy control Chinese children to search association of VDR with MetS. They

found significantly increased risk of MetS with the FokI AA genotype, and ApaI AA genotype was correlated with overweight/obesity [20]. Cobayashi et al. [21] studied the same gene polymorphisms in the VDR gene of Brazilian children as did Wang et al. [20] and they found only VDR BsmI gene polymorphism was associated with metabolic parameters [21]. These reported contradictory results between studies may be due to the diversity of genetic backgrounds even in the same population, sample size, age range, environmental factors, and methodology.

How VDR polymorphisms are related to the disease was studied by many investigators and BsmI polymorphism of the VDR gene was shown to affect the target cells by changing the VDR length and configuration, altering the intronic regulatory elements, changing mRNA stability, or disrupting mRNA transcription splice sites [22]

The major limitations of the current study are as follows; first, the number of samples size may not be sufficient to generalize the results to the population. Second, we did not determine the serum 25 (OH) D level. Third, we didn't include healty control and obese patients under ten years old. Fourt and last, all VDR polymorphisms were not investigated.

In conclusion, this study showed that BsmI polymorphisms of the VDR gene were strong risk factors for obesity, metabolic syndrome and hepatosteatosis respectively. Children who carry risk factors for childhood obesity such as birthweight abnormality, paternal obesity, gestational diabetes, sleep disturbances, and sedentary lifestyle could be screened before the development of obesity and associated metabolic complications using BsmI polymorphisms of the VDR gene and this information might be used to identify and timely management of children at risk of obesity. To validate the results, further studies with larger multiethnic cohorts would be required.

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

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Author contributions

B.O. and E.B. designed the study, performed the data collection, wrote the draft, and created the tables for the manuscript. G.O.C. and K.A. designed the study, performed data collection and analysis, and revised the article. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

Research Article

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Investigation of the possible relation of apelin, kynurenine and IL-4/IL-10/IL-12/ Tnf-α levels with clinical and metabolic parameters in obesity

Obezitede apelin, kinürenin ve IL-4/IL-10/IL-12/Tnf-α düzeylerinin klinik ve metabolik parametrelerle olası ilişkisinin araştırılması

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Abstract

Purpose: Obesity is a medical condition caused by excess body fat that accumulates at a level that can have a detrimental impact on health. Altered glucose and lipid metabolism, low-grade chronic inflammation contributes to the pathogenesis of obesity and obesity-related metabolic dysfunction. In our study, we purposed to specify the apelin, kynurenine, IL-4, IL-10, IL-12, TNF-α protein levels in obese individuals and healthy control groups, and to research the possible relationship between clinical parameters with the data to be obtained.

Materials and methods: The levels of apelin, kynurenine, IL-4, IL-10, IL-12, TNF-α in serum/plasma samples were determined with enzyme-linked immunosorbent assay. Absorbance of the samples were measured on a microplate reader spectrophotometrically at a wavelength of 450 nm.

Results: The levels of kynurenine, IL-4, and IL-12 in the serum were higher in control group than in obese patients (p=0.009, p=0.004, p=0.002, respectively). The levels of TNF- α, IL-10, and apelin did not differ substantially between the obese patients and the control group (p=0.277, p=0.711, p=0.472, respectively).

Conclusion: Inflammation and altered immune response are two important components of obesity. They play a significant part in the emergence of obesity-related metabolic disorders. Alterations in adipokine levels may lead to the occurence and maintenance of insulin resistance and systemic inflammation in obesity. The results demonstrate that kynurenine, IL-4, and IL-12 have a complex role in obesity and can be used as therapeutic targets.

Key words: Obesity, apelin, kynurenine, TNF-α, IL-4/IL-10/IL-12.

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Öz

Amaç: Obezite, sağlığı olumsuz etkileyebilecek düzeyde biriken aşırı vücut yağının neden olduğu tıbbi bir durumdur. Değişen glikoz ve lipid metabolizması, düşük dereceli kronik inflamasyon, obezite ve obezite ile ilişkili metabolik disfonksiyonun patogenezinde rol oynar. Çalışmamızda obez hastalarda ve sağlıklı kontrol gruplarında apelin, kinurenin, IL-4, IL-10, IL-12, TNF-α serum protein düzeylerini belirlemeyi ve klinik parametreler ile arasındaki olası ilişkiyi araştırmayı amaçladık.

Gereç ve yöntem: Serum/plazma örneklerindeki apelin, kinurenin, IL-4, IL-10, IL-12, TNF-α seviyeleri, enzim bağlantılı immunosorbent testi ile ölçüldü. Numunelerin absorbansı, 450 nm dalga boyunda spektrofotometrik olarak bir mikroplaka okuyucu ile belirlendi.

Bulgular: Kontrol grubunun serumlarındaki Kinurenin, IL-4 ve IL-12 düzeylerinin obez hastalara oranla anlamlı düzeyde daha yüksek olduğu belirlenmiştir (sırasıyla p=0,009, p=0,004, p=0,002). TNF-α, IL-10 ve apelin düzeyleri değerlendirildiğinde ise obez hastalar ve kontrol grubu arasında anlamlı bir fark olmadığı tespit edilmiştir (sırasıyla p=0,277, p=0,711, p=0,472).

Sonuç: Enflamasyon ve değişmiş bağışıklık yanıtı obezitenin iki önemli bileşenidir. Bu bileşenler obeziteye bağlı metabolik hastalıkların oluşumunda önemli rol oynamaktadırlar. Adipokin düzeylerindeki değişiklikler, obezitede sistemik inflamasyonun gelişimine ve insülin direncinin sürdülmesine yol açabilir. Elde edilen sonuçlar, kinurenin, IL-4 ve IL-12'nin obezitede karmaşık bir role sahip olduğunu ve terapötik hedefler olarak kullanılabileceğini göstermektedir.

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Anahtar kelimeler: Obezite, apelin, kinurenin, TNF-α, IL-4/IL-10/IL-12.

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Introduction

The World Health Organization (WHO) claims that, obesity is a condition that occurs as a result of aberrant and excessive fat accumulation that can impair human health. Obesity is a very serious problem today. Unfortunately, every year the number of deaths due to obesity is at least 2.8 million people in worldwide [1].

The etiology of obesity involves altered glucose and lipid metabolism. Recent studies suggest evidence that tryptophan, an essential amino acid, is preferentially catabolized via the kynurenine pathway in obese patients, and therefore the level of circulating kynurenine increases (Kyn) [2]. Apelin is a regulatory peptide and the G-protein-coupled receptor's ligand (APJ). Apelin and APJ are largely expressed in many tissues and organs, including brain, heart, lung, liver, kidney, blood plasma, gastrointestinal tract, endothelial and adipose tissues [3]. Many studies have reported that increased plasma apelin is associated with metabolic pathologies. Recent studies show that apelin peptide may be a useful adipokine in metabolic disorders and it can be a potential therapeutic target for obesity and antidiabetic drugs [4, 5]. Apelin is upregulated in obesity. In clinical and experimental studies, serum apelin level or adipose tissue apelin expression increases in obesity and insulin resistance [6].

There are studies demonstrate that low-grade chronic inflammation plays a substantial role in the pathogenesis of obesity and obesity-related metabolic dysfunction. Chronic inflammatory alterations have been determined to be associated with the function of immune cells in many tissues such as hypothalamus, muscle, adipose tissue, liver, and pancreatic islet.

Regulatory T (Treg) cells, eosinophils, natural killer T cells (iNKT), and M2-like macrophages are commonly resided in adipose tissue. These cells also secrete T helper (Th) 2 cytokines and IL-10, which are anti-inflammatory cytokines that inhibit inflammation in adipose tissue. Chronic systemic inflammation brought on by altered cytokine activation and inflammatory signaling is linked to obesity. Numerous studies have attributed increased manufacture of inflammatory cytokines such as IL-6, TNF and some adipokines to obesity and increased insulin resistance during the inflammatory process. It is less clear how anti-inflammatory cytokines like IL-4 affect the emergence of insulin resistance or obesity. Insulin sensitivity and local immune response are controlled by IL-4 produced by adipocytes and hepatocytes. These findings suggest that IL-4 may attend in diet-induced obesity and metabolism processes. Additionally, IL-12 is crucial for the pathophysiology of type 1 diabetes [7].

In our study, we purposed to determine the apelin, kynurenine, TNF-α/IL-4/IL-10/IL-12 protein levels in obese individuals and healthy control groups, and to investigate the possible relationship between body fat parameters, glycolipid metabolism, insulin resistance and clinical parameters with the data to be obtained.

Materials and methods

Serum/plasma of blood samples taken from patients who applied to Internal Medicine Endocrinology outpatient clinic of Pamukkale University Hospital and diagnosed with obesity (n=31) and age-sex matched healthy control group (n=33) were studied. Demographic characteristics, history and examination findings of all cases included in the study were recorded. Since the demographic data of 2 people in the control group were not available, they could not be evaluated in the Pearson correlation analysis. Height and weight were measured using standard techniques in the patient group and healthy control group.

Body mass index was calculated as the ratio of weight to height squared (kg/m^2). Percentage of body fat distribution was measured by BIA (Bioelectrical impedance analysis) in PAU Endocrinology outpatient clinic. Concentrations of apelin, kynurenine, IL-4, IL-10, IL-12, TNF-α were measured in serum/plasma samples

with ELISA kits using the directions provided by the manufacturer (Bioassay Technology Laboratory). All samples and standards were run in duplicate. Absorbance of the samples were measured on a microplate reader spectrophotometrically at a wavelength of 450 nm.

Written informed consent was provided from all patients and the Pamukkale University ethics committee approved the study's design.

Enzyme-linked immunosorbent assay

Blood samples taken from the control and patient groups were centrifuged at 2500 rpm for 10 minutes to obtain serum. Apelin, kynurenine, TNF-α, IL-4, IL-10, IL-12 levels of serum samples were determined by applying ELISA kit protocols. In this context, the relevant protocol is as follows:

a. Standard preparation followed the kit protocol and to the appropriate wells, 50 µl of the standards were added.

b. The wells designated for the blank received 100 µL of Sample Diluent solution., and 50 µL of Sample Diluent solution for samples. Each sample was put to the appropriate wells in 50. Each well received 50 µL of Biotin-Conjugate solution. The plate was sealed, and it was allowed to rest for two hours at room temperature.

c. The sealer was removed, the wells were decanted and washed with wash buffer.

d. Each well received 100 µL of the streptavidin-HRP solution. After sealing the plate, it was left at room temperature for an hour. The wells were decanted and cleansed with wash buffer once the sealant was removed. The substrate solution was put into each well at a volume of 100 µL. At room temperature, the plate was incubated for 10 minutes.

e. To each well, 100 µL of Stop Solution were added.

f. The samples' absorbance was determined spectrophotometrically on a microplate reader at a wavelength of 450 nm.

Statistical analysis

Using SPSS 23.0 version, the study's data were statistically analyzed. All data were presented as mean ± standard deviation. To examine differences between independent group, a one-way ANOVA technique was utilized and for the correlation between continuous variables, Pearson correlation analysis was employed. $P<0.05$ was considered statistically significant.

Results

The levels of Kynurenine, IL-4, and IL-12 in the serum were higher in control group than in obese patients ($p=0.009$, $p=0.004$, $p=0.002$, respectively) (Figure 1). The levels of TNF-α, IL-10, and Apelin did not differ substantially between the obese patients and the control group ($p=0.277$, $p=0.711$, $p=0.472$, respectively).

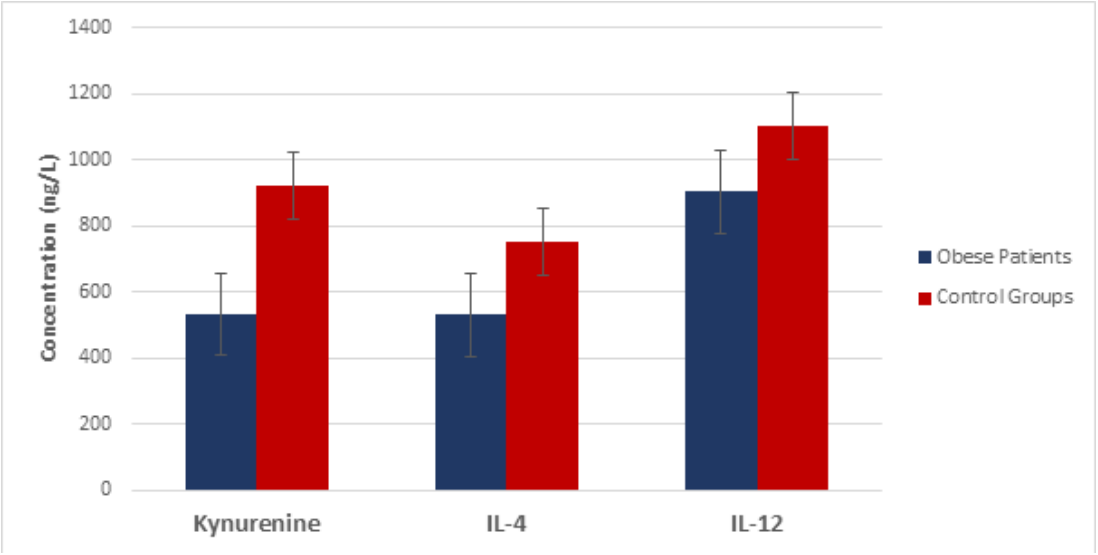

Figure 1. Kinurenin, IL-4, and IL-12 levels in serum samples of obese patients and control group

These serum protein levels were also compared with age, gender, body mass index (BMI), fat ratio, basal metabolic rate (BMR), (Table 1). When the correlation between kynurenine and gender, body mass index, age, basal metabolic rate and fat ratio was evaluated, it was specified that there was a negative correlation between kynurenine and age, BMI and fat ratio (p=0.002, p=0.033, p=0.014, respectively). Even though that there was no correlation between the levels of TNF-α and IL-10 in the obese patients and the control group there was a negative correlation between TNF-α and IL-10 with age (p=0.010, p=0.006). It was found that there was a negative correlation between IL-4 and age, BMI and fat ratio (p=0.002, p=0.010, p=0.006, respectively). In addition, it was specified that there was a negative correlation between IL-12 and age, BMI and fat ratio (p=0.019, p=0.014, p=0.018, respectively).

Table 1. Correlations between the apelin, kynurenine, IL-4/IL-10/IL-12/TNF-α levels and clinical parameters in total groups

	Gender	Age	BMR	BMI	%Fat
Kynurenine					
Pearson Correlation	-.126	-.392**	-.078	-.271*	-.310*
Sig (2-tailed)	.321	.002	.547	.033	.014
N	64	62	62	62	62
TNF-α					
Pearson Correlation	-.048	-.326**	-.031	-.136	-.157
Sig (2-tailed)	.707	.010	.809	.293	.222
N	64	62	62	62	62
IL-4					
Pearson Correlation	-.115	-.381**	-.114	-.323*	-.345**
Sig (2-tailed)	.368	.002	.378	.010	.006
N	64	62	62	62	62
IL-12					
Pearson Correlation	-.039	-.298*	-.187	-.309*	-.299*
Sig (2-tailed)	.759	.019	.146	.014	.018
N	64	62	62	62	62
IL-10					
Pearson Correlation	-.154	-.347**	.080	-.075	-.110
Sig (2-tailed)	.223	.006	.538	.563	.395
N	64	62	62	62	62

*/** Statistically significant against other groups (**p<0.01 *p<0.05)

Discussion

Obesity is a chronic condition characterized by an increase in body fat mass relative to lean body mass and is brought on by the body consuming more energy than it expends through meals. Obesity, a public health issue whose prevalence has been increasing in recent years. It is responsible for various factors such as different dietary habits, gender, smoking, age, marital status, education level, and alcohol consumption, and lack of physical activity. While the World Health Organization (WHO) accepts obesity as one of the 10 riskiest diseases, it has been determined that obesity is closely related to cancer in the latest studies conducted by the same organization.

Obesity is associated with a chronic systemic inflammation and immune activation. Therefore, it can be classified as an inflammatory disease. If the daily energy intake is more than the consumed energy, the energy that cannot be consumed is stored as fat in the body and causes obesity [8]. In general, obesity is accompanied by abnormal metabolism of amino acids [9].

Previous studies suggest evidence that tryptophan, an essential amino acid, is preferentially catabolized via the kynurenine pathway (KP) in obese patients, and therefore increases in circulating Kynurenine (Kyn) [2]. In our findings, we observed that kynurenine levels in serum samples of the control group were pointedly higher than those of obese patients (p=0.009). Mangge et al. [10] determined that the ratio of Kyn and Kyn/Trp in serum samples of obese adult patients was significantly higher than the healthy group, but the ratio of Kyn and kyn/Trp was significantly lower in individuals

under the age of 18 compared to the control group. Kyn is generally considered to be an immunosuppressive factor, and obesity is always accompanied by low-grade chronic inflammation. Therefore, the increase in circulating Kyn is thought to be a compensatory effect [11, 12]. However, it also appears that this increase in Kyn does not improve the inflammatory microenvironment in obese individuals [13, 14]. This suggests that Kyn has a complex role in obese individuals. The level of serum Trp and its metabolites, such as Kyn, Kynurenic acid (Kyn A), 3-hydroxykynurenine (3HKyn), xanthurenic acid (XA), quinolinic acid(QA) and KP enzymes were determined to be associated with type 2 diabetes [15-17]. Alteration in the levels of circulating KP metabolites has also been described in obesity and obesity-related metabolic disorders [18, 19]. However, according to studies, the relationship of these metabolites with obesity is controversial [20, 21]. The regulation of KP is affected by various factors such as age, gender, body mass index, inflammatory state. These different factors can affect the results of human studies, particularly in those with small sample size. Therefore, kyn levels should be evaluated in a larger sample group among the groups in which variables such as age and gender are classified. In our findings, it was specified that there was a negative correlation between kynurenine and age, BMI and fat ratio (p=0.002, p=0.033, p=0.014, respectively).

Apelin is a regulatory peptide and the G-protein-coupled receptor's ligand (APJ). Apelin and APJ are largely expressed in many tissues and organs, including brain, heart, lung, liver, kidney, blood plasma, gastrointestinal tract, endothelial and adipose tissues [3]. A significant number of clinical studies investigating apelin levels in body fluids, both in healthy controls and in patients with different pathologies, have been reported. Recent research shows that apelin peptide may be a useful adipokine in metabolic disorders and be used as a promising therapeutic target for obesity and antidiabetic drugs [4, 5]. Apelin is upregulated in obesity. In clinical and experimental studies, serum apelin level or adipose tissue apelin expression increases in obesity and insulin resistance [6]. Apelin supplementation is reported to improve in vitro insulinotropic activity, glucose uptake by adipocyte, glucose elimination, and insulin release in obese mice [22]. After 28 days of apelin administration, obese and insulin-resistant rats showed a significant improvement in insulin sensitivity as well as a decrease in body fat [23]. In our findings, we could not reach a significant result when the apelin levels of the obese patients were compared with the apelin levels of the control group.

Increased production of inflammatory cytokines including IL-6 and TNF has been related in numerous studies and some adipokines have been linked to insulin resistance development and obesity throughout the inflammatory process. It is less clear how anti-inflammatory cytokines like IL-4 affect the emergence of insulin resistance or obesity. In a study, it was observed that IL-4 production by splenic lymphocytes from diet-induced obese mice increased and serum IL-4 amount decreased in Sprague-Dawley rats after visceral fat removal surgery [24]. Insulin sensitivity and local immune response are controlled by IL-4 produced by adipocytes and hepatocytes. In our findings, we specified that the IL-4 level in the serum was pointedly lower in obese individuals compared to the healthy control group (p=0.004). Additionally, it was specified that there was a negative correlation between IL-4 and age, BMI and fat ratio (p=0.002, p=0.010, p=0.006, respectively). These results imply that IL-4 may involve in diet-induced obesity and metabolism processes.

Chronic inflammatory changes; It has been showed that it is associated with the function of immune cells in many tissues such as hypothalamus, adipose tissue, muscle, liver and pancreatic islet. Regulatory T (Treg) cells, eosinophils, invariant natural killer T cells (iNKT), and M2-like resident macrophages are commonly found in adipose tissue. These cells also secrete T helper (Th) 2 cytokines and IL-10, which are anti-inflammatory cytokines that inhibit inflammation in adipose tissue. In a study conducted by Calcaterra et al. [25] on obese children and adolescents, they found that IL-10 protein levels were high in obese individuals and stated that metabolic syndrome was not associated with low levels of IL-10. In a study by Pereira et al. [26], protein levels of IL-10 were also determined to be higher in obese individuals. In a study by Schmidt et al. [27] conducted with 117 obese and 83 healthy controls, the serum level of IL-10 was determined to be pointedly

higher in obese patients. In a study conducted by Arismendi et al. [28] with 129 morbidly obese individuals, the serum levels of IL-10 in the obese and control groups were examined and they showed that the serum level of IL-10 was higher in obese individuals than in controls. They observed that IL-10 levels decreased in the same patients when bariatric surgery was subsequently performed. Esposito et al. [29] also discovered that the IL-10 level in obese women was higher than in non-obese women, but the IL-10 protein level was lower in both obese and non-obese women with metabolic syndrome. In another study, the effect of IL-10 on the metabolic syndrome was investigated and it was revealed that the IL-10 protein level was significantly reduced in both men and women with metabolic syndrome [30]. In a study on childhood obesity, it was determined that the IL-10 level in obese individuals was lower than in controls. It has also been reported to have a role in increased inflammation, tissue damage and obesity [31]. These results show that IL-10 has a protective role in inflammation. In our study, we showed that the serum IL-10 level of the obese patient group was lower than the serum IL-10 level of the healthy control group. But the difference was insignificant. Although there was no meaningful correlation between IL-10 levels and obese patients and control group, there was a negative correlation between IL-10 with age (p=0.006).

Additionally, IL-12 is crucial for the pathophysiology of type 1 diabetes [32]. According to studies, IL-12 may contribute to the emergence of insulin resistance in obese mice. Suárez Álvarez et al. [33] found that IL-12 levels were pointedly higher in overweight and obese individuals compared to the control group. Nikolaju et al. [34] indicated that there was a statistically significant positive correlation between IL-12 levels and total cholesterol levels in overweight and obese women. In our study, we specified that the serum IL-12 level of the healthy control group was pointedly higher than the serum IL-12 level of the patients with obesity (p=0.002). Additionally, it was signified that there was a negative correlation between IL-12 and age, BMI and fat ratio (p=0.019, p=0.014, p=0.018, respectively).

In conclusion, inflammation and altered immune response are two important components of obesity. They play a significant role in the development of metabolic diseases associated with obesity. Changes in adipokine levels may lead to the development and maintenance of insulin resistance and systemic inflammation in obesity. The results demonstrate that kynurenine, IL-4, and IL-12 have a complex role in obesity and can be used as therapeutic targets.

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Contributions of the authors to the article

I.C.B.M. and Y.D. constructed the main idea and hypothesis of the study. I.C.B.M., Y.D., G.F.Y., E.P. and Z.A. developed the theory and organized the material and method section. Data collection was done by G.F.Y. and E.P., and data analysis was done by I.C.B.M. and Y.D. evaluated the data in the results section. The discussion section of the article was written by I.C.B.M. and Y.D. reviewed the article and made the necessary corrections and approved it. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Urinary tract infections caused by carbapenem-resistant Klebsiella pneumonia: monotherapy or combined therapy?

Karbapenem dirençli Klebsiella pneumonia'nın neden olduğu idrar yolu enfeksiyonları: monoterapi ya da kombineterapi?

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Abstract

Purpose: In this study, we evaluated healthcare-associated urinary tract infections caused by carbapenem-resistant Klebsiella pneumoniae.

Materials and methods: The study included 134 patients, diagnosed with healthcare-associated urinary tract infection caused by carbapenem-resistant Klebsiella pneumoniae. Demographic features, initial clinical conditions, comorbidities, and Charlson's comorbidity index of the patients were recorded. In addition, the MIC values of meropenem on the CR-Kp isolates, treatment regimens, clinical and microbiological responses to the treatment, as well as 14- and 28-day mortality rates of the patients, were reviewed.

Results: The 14-day mortality rate was 34.3%, and the 28-day mortality rate was 42.5%. The mean age of the patients who died was significantly higher (p=0.03). Similarly, Charlson's comorbidity index (p=0.03) and the qSOFA values (p=0.00) were significantly higher in the patients who died. The microbiological response rate was higher in the patients who survived (p=0.01) with no difference in bacteremia between the groups (p=0.29). It was found that combined antibiotherapy provided significantly better 14- and 28-day mortality rates compared to monotherapy in the group of patients with sepsis (p=0.00 and p=0.04, respectively). However, monotherapy and combination therapy in groups of patients without sepsis were insignificant (p=0.72 and p=0.36, respectively).

Conclusion: Our study supports the use of combination therapy in patients with sepsis, and monotherapy with an in-vitro active agent may be used for patients without sepsis in the treatment of urinary tract infections caused by CR-KP.

Key words: Klebsiella pneumoniae, carbapenem-resistant, healthcare-associated infection, urinary tract infection.

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Öz

Amaç: Bu çalışmada karbapenem dirençli Klebsiella pneumoniae'nın neden olduğu sağlık hizmeti ilişkili üriner sistem enfeksiyonlarını değerlendirildi.

Gereç yöntem: Çalışmaya karbapenem dirençli Klebsiella pneumoniae'nın (CR-Kp) neden olduğu sağlık hizmeti ilişkili idrar yolu enfeksiyonu tanısı almış 134 hasta dahil edildi. Hastaların demografik özellikleri, başlangıç klinik durumları, komorbiditeleri ve Charlson komorbidite indeksi kaydedildi. Ayrıca meropenemin CR-Kp izolatları üzerindeki MİK değerleri, tedavi rejimleri, tedaviye klinik ve mikrobiyolojik yanıtları ile hastaların 14 ve 28 günlük mortalite oranları incelendi.

Bulgular: 14 günlük mortalite oranı %34,3, 28 günlük mortalite oranı ise %42,5 bulundu. Ölen hastaların yaş ortalaması anlamlı olarak daha yüksekti (p=0,03). Benzer şekilde ölen hastalarda Charlson komorbidite indeksi (p=0,03) ve qSOFA değerleri (p=0,00) anlamlı olarak yüksekti. Yaşayan hastalarda mikrobiyolojik yanıt oranı daha yüksekti (p=0,01) ve bakteriyemi açısından gruplar arasında fark yoktu (p=0,29). Sepsisli hasta grubunda kombine antibiyoterapinin 14 ve 28 günlük mortalite oranlarını monoterapiye göre anlamlı olarak daha üstün olduğu saptandı (sırasıyla p=0,00 ve p=0,04). Ancak sepsis olmayan hasta gruplarında monoterapi ve kombinasyon tedavisi arasında anlamlı fark yoktu (sırasıyla p=0,72 ve p=0,36).

Sonuç: Çalışmamız, CR-Kp'nin neden olduğu üriner sistem enfeksiyonlarının tedavisinde sepsisli hastalarda kombinasyon tedavisinin, sepsis olmayan hastalarda ise in vitro aktif bir ajanla monoterapinin kullanılabileceğini desteklemektedir.

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Anahtar kelimeler: Klebsiella pneumonia, karbapenem dirençli, sağlık hizmetiyle ilişkili enfeksiyon, idrar yolu enfeksiyonu.

Temoçin F, Kayhan ŞB, Şensoy L, Kuruoğlu T, Atilla A, Tanyel E. Karbapenem dirençli Klebsiella pneumonia'nın neden olduğu idrar yolu enfeksiyonları: monoterapi ya da kombineterapi? Pam Tıp Derg 2023;16:290-297.

Introduction

Klebsiella pneumoniae is one of the most common causative bacteria of hospital infections, particularly urinary tract infections (UTI) [1]. Until recently, carbapenems were the last-resort antibiotics used against Enterobacterales, including *K. pneumoniae*; however, the antimicrobial activity decreased considerably with the emergence of carbapenemase-producing isolates [2]. Carbapenem-resistant *K. pneumoniae* (CR-KP) is an important cause of mortality with rising frequency due to limited treatment options and hospital outbreaks. Currently, outbreaks of carbapenem-resistant *Enterobacterales* (CR-E) infections are reported worldwide, particularly CR-KP infections, and this has made the treatment a global priority [3-5]. In 2017, the World Health Organization published for the first time the list of bacteria with the urgent need for new antibiotics and included CR-KPs in the group of bacteria of critical importance [6].

CR-KPs are resistant to first-line antibiotics, including carbapenems [7]. Currently, several alternative antibiotics (e.g., colistin, aminoglycosides, fosfomycin, and tigecycline) are used in the treatment of these infections in a wide geography of the world [8]. However, these antibiotics have several disadvantages, such as low urinary concentrations (tigecycline), nephrotoxicity (colistin, aminoglycosides), and poor efficacy (fosfomycin) [9, 10]. Although new antibiotics in development or recently approved antibiotics are unavailable in many regions of the world, the development of resistance to these new antibiotics is inevitable [11, 12].

In this view, available data should be reviewed to aid the clinicians in planning the treatment of infections associated with resistant bacteria. Thus, we evaluated healthcare-associated urinary tract infections (HA-UTI) caused by CR-KPs belonging to the Enterobacterales family. This study aimed to determine the preferred treatment strategies as well as clinical and microbiological responses to these treatments, 14- and 28-day mortality rates, and factors affecting mortality.

Materials and methods

Design and data collection

The study was conducted by the Infectious Diseases and Clinical Microbiology Department of Ondokuz Mayis University, Medical Faculty Hospital. This regional hospital, situated in the Black Sea Region of Türkiye, has a main building with 1,100 beds, an additional Hematology-Oncology building with 120 beds, and an adult intensive care unit with 100 beds. The study included all patients aged 18 years and older, diagnosed with healthcare-associated urinary tract infection caused by culture-confirmed carbapenem-resistant *Klebsiella pneumoniae* from January 2010 to August 2020. Healthcare-associated UTI (HA-UTI) was defined according to the CDC recommendations.

Patients were included by searching the clinic's archive, hospital digital archive, and the surveillance records of the Infection Control Committee.

Patients were excluded under 18 years of age or if they had bacteria susceptible to any carbapenem antibiotics, lower urinary tract infections, or treatment duration of less than 48 h.

Demographic features, initial clinical conditions, comorbidities, and Charlson's comorbidity indices of the patients were recorded. In addition, the MIC values of meropenem on the CR-KP isolates, antibiotic treatment regimens used, clinical and microbiological responses to the treatment, as well as 14- and 28-day mortality rates of the patients were reviewed and evaluated using statistical analysis. The presence of sepsis at the beginning of the treatment was determined using the quick Sequential Organ Failure Assessment (qSOFA) score. The qSOFA score is a score consisting of three items: respiratory rate (RR) ≥22 breaths per minute, altered mentation (Glasgow Coma

Scale [GCS] <15), and systolic blood pressure (SBP) <100 mmHg.

Microbiological analysis

We performed species-level identification and antibiotic susceptibility testing with automated VITEK® 2 Systems 8.01 (bioMérieux, Inc., Marcy l'Etoile, France). The in-vitro antibiotic susceptibility of the isolates was determined based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. Colistin susceptibility testing was performed using 96-well broth microdilution (BMD) panels following the recommendations of EUCAST.

Statistical analysis

Statistical analysis was performed using the SPSS v.21 software (Armonk, NY: IBM Corp., USA). Pearson's Chi-square and Fisher's exact test was used for categorical variables. An independent t-test was used for non-categorical variables with normal distribution, and the results were presented as mean±standard deviation. Mann-Whitney U test was used for the groups without normal distribution, and the results were presented as the median (minimum-maximum). All tests were performed with a confidence interval of 95%, and a *p*-value of <0.05 was considered statistically significant. The study was approved by the Ondokuz Mayis University, Medical Faculty Clinical Research Ethics Committee.

Results

Sixty-eight male (50.7%) and 66 (49.3%) female patients meeting the inclusion criteria and previously diagnosed with healthcare-associated urinary tract infection (HA-UTI) caused by CR-KP were evaluated during the study period. In the study, the 14-day mortality rate was 34.3% (46/134), and the 28-day mortality rate was 42.5% (57/134). The mean age of the patients who died was significantly higher than that of the surviving patients (p=0.03). Similarly, Charlson's comorbidity index (p=0.03) and the qSOFA values (p=0.00) at the start of treatment were significantly higher in the patients who died. The microbiological response rate was higher in the patients who survived (p=0.01) with no difference in bacteremia between the groups (p=0.29). The distribution and statistical analysis of the surviving and dying patients are tabulated (Table 1).

Table 1. Comparison of surviving and dying patients with urinary tract infections caused by carbapenem-resistant *Klebsiella pneumoniae*

	All patients (n=134)	Surviving patients (n=77)	Dying patients (n=57)	*p*-value
Gender (M/F)	68/66	37/40	31/26	0.46
Age (mean ± standard deviation)	64.9±14.2	62.7±13.9	68±14.3	**0.03**
Ward				
Internal medicine ward	75 (56%)	51 (68%)	24 (32%)	**0.01**
Surgery ward	24 (17.9%)	13 (54.2%)	11 (45.8%)	**0.01**
Intensive care unit	35 (26.1%)	13 (37.1%)	22 (62.9%)	**0.01**
Charlson's comorbidity index median (minimum-maximum)	6 (0-13)	5 (0-9)	6 (1-13)	**0.03**
Meropenem MIC <32	68 (50.7%)	39 (57.4%)	29 (42.6%)	0.5
Meropenem MIC <32	66 (49.3%)	38 (57.6%)	28 (42.4%)	0.5
Sepsis at the start of treatment	56 (41.8%)	12 (15.5%)	44 (77.2%)	**0.00**
Bacteremia	36 (26.9%)	18 (23.4%)	18 (31.6%)	0.29
Microbiological cure	98 (73.1%)	63 (81.8%)	35 (61.4%)	**0.01**
Treatment				
Monotherapy	56 (41.8%)	35 (62.5%)	21 (37.5%)	0.31
Combination therapy	78 (58.2%)	42 (54.5%)	36 (46.2%)	0.31

Among the patients, 56 (41.7%) received monotherapy with an in-vitro active agent, while 78 (58.2%) received combination therapy. No difference was found in 14- and 28-day mortality rates between the monotherapy and combination therapy groups (p=0.77 and p=0.31, respectively). During the sub-group analysis of the initial treatment regimens of patients, it was found that combined anti-biotherapy provided significantly better 14- and 28-day mortality rates compared to monotherapy in the group of patients with sepsis (p=0.00 and p=0.04, respectively). However, monotherapy and combination therapy in groups of patients without sepsis at the start of treatment were insignificant (p=0.72 and p=0.36, respectively) (Table 2).

Meropenem and colistin combination was the preferred combination in patients treated with combination therapy, with 34.6% of patients receiving this regimen, followed by a combination of meropenem and aminoglycoside, received by 21.7% patients. No significant difference was found between the preferred combination regimens in terms of mortality and microbiological cure. Conversely, only the combinations containing tigecycline showed a significantly worse microbiological response. Among all combination therapies, meropenem was preferred by 65.3% of the patients. There was no significant difference in 14-day mortality, 28-day mortality, and microbiological response between the patients receiving combination therapy with or without meropenem (Table 3).

The MIC values of meropenem for 68 strains of CR-KPs was ≥16 and ≥32 for 66 strains. Comparison of patients with a meropenem MIC of ≥16 and ≥32 showed no significant difference in terms of 14-day mortality, 28-day mortality, and microbiological cure irrespective of the treatment regimen (Table 4). In addition, no difference was observed in the mortality and microbiological cure that included only the 51 patients receiving meropenem in combination therapy (Table 5). CR-KP strains showed 37.2% colistin resistance, and 55.9% amikacin resistance, presented in Table 6, along with other antibiotic resistance rates.

Table 2. Comparison of treatments of patients with or without sepsis at the start of treatment

	Total	Monotherapy	Combination therapy	*p*-value
All patients	n=134	n=56	n=78	
14-day mortality	46 (34.3%)	20 (35.7%)	26 (33.3%)	0.77
28-day mortality	57 (42.2%)	21 (37.5%)	36 (46.2%)	0.31
Patients with sepsis at the start of treatment	n=56	n=17	n=39	
14-day mortality	37 (66.1%)	16 (94.1%)	21 (53.8%)	**0.00**
28-day mortality	44 (78.6%)	16 (94.1%)	28 (71.8%)	**0.04**
Patients without sepsis at the start of treatment	n=78	n=39	n=39	
14-day mortality	9 (11.5%)	4 (10.3%)	5 (12.8%)	0.72
28-day mortality	13 (16.7%)	5 (12.8%)	8 (20.5%)	0.36

Table 3. Comparison of combination regimens with and without meropenem

	Combination with meropenem n=51	Combination without meropenem n=27	*p*-value
14-day mortality	17 (33.3%)	9 (33.3%)	1
28-day mortality	23 (45.1%)	13 (48.1%)	0.7
Microbiological cure	13 (25.5%)	7 (25.9%)	0.9

Table 4. The 14- and 28-day mortality rates of all patients by meropenem MIC values

	Meropenem MIC ≥16 (n=68)	Meropenem MIC ≥32 (n=66)	*p*-value
14-day mortality	25 (36.8%)	21 (31.8%)	0.5
28-day mortality	29 (42.6%)	28 (42.4%)	0.9
Microbiological cure	22 (32.4%)	14 (21.2%)	0.1

Table 5. The 14- and 28-day mortality rates of the patients receiving meropenem in combination therapy by meropenem MIC values

	Meropenem MIC ≥16 (n=22)	Meropenem MIC ≥32 (n=29)	*p*-value
14-day mortality	8 (36.4%)	9 (31.0%)	0.6
28-day mortality	10 (45.5%)	13 (44.8%)	0.9
Microbiological cure	14 (63.6%)	24 (82.8%)	0.2

Table 6. Rates of resistance against other antibiotics in carbapenem-resistant *Klebsiella pneumoniae* strains

	Amikacin (n=134)	Fosfomycin (n=118)	Colistin (n=51)	Tigecycline (n=47)
Susceptible	59 (44%)	73 (61.8%)	32 (62.7%)	23 (48.9%)
Resistant	75 (55.9%)	45 (38.2%)	19 (37.2%)	24 (50%)

Discussion

In the present study, the 14-day mortality rate was 34.3%, and the 28-day mortality rate was 42.5% in HA-UTIs caused by carbapenem-resistant *Klebsiella pneumoniae*. These high mortality rates are noteworthy and emphasize the significance of HA-UTIs caused by CR-Kp. Thus, these infections should be evaluated carefully. Xu et al. [7] evaluated 62 studies by meta-analysis and reported a 42% mortality rate in infections caused by CR-Kp, with lower mortality in urinary tract infections. The meta-analysis also suggested that older age, the need for intensive care, and the presence of underlying diseases are variables increasing the mortality. Concordant to literature data, our study also found that mortality increased with age. In addition, high Charlson's comorbidity index scores, the presence of sepsis, and the need for intensive care were found to increase mortality. Thus, patients with comorbidities and/or elderly patients should be closely monitored.

It is challenging to define the standard treatment regimens, as the first-line antibiotics are not used in the majority of the cases of CR-Kp infection [7]. Antibiotics, including colistin, aminoglycosides, fosfomycin, and tigecycline, used in monotherapy or combination therapy of these infections, are sometimes associated with side effects, and uncertainty in its efficacy [8]. However, the impact of monotherapy and combination therapies on survival outcomes is still unclear [13, 14]. Paul et al. [15] showed that combination therapy, including at least one *in-vitro* active agent, decreased mortality in patients with sepsis and septic shock in their study on 205 patients with CR-Kp bacteremia. Tumbarello et al. [16] found no difference between monotherapy and combination therapy in urinary tract infections caused by KPC-producing *Klebsiella pneumoniae*. Moreover, they also reported that combination therapy was associated with higher survival compared to monotherapy in patients with septic shock. Combination therapy was found to be significantly superior to monotherapy in urinary tract infections caused by CR-Kp in terms of 14- and 28-day survival in patients with sepsis, similar to the literature. However, no significant differences were found between combination and monotherapy in the group without sepsis.

Thus, monotherapy with an *in-vitro* active agent could be administered to patients without sepsis/septic shock at the start of treatment for urinary tract infections caused by CR-Kp, and combination therapy could be initiated in patients with sepsis.

There is inadequate evidence to propose specific antibiotic combinations for the treatment of CR-Kp infections [17]. Papst et al. [18] reviewed the treatment strategies in a cross-sectional study used for CR-E infections in all hospitals (France, Greece, Israel, Italy, Kosovo, Spain, and Slovenia) and some selected hospitals in the U.S. with more than 800 acute care beds. It was observed that the carbapenem-colistin combination was used in 50.5% of patients with HA-UTI caused by CR-Kp. In our study, the most preferred antibiotic pair was meropenem and colistin, followed by meropenem and aminoglycoside. Although several studies reported that colistin-containing combinations are associated with lower mortality [19, 20], our study showed no significant difference between the combinations.

Despite carbapenem-resistance, adding carbapenem in combination regimens and evaluating meropenem MIC values decreases mortality and speeds up clinical recovery. In a multicenter study assessing the efficacy of different antibiotic combinations on infections caused by CR-Kp, combination regimens containing meropenem provided considerable therapeutic benefits when MIC was ≤8 mg/L while proving ineffective with MIC over 32 mg/L. Thus, emphasizing that further studies are required to define the potential of carbapenem-containing combinations for isolates with a meropenem MIC of 16 [16]. A similar study demonstrated that in septicemia by CR-Kp, the slow addition of infused high-dose meropenem to the combination also decreased mortality in patients with a MIC of ≥16 [21]. Another study reported that combination regimens, including carbapenem with at least one active drug (commonly colistin), provides a significantly higher success rate compared to non-carbapenem-containing regimens [22]. In our present study, meropenem was added to an *in-vitro* active agent in 51 of the 78 patients (65.3%). However, no difference was found in mortality between combinations with and without meropenem. Analysis based on the meropenem MIC against *Klebsiella pneumoniae*, following treatment regimens with or without meropenem, showed insignificant differences between patients with a MIC of ≥16 and ≥32 in terms of mortality and microbiological cure. However, our study did not include strains with lower MIC values, since there were no isolates with MIC values of <16. Moreover, an inadequacy of our study was that meropenem doses and infusion times used in patients were not evaluated. In most studies, meropenem was used in high doses and prolonged infusion regimens proposing its addition to combination regimens on strains with high MIC values in CR-Kp infections. Furthermore, the contribution of high-dose, prolonged infusion of meropenem to the combination regimens in HA-UTIs caused by CR-Kp should be investigated.

The resistance rates of the CR-Kp strains to colistin, aminoglycosides, tigecycline, and fosfomycin were 37.2%, 55.9%, 50%, and 38.2%, respectively. The 37% resistance to colistin is particularly alarming. A joint recommendation by the CLSI and EUCAST released in 2016 recommended the ISO-20776 standard broth microdilution (BMD) method for MIC testing of colistin [23, 24]. Other testing methods, such as agar dilution, disk diffusion, and gradient diffusion, are not currently recommended. Our high colistin resistance rates may be due to the use of disc diffusion for susceptibility testing in the past. But it is a fact that colistin resistance is dramatically increasing over the years [25]. Resistance rates vary depending on regions, similar to the literature. Consequently, we risk losing all these treatment options in the near future until strict policies for antibiotic use and infection control measures are implemented.

In conclusion, our study aimed to contribute by providing an analysis of data on urinary tract infections caused by CR-Kp, and its increasing frequency, which is a significant health problem due to high mortality rates and limited treatment. Our study supports the use of combination therapy in patients with sepsis, and monotherapy with an in-vitro active agent may be used for patients without sepsis in treating urinary tract infections. An additional benefit of adding meropenem to combination therapy was not established. However, it should be kept in mind that meropenem MIC values were ≥16 for 68 strains and ≥32 for 66 isolates.

More studies are needed to evaluate the treatment and follow-up options, considering the high morbidity and mortality rates of carbapenem-resistant *Klebsiella pneumoniae* infections. This can be achieved if each center could contribute by presenting their data. Thus, there is a need for data and recommendations with high-level evidence from randomized controlled studies on this subject.

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Author contributions

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Closure of the appendix stump with two different suture materials in laparoscopic appendectomy in children

Çocuklarda laparoskopik appendektomide iki farklı sütür materyali ile appendiks güdüğünün kapatılması

Osman Uzunlu

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Abstract

Purpose: To evaluate the outcomes of the closure of the appendix base using two different suture materials that we used in our clinic with those published in the literature.

Materials and methods: The study retrospectively reviews 36 cases of children who underwent laparoscopic appendectomies. The patients were divided into two groups: The Vicryl suture group and the silk suture group. Laboratory results, diagnostic algorithms, surgical techniques, and complications were investigated.

Results: Sixteen (44%) of the cases were male, and twenty (56%) were female. All patients' mean hospital admission time was 2.22±1.6 days (range: 1-7 days). The mean WBC and CRP levels were 14.67±5.8x109/L, 139.3±120 mg/L in the vicryl group and 15.36±6.4x109/L and 86±80 mg/L in the silk group. Small bowel obstruction was observed in nine patients in the vicryl group and six patients in the silk group. Ultrasonography was performed in 31 of the cases, the appendix was visualized in 21 cases (67%).Intravenous contrast-enhanced CT imaging was applied in 25 cases. At laparoscopic exploration, 20 cases were classified as complicated—17 due to perforation, and 3 due to gangrene. All cases were treated with broad-spectrum antibiotics. The mean follow-up period was 43 months (range: 10-117 months); none of the patients experienced intraoperative complications, and only two patients had minor complications in the postoperative period.

Conclusion: In all circumstances and regardless of the appendix's diameter, the appendix stump can be securely closed with both silk and vicryl suture material.

Key words: Laparoscopic appendectomy, child, stump closure, complications.

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Öz

Amaç: İki farklı dikiş materyali kullanılarak apendiks güdüğünün kapatılması sonuçlarının literatür verileri ile karşılaştırılması.

Gereç ve yöntem: Bu çalışmada laparoskopik apendektomi uygulanan 36 çocuk vakası retrospektif olarak incelendi. Hastalar iki gruba ayrıldı: Vicryl sütür grubu ve ipek sütür grubu. Laboratuvar sonuçları, tanı algoritmaları, cerrahi teknikler ve komplikasyonlar araştırıldı.

Bulgular: Olguların 16'sı (%44) erkek, 20'si (%56) kız idi. Tüm hastaların ortalama hastaneye başvuru süresi 2.22±1,6 gün (1-7 gün) idi. Ortalama WBC ve CRP seviyeleri vicryl grubunda 14.67±5.8x109/L, 139.3±120 mg/L ve ipek grubunda 15,36±6,4x109/L ve 86±80 mg/L idi. Vicryl grubunda 9, ipek grubunda 6 hastada ince barsak obstrüksiyonu görüldü. Olguların 31'ine ultrasonografi yapıldı, 21'inde (%67) apendiks görülebildi. 25 olguya intravenöz kontrastlı BT uygulandı. Laparoskopik eksplorasyonda 20 vaka komplike olarak sınıflandırıldı - 17'si perforasyon ve 3'ü gangrenöz. Tüm olgular geniş spektrumlu antibiyotiklerle tedavi edildi. Ortalama takip süresi 43 aydı (10-117 ay); hiçbir hastada intraoperatif komplikasyon görülmedi ve sadece iki hastada postoperatif dönemde minör komplikasyon gelişti.

Sonuç: Her koşulda ve apendiksin çapından bağımsız olarak apendiks güdüğü hem ipek hem de vikril sütür materyali ile güvenli bir şekilde kapatılabilir.

Anahtar kelimeler: Laparoskopik apendektomi, çocuk, güdük kapatılması, komplikasyonlar.

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Introduction

The most frequent intra-abdominal surgical operation in children is an appendectomy [1]. The laparoscopic appendectomy has become a gold standard method for acute appendicitis cases. The base of the appendix is secure with many different methods, techniques, and equipment [2-6]. The best strategy for securing the appendix's base has not yet been agreed upon in a standard manner. The most crucial step in this operation is to seal the appendiceal stump securely, that is why research on the subject is still being done [7]. The base of the appendix can be secured using a variety of techniques, including energy-based devices, metallic or plastic clips, staplers, or suture materials (extracorporeal or intracorporeal) [8]. All method has advantages and disadvantages of their own. The purpose of this study was to evaluate the outcomes of the closure of the appendix base using two different suture materials that we used in our clinic and compare with those published in the literature.

Materials and methods

At our clinic, 36 cases of acute appendicitis were treated laparoscopically between March 2017 and July 2019. Laparoscopic surgery was our first choice of treatment in all appendicitis cases and surgical procedure was performed by a single surgeon in all cases. In the early years of our surgical approach, the base of the appendix was secured with vicryl sutures; however, in the following years, silk sutures were chosen. The patients were divided into two groups: The Vicryl suture group and the silk suture group. Ethical approval for this study was obtained from University Ethics Committee.

Diagnosis of acute appendicitis

The diagnosis of acute appendicitis was based on clinical history, physical examination, laboratory results, and radiological evaluation. Cases pre-defined as appendicitis were subsequently scanned by routine radiological examinations. Abdominal radiography and abdominal ultrasonography (US) were the initial radiologic evaluations. In the US findings, an appendix diameter of longer than six millimeters, an uncompressible appendix, and echogenicity of tissue around the appendix confirmed acute appendicitis. Computed abdominal tomography (CT) was applied to late-admitted and obese patients and cases where US failed to confirm appendicitis.

Surgical procedure

Laparoscopic appendectomy was performed using a standard three-port technique. A 10-millimeter 30° port for the camera was used for abdominal exposure, placed transumbilical using the open technique. After carbon dioxide insufflation (maximum pressure: 10-12 mmHg), an additional two working ports were inserted from the suprapubic and left lower quadrants. The mesentery of appendix was sectioned using a surgical energy device and hook cautery. An intracorporal suturing technique with 1-0 silk or vicryl suture material secured the base of the appendix and the appendectomy was performed. Removal of appendix specimens was accomplished through the first port site without any retrieval bags. The peritoneal cavity was irrigated and aspirated with saline solution and dried.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc. Chicago, IL). The Kolmogorov–Smirnov test was performed to assess normal distribution. The homogeneity of variance was determined by the Levene test. Parametric variables were analyzed by independent *t*-test and non-parametric variables by Mann-Whitney *U* test. To compare qualitative variables Chi-square test with Fisher exact test correction was used. The level of statistical significance for descriptive statistics was set at $p<0.05$.

Results

Sixteen (44%) of the cases were male, and twenty (56%) were female. The male-to-female ratio was 0.8. All patients' mean hospital admission time was 2.22±1.6 days (range: 1-7 days). The mean admission time for the 15 vicryl group was 1.94±1.2 days (range: 1-5 days), and the mean admission time for the 21 silk group was 2.5±1.9 days (range: 1-7 days). Admission time in the groups was not significant ($p=0.077$). Mean WBC and CRP levels were 14.67±5.8x10^9/L, 139.3±120 mg/L in the vicryl group and 15.36±6.4x10^9/L and 86±80 mg/L in the silk group. Small bowel

obstruction was observed in nine patients in the vicryl group and six patients in the silk group (Table 1). Ultrasonography was performed in 31 of the cases, the appendix was visualized in 21 cases (67%), all of which presented acute appendicitis. The mean appendix diameter was 9.6±3 mm (range: 7-15 mm). No cases presented additional abdominal pathologies (e.g, tuba-ovarian pathology). Intravenous contrast-enhanced CT imaging was applied in 25 cases. In four cases, the appendix could not be identified due to its perforated structure. In the 21 cases where the appendix was identified, it appeared inflamed, and enlarged, peri appendiceal fat stranding was observed, and the mean appendix diameter was 10.3±2 mm (range: 7-16 mm). At laparoscopic exploration, 20 cases were classified as complicated - 17 due to perforation, and 3 due to gangrene. All cases were treated with broad-spectrum antibiotics. All patients were discharged with an oral antibiotic regimen of amoxicillin, clavulanic acid and metronidazole.

Table 1. General characteristics of vicryl and silk group

	Vicryl group n:16	**Silk group n:20**	***p-value***
Symptom duration (day)	1.94±1.2	2.5±1.9	0.077
WBC x10^9/L	14.67±5.8	15.36±6.4	0.739
CRP mg/L	139.3±12.0	86±80	0.132
Ileus sign (x-ray) n (%)	9 (56)	6 (30)	0.001*
LOS (day)	5.31±2.2	5.9±2.7	0.498

LOS: Lenght of hospital stay, WBC: White blood cell, CRP: C-reactive protein, *p<0.05 is significant

The mean follow-up period was 43 months (range: 10-117 months); none of the patients experienced intraoperative complications, and only two patients had minor complications in the postoperative period. An intra-abdominal abscess occurred 15 days after surgery in one of these cases, and a surgical site infection (transumbilical port insertion site) occurred in the other. The case which had intraabdominal abscess was managed conservatively by antibiotic therapy.

Discussion

In both children and adults, laparoscopic appendectomy is one of the most common surgical procedures. The increase in scientific and technological advancements since the first description has led to changes in the surgical procedure's use [9]. The mesentery of appendix is generally dissected in a standard manner using energy-based instruments, however, there are many techniques for securing the base of the appendix [3, 6]. Due to the potential for serious postoperative complications, such as postoperative peritonitis, sepsis, fistulas, and reoperations, associated with the appendiceal stump's improper management, the secure closure of the stump is believed to be the most crucial step of the procedure [10-12].

In open surgery, the method for securing the appendix base is standard, whereas laparoscopic methods' approaches (devices, materials, etc.) depend on the surgeon's preference and level of skill [4]. In experimental studies, techniques for securing the base of the appendix, including energy-based devices, clips, and suturing techniques, have been investigated [12, 13]. Though these devices are not always accessible, they are expensive, and their use is not always feasible in all circumstances, there is adequate experimental and clinical research on the closure of the appendix base with energy devices [4, 14].

According to reports, the usage of energy devices, clips, and staplers throughout the healing process may lead to complications including intestinal obstruction, abscess, and clip migration [15, 16]. In our study, we observed no complications such as intestinal obstruction, fistula, leakage, reoperation, etc. over the period of the mean 43-month follow-up period.

Recent studies have shown that stump leaking was not detected during laparoscopic appendectomies performed using various techniques; in our research, we also used two different suture materials and reported no leakage [3, 14, 17].

It is crucial for leakage and local infection to seal the stump closed, particularly in the inflammatory and perforated structure of the appendix. Despite the fact that 50% of the cases in our study had perforations, both the vicryl suture and the silk suture material had no issues with stump closure. The suture materials did not differ significantly from one another.

Among the factors that significantly affect stump closure are the appendix's diameter and its degree of inflammation. Use of staplers is advised, particularly in cases of inflamed or appendicitis with a diameter of more than 10 mm [10, 18]. The stump closure technique was carried out safely using the conventional method even though the appendix diameter was greater than 10 mm in 52% (19/36) of the cases in our study.

The choice of stump closure is known to be influenced by the surgeon's experience and the cost of the materials [3, 4, 7]. The intracorporeal suturing technique's main drawback is that it necessitates prior surgical training and experience. One of the most fundamental applications of laparoscopic surgery training is an appendectomy, and this study demonstrates that both suture materials can be used in this procedure safely (especially for surgeons who are in the training phase). The retrospective, non-randomized, small sample size and the fact that the surgeries were performed by a single surgeon with experience in minimally invasive surgery are among the study's limitations.

In conclusion; It is well known that laparoscopic surgery reduces the length of hospital stays, dependence on pain medication, and many other complications. In fact, laparoscopic appendectomy is the main therapeutic approach for appendicitis. We propose that, in all circumstances and regardless of the appendix's diameter, the appendix stump can be securely closed with both silk and vicryl suture material.

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Research Article

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Investigation of the effect of perineural invasion on survival rates in rectal tumors

Rektum tümörlerinde perinöral invazyonun sağkalım oranlarına etkisinin araştırılması

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Abstract

Purpose: Colorectal cancers compose the third most common cancer group leading to death, with increasing rates in developed countries after lung and breast cancers. Rectal cancers make up about 30% of all colorectal cancers. Although many factors affecting recurrence and survival in rectal cancer have been identified, the stage of the tumor is the most important prognostic factor. However, many additional parameters that can be associated with survival and affect the adjuvant treatment plan have been identified besides the stage. Perineural invasion (PNI) is one of the parameters that can be associated with survival and affect the adjuvant treatment plan in colorectal cancers. The aim of this study is to investigate the effects of various histopathological prognostic factors, including PNI on disease-free survival, overall survival, and recurrence rates in the study group.

Materials and methods: The clinical records of patients diagnosed with rectal cancer and operated on at the Department of General Surgery, Faculty of Medicine, Pamukkale University between 2008-2014 were reviewed. To investigate the factors affecting disease-free survival, recurrence rates, and overall survival, the presence of PNI, serum CEA levels, cTNM stage, pT stage, pN stage, number of metastatic LNs, tumor budding status, histopathological grades, lymphocyte infiltration, and presence of desmoplasia were evaluated in patients.

Results: A total of 124 patients were included in the study. 42 patients (33.9%) had PNI while 82 patients (66.1%) did not. Of the 45 patients with recurrence, 26 had PNI while 19 did not have PNI. Of the 79 patients without recurrence, 16 had PNI while 63 did not exhibit any evidence of PNI. Similarly, PNI was seen more frequently in deaths related to the disease. The 3-year average survival of patients with and without PNI was 55% and 89%, respectively (p<0.05). The 5-year survival was 38% and 83% respectively (p<0.05). Similarly, there was a significant relationship between the presence of PNI and 3-year disease-free survival (46.8% and 84.1% respectively, p<0.05) and 5-year disease-free survival (22.3% and 80.4% respectively, p<0.05). In the multivariate analysis in which other prognostic factors were also evaluated, the presence of PNI was found to be significantly associated with both overall survival (HR:3.4, 95% CI:1.7-7.2) and disease-free survival (HR:3.0, 95% CI:1.6-5.6).

Conclusion: PNI presence is a strong and independent prognostic indicator for survival. Large-scale, randomized, prospective studies are needed to determine whether PNI presence should be taken into account in the staging of colorectal cancer or play a role in choosing adjuvant therapy.

Key words: Rectal cancer, perineural invasion, survival.

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Öz

Amaç: Kolorektal kanserler insidansı gelişmiş ülkelerde giderek artan oranlarda akciğer ve meme kanserlerinden sonra en sık ölüme yol açan üçüncü kanser grubunu oluşturmaktadır. Tüm kolorektal kanserlerin %30 kadarını ise rektum kanserleri oluşturmaktadır. Rektum kanserlerinde nüks gelişimi ve sağkalım üzerine etkisi olabilecek birçok faktör tanımlanmış olmakla birlikte en önemli prognostik faktör tümörün evresidir. Ancak evre ile birlikte sağkalımla ilişkili olabilecek ve adjuvan tedavi planını etkileyebilecek birçok ek parametre tanımlanmıştır. Perinöral invazyon (PNİ) da kolorektal kanserlerde sağkalımla ilişkili olabilecek ve adjuvan tedavi planını etkileyebilecek parametrelerden birisidir. Bu çalışmanın amacı rektum kanseri nedeniyle opere edilen hastaların başta PNİ olmak üzere çeşitli histopatolojik prognostik faktörlerin hastalıksız sağkalıma, genel sağkalıma, rekürrens oranlarına etkilerinin araştırılmasıdır.

Gereç ve yöntem: Pamukkale Üniversitesi Tıp Fakültesi Hastanesi'nde 2008-2014 yılları arasında rektum kanseri tanısı koyularak Genel Cerrahi Anabilim Dalı tarafından opere edilen hastaların klinik kayıtları incelendi. Olgularda hastalıksız sağkalıma, nüks oranlarına ve genel sağkalıma etki eden faktörleri araştırmak amacıyla başta PNİ olmak üzere serum CEA düzeyleri, tümör evresi (cTNM, pT, ve pN evresi), metastatik LN sayıları, tümör budding durumu, tümör gradeleri, lenfosit infiltrasyonu ve desmoplazi varlığı değerlendirilmiştir.

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Bulgular: Bu çalışma toplam 124 hasta analiz edilmiştir. Hastaların 42 tanesinde (%33,9) PNİ saptanırken kalan 82 hastada (%66,1) PNİ saptanmadı. Nüks saptanan 45 hastanın 26 tanesinde PNİ saptanırken 19 tanesinde saptanmamıştır. Nüks saptanmayan 79 hastanın 16 tanesinde PNİ varken 63 tanesinde PNİ saptanmamıştır. Benzer şekilde hastalığa bağlı ölümlerde PNİ anlamlı olarak daha sık görülmektedir. PNİ olan ve olmayan hastaların 3 yıllık ortalama sağkalımları sırası ile %55 ve %89 olarak saptandı (p<0,05). 5 yıllık sağkalımları ise sırası ile %38 ve %83 olarak saptandı (p<0,05). Benzer şekilde PNİ varlığı ile 3 yıllık hastalıksız sağkalım (sırası ile %46,8 ve %84,1, p<0,05) ve 5 yıllık hastalıksız sağkalımları (sırası ile %22,3 ve %80,4, p<0,05) arasında anlamlı ilişki bulundu. Diğer prognostik faktörlerin de incelendiği multivariate analizde PNİ varlığı hem genel sağkalım için (HR:3,4; 1,7-6,7; %95CI, p<0,001) hem de hastalıksız sağkalım için (HR:2,5; 1,2-4,9; %95 CI, p<0,001) bağımsız prognostik faktör olarak saptanmıştır.
Sonuç: PNİ varlığının sağkalım için güçlü ve bağımsız bir prognostik belirteç olduğu saptanmıştır. PNİ varlığının kolorektal kanser evrelemesinde dikkate alınması ya da adjuvan tedavi seçiminde rol oynayabilmesi için geniş kapsamlı randomize prospektif olarak dizayn edilmiş çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Rektal kanser, perinöral invazyon, sağkalım.

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Introduction

Colorectal cancers are the third most common cancers leading to death in developed countries following lung and breast cancers [1]. Approximately 800,000 new colorectal cancer patients are diagnosed worldwide each year, which is about 10% of all cancers with an increasing incidence. Among all, rectal cancers forms over one-third of colorectal cancers [2]. Rectal cancer is a complex disease, primarily due to its location with relatively high recurrence rates generally ranging from 20% to 50% [3], with higher rates in patients with a more advanced initial tumor stage. Although many factors affecting recurrence and survival rates in rectal cancer have been identified, the stage of the tumor is the most important prognostic factor [3]. The five-year survival rates for patients vary according to the stages in the TNM system [3]. However, many additional parameters that can be associated with survival and the adjuvant treatment decision have been identified besides stage.

Perineural invasion (PNI) is one of the parameters that can be associated with survival and affect the adjuvant treatment plan in colorectal cancers [4]. The invasion of tumor cells into the lymphatic or blood vessels is an important indicator in the metastatic process. Perineural invasion (PNI) is not a subcategory of lymphovascular space invasion (LVSI), and there are no lymphatic or vascular vessels in the perineural space. PNI is directly related to survival in colorectal cancers [4]. In a study by Liebig et al. [5], the five-year disease-free survival rates were reported as 16% and 65% for patients with and without PNI, respectively, while the general survival rates were 25% and 72% for the same patients.

The aim of our study is to evaluate the effect of perineural invasion (PNI) along with serum Carcinoembryonic antigen (CEA) levels and the other histopathological prognostic factors (The TNM Classification of Malignant Tumours (TNM) stage, primary tumor invasion (pT stage), regional lymph nodal metastasis (pN stage) pT stage, metastatic LN count, tumor budding condition, grade, lymphocyte infiltration and presence of desmoplasia) on disease-free survival, overall survival, and recurrence rates in patients who underwent surgery for rectal tumor.

Material and method

The clinical records of patients diagnosed with rectal cancer who underwent surgery at a tertiary referral center between 2008 and 2014 were obtained from the patient database. The study design was approved by the University Ethics Committee (07.03.2017/04). All patients had biopsy-confirmed rectal adenocarcinoma. Demographic information, forms of neoadjuvant therapy, surgical methods, clinicopathological data, and survival times were collected through direct patient interviews. Data collection was terminated at August 2017. Cases who have died or dropped out were not considered as “at risk”. Patients were labeled as “censored” if they were lost before the cencoring time. The available time-to-event was censored as the date patient’s death or dropout. Otherwise,

all outcome parameters were recruited for all "non-censored" subjects at the date of study termination.

The assessment included PNI and serum CEA levels, cTNM stage, pT stage, pN stage, metastatic LN count, tumor budding, histopathological grades, lymphocyte infiltration, and the presence of desmoplasia. Patients with distal organ metastasis, synchronous malignancy in another system, previously operated rectal cancer, or Adenomatous polyposis coli syndrome (APC) were excluded, leaving 124 patients in the study.

Tumor localization was classified as lower rectum (tumors up to 5 cm from the anal canal), middle rectum (tumors between 5 to 10 cm from the anal canal), and upper rectum (tumors farther than 10 cm from the anal canal). Standard surgery was performed based on the principles of total mesorectal excision for middle and lower rectal tumors. The TNM staging system defined by the Union for International Cancer Control (UICC), American Joint Committee on Cancer, and Union Internationale Contre Le Cancer workgroups was used for staging [6].

Decision on neoadjuvant chemotherapy, radiotherapy, or chemoradiotherapy was discussed in a multidisciplinary team. Stage 2-3 diseases were treated with first-step chemotherapy. Patient condition and acceptance of therapy were considered during the decision-making process. The disease-free survival period was defined as the time between the first operation and the date of clinical or histopathological recurrence. The overall survival period was defined as the time between the first operation and the date of last contact, either in person or by phone, or the date of death.

Patients were invited for follow-up visits every three months for the first two years after surgery, every six months for the next three years, and once a year thereafter. Patients who missed scheduled visits were contacted by phone for clinical information. In addition to symptoms, patients underwent physical examination, serum CEA level testing, chest radiography, and computerized and positron emission tomography (PET) imaging in case of doubt. Recurrence was diagnosed based on clinical findings, imaging, and histopathological results, if applicable. Recurrence was classified into three categories: local recurrence, distal recurrence, and both local and distal recurrence.

Resection specimens were analyzed for invasion depth, LN retention and count, histological type and grade, LVSI, PNI, lymphocyte infiltration, and presence of desmoplastic reaction. LVSI was considered positive when there were tumoral cells in endothelium-filled gaps or invasion of the lymphovascular wall by tumor cells. PNI was indicated by the presence of tumor cells around neural tissues that surrounded at least 1/3 of the neural tissue but did not invade the epineurium or nerve sheath. Tumor budding was defined as the presence of individual tumor cells or clusters of less than five tumor cells on the resection margin. Paraffin blocks were stained with standard hematoxylin and eosin stain and examined under a light microscope.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). Descriptive statistics were presented as mean, and standard deviation. The normal distribution was tested using the Kolmogorov-Smirnov test. The student t-test was used to compare the mean of the variables that match the normal distribution, and the Mann–Whitney U test was used for variables that do not match the normal distribution. The chi-square test was used to compare the qualitative data. Survival analyses were performed with Kaplan-Meier curves. The log-rank test was used to compare the survival curves. First, univariate analysis was conducted to examine which variables had an impact on survival. Cox regression was then used in the multivariate analysis of the variables, which were statistically significant ($p<0.05$) in the univariate analysis. The results were assessed at a confidence interval of 95 percent. $P<0.05$ was considered statistically significant.

Results

One hundred twenty-four patients were operated with the diagnosis of rectal cancer. The mean age of the patients was 62 years (26 to 93 years), 42 patients were female (33.9%) and 82 patients were male (66.1%). Neoadjuvant therapy was administered to 87 patients

(70.2%). 99 patients (79.8%) received adjuvant chemotherapy, and only seven patients (5.6%) received adjuvant radiotherapy. Recurrence was observed in 45 patients (36.3%) in total. Recurrence arise as local in three patients, as distal metastasis in 31 patients, and as both local and distal recurrence in 11 of the patients. In total 47 patients (37.9%) died for a reason attributable to the disease. The localization of the tumor was recorded as the lower rectum for 31 patients (25%), the mid-rectum for 35 patients (28.2%), and the upper rectum for 58 patients (46.8%). CEA readings were available for 123 patients, and nine of them had a high CEA. When prognostic factors of the histopathological specimens were considered, 42 patients (33.9%) had PNI while 82 patients (66.1%) did not have PNI. Other clinical and histopathological features of the patients are given in Table 1.

Table 1. Demographic and clinicopathological features of the patients

	N	%
Sex	124	
Female	42	33.9
Male	82	66.1
Age (average=62)		
<62	65	52.4
≥62	59	47.6
Neoadjuvant therapy		
Administered	87	70.2
Not administered	37	29.8
Adjuvant therapy		
Chemotherapy	99	79.8
Radiotherapy	7	5.6
Recurrence		
Present	45	36.3
Not present	79	63.7
Localization of recurrence	(N=45)	
Local recurrence	3	6.7
Distal metastasis	31	68.9
Local + distal recurrence	11	24.4
Disease-related death		
Yes	47	37.9
No	77	62.1
Tumor localization		
Lower rectum	31	25.0
Middle rectum	35	28.2
Upper rectum	58	46.8
Elevated CEA levels	(N=123)	
Present	9	7.3
Not present	114	92.7
cTNM stage		
0	11	8.9
1	0	0
2	1	0.8
3	97	78.2
4	15	12.1
pT stage		
0	38	30.6
1	8	6.5
2	21	16.9
3	46	37.1
4	11	8.9

Table 1. Demographic and clinicopathological features of the patients (continued)

	N	%
pN stage		
0	81	65.3
1	25	20.2
2	18	14.5
Histopathology		
No residual tumor	7	5.6
Adenocarcinoma	24	19.4
Well-differentiated adenocarcinoma	5	4.0
Moderately differentiated adenocarcinoma	60	48.4
Poorly differentiated adenocarcinoma	12	9.7
Signet ring cell	1	0.8
Mucinous adenocarcinoma	15	12.1
Tumor budding		
Present	39	31.5
Not present	85	68.5
LVSI		
Present	47	37.9
Not present	77	62.1
Lymphocyte infiltration		
Present	56	45.2
Not present	68	54.8
Desmoplasia		
Present	55	44.4
Not present	69	55.6
PNI		
Present	42	33.9
Not present	82	66.1

CEA: carcinoembryonic antigen, LVSI: lymphovascular space invasion, PNI: perineural invasion

Classification of the clinicopathological features of the patients by the presence of PNI is summarized in Table 2. PNI was detected in 26, and not detected in 19, out of 45 patients with recurrence. Similarly, PNI is observed significantly more often in disease-related deaths. The pN stage tumor budding, LVSI and the number of metastatic lymph nodes are significantly higher in the patients with PNI. Lymphocyte infiltration and desmoplasia however, were significantly lower in patients with PNI. Additionally, there was no significant difference between the patients with PNI and without PNI in terms of sex, age, neoadjuvant treatment, recurrence patterns, localization of the primary tumor, CEA elevation, cTNM stage, pT stage, histopathological subtypes and the average number of lymph nodes extracted (Table 2).

Three-year survival rate was 89% for patients without PNI and 55% for patients with PNI (p<0.05). Five-year survival rates were 83% and 38% respectively. The mean survival was 153.6 months (134.8-172.4±9.6) for those without PNI, and 59.2 months (42.6-75.9±8.5) for those with PNI (p<0.05). Median survival was 48 months for the patients with PNI, and was not applicable for the patients without PNI since fewer than 50 percent of them were lost. Figure 1 shows the Kaplan–Meier curves of overall survival rate of the patients based on the presence of PNI.

Considering the factors that contribute to overall survival; neoadjuvant treatment, tumor localization, CEA elevation, cTNM stage, pT stage, pN stage, tumor budding, presence of LVSI, presence of PNI, presence of lymphocyte infiltration, and presence of desmoplasia were analyzed in univariate analysis. Of those variables, pN stage, tumor budding, LVSI, PNI, presence of lymphocyte infiltration and desmoplasia were found to affect the overall survival (Table 3). The factors that affected overall survival in univariate analysis were then analyzed by multivariate analysis using cox regression. The presence of PNI (HR 3.4; 1.7-6.7; 95 percent CI), lymphocyte infiltration (HR 0.41; 0.21-0.80; 95 percent CI) and desmoplasia (HR: 0.47; 0.23-0.94; 95 percent CI) were identified as independent variables that affected overall survival (Table 4).

Table 2. Comparison of clinicopathological characteristics of patients by PNI status

	PNI present (N=42)	PNI not present (N=82)	*p*
Sex			
Female	15 (35.7)	27 (32.9)	>0.05
Male	27 (64.3)	55 (67.1)	
Age (average=62)	61	62	
<62			>0.05
≥62			
Neoadjuvant therapy			
Administered	30 (71.4)	57 (69.5)	>0.05
Not administered	12 (28.6)	25 (30.5)	
Recurrence (percentages relate to the column)			
Present	26 (61.9)	19 (23.2)	**<0.001**
Not present	16 (38.1)	63 (76.8)	
Localization of recurrence (N=45)			
Local recurrence	2 (7.7)	1 (5.3)	>0.05
Distal metastasis	15 (57.7)	16 (84.2)	
Local + distal recurrence	9 (34.6)	2.4 (10.5)	
Disease-related death			
Yes	30 (71.4)	17 (20.7)	**<0.001**
No	12 (28.6)	65 (79.3)	
Tumor localization			
Lower rectum	10 (23.8)	21 (25.6)	>0.05
Middle rectum	17 (40.5)	18 (22.0)	
Upper rectum	15 (35.7)	43 (52.4)	
Elevated CEA			
Present	4 (9.5)	5 (6.1)	>0.05
Not present	38 (90.5)	76 (92.7)	
cTNM stage			
0	0	11 (13.4)	
1	0	0	>0.05
2	0	1 (1.2)	
3	36 (85.7)	61 (74.4)	
4	6 (14.3)	9 (11)	
pT stage			
0	12 (28.6)	26 (31.7)	
1	1 (2.4)	7 (8.5)	>0.05
2	4 (9.5)	17 (20.7)	
3	18 (42.9)	28 (34.1)	
4	7 (16.7)	4 (4.9)	
pN stage			
0	15 (35.7)	66 (80.5)	**<0.001**
1	10 (23.8)	15 (18.3)	
2	17 (40.5)	1 (1.2)	
Histopathology			
No residual tumor	0	7 (8.5)	
Adenocarcinoma	5 (11.9)	19 (23.2)	
Well-differentiated adenocarcinoma	1 (2.4)	4 (4.9)	>0.05
Moderately differentiated adenocarcinoma	22 (52.4)	38 (46.3)	
Poorly differentiated adenocarcinoma	5 (11.9)	7 (8.5)	
Signet ring cell	1 (2.4)	0	
Mucinous adenocarcinoma	8 (19)	7 (8.5)	
Tumor budding			
Present	26 (61.9)	13 (15.9)	**<0.001**
Not present	16 (38.1)	69 (84.1)	

Table 2. Comparison of clinicopathological characteristics of patients by PNI status (continued)

	PNI present (N=42)	PNI not present (N=82)	*p*
LVSI			
Present	29 (69.0)	18 (22.0)	**<0.001**
Not present	13 (31.0)	64 (78.0)	
Lymphocyte infiltration			
Present	13 (31.0)	43 (47.6)	**<0.05**
Not present	29 (69.0)	39 (52.4)	
Desmoplasia			
Present	13 (31.0)	42 (51.2)	**<0.05**
Not present	29 (69.0)	40 (48.8)	
Average number of LN extracted	10.3	7.9	>0.05
Metastatic LN count	3.4	0.5	**<0.001**

CEA: carcinoembryonic antigen, LVSI: lymphovascular space invasion, PNI: perineural invasion, $p<0.05$ revealed statisticaly significant

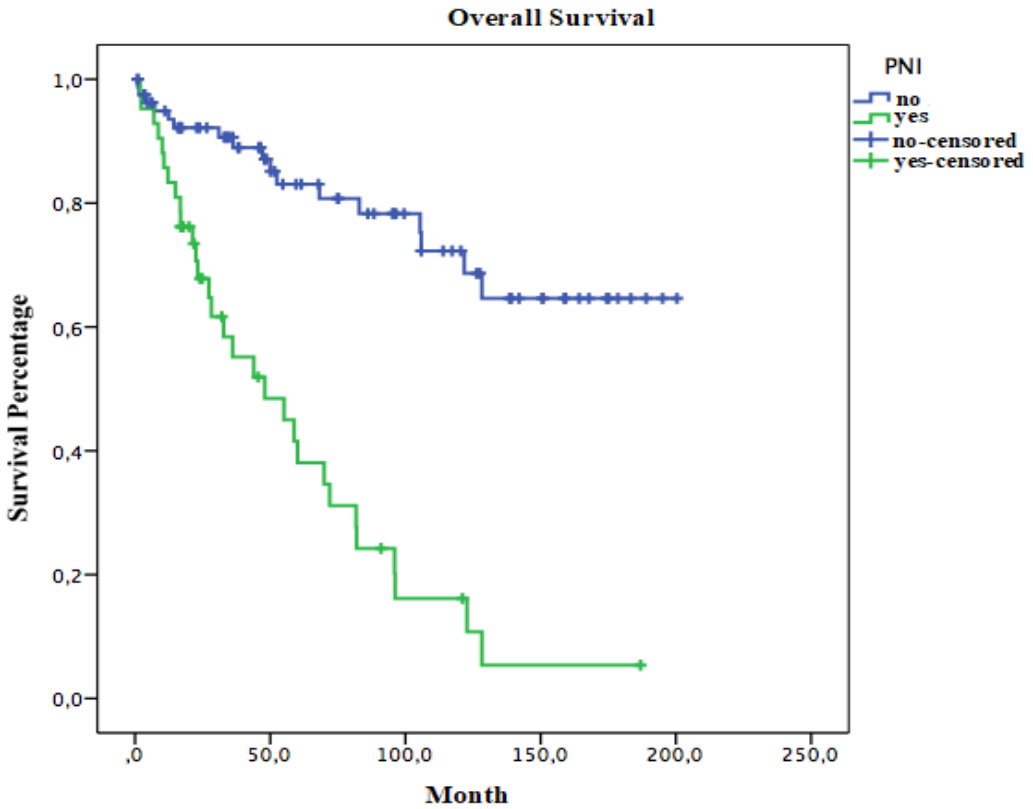

Figure 1. The Kaplan–Meier curve that shows the overall survival rates of patients by PNI status (Log rank test $p<0.05$)

Table 3. Univariate analysis of the factors that affect overall survival

Factor	*P*
Neoadjuvant therapy	>0.05
Tumor localization	>0.05
Elevated CEA	>0.05
cTNM stage	>0.05
pT stage	>0.05
pN stage	**<0.01**
Tumor budding	**<0.01**
LVSI	**<0.01**
PNI	**<0.01**
Lymphocyte infiltration	**<0.05**
Presence of desmoplasia	**<0.05**

CEA: carcinoembryonic antigen, LVSI: lymphovascular space invasion, PNI: perineural invasion
p<0.05 revealed statisticaly significant

Table 4. Multivariate analysis of the factors that affect overall survival

Factor	*P*	HR	95% CI
pN stage	>0.05		
Tumor budding	>0.05		
LVSI	>0.05		
PNI	**<0.001**	3.4	1.7-6.7
Lymphocyte infiltration	**<0.05**	0.4	0.2-0.8
Presence of desmoplasia	**<0.05**	0.5	0.2-0.9

LVSI: lymphovascular space invasion, PNI: perineural invasion, *p*<0.05 revealed statisticaly significant
HR:Hazard ratio, CI:confidence

The mean disease-free survival for the patients without PNI was 146.9 months (127.1-166.7±10) and the mean disease-free survival for the patients with PNI was 56.2 months (33.0-79.3±11.8) (*p*<0.05). Median disease-free survival was 32 months for the patients with PNI, and was not applicable to the patients without PNI since fewer than 50 percent of them had recurrence. Figure 2 shows the Kaplan–Meier curves of disease-free survival of the patients based on the presence of PNI.

While LN metastasis was not detected in 81 patients, it was present in 43 patients. Association of positive PNI with an increased risk of LN metastasis was shown above. The effect of the presence of PNI on the patients with and without LN metastasis was also investigated. Mean three-year survival rate was 87%, and mean survival was 148 months (127-170±10 months) for the patients without LN metastasis and PNI. Since less than half of the patients in this group died, the median survival value was not available. Mean three-year survival rate was 76%, and mean survival was 58 months in the presence of PNI for the patients without LN metastasis. Overall survival decreases significantly even in the absence of LN metastasis when PNI is present (*p*<0.01). Figure 3 shows the correlation between the presence of PNI and overall survival for the patients without LN metastasis.

Mean three-year survival rate was 92.3%, and average survival was 163 months (130-195±16 months) for the patients with LN metastasis without PNI. Since less than half of the patients in this group died, the median survival value was not available. Average three-year survival rate was 48%, and mean survival was 36 months in the presence of PNI for the patients with LN metastasis. Presence of PNI significantly reduces overall survival for the patients with LN metastasis as well (*p*<0.01). Figure 4 shows the correlation between the presence of PNI and overall survival for the patients with LN metastasis.

As presented in the figures above, three-year and five-year survival rates of the patients with PNI and without LN metastasis (76% and 46%, respectively) are lower than the three-year and five-year survival rates of the patients with LN metastasis and without PNI (92% and 82% respectively). In this respect, the presence of

PNI appears to be a more significant prognostic factor than LN metastasis for the survival of patients. It is seen that similar outcomes also apply to the disease-free survival periods.

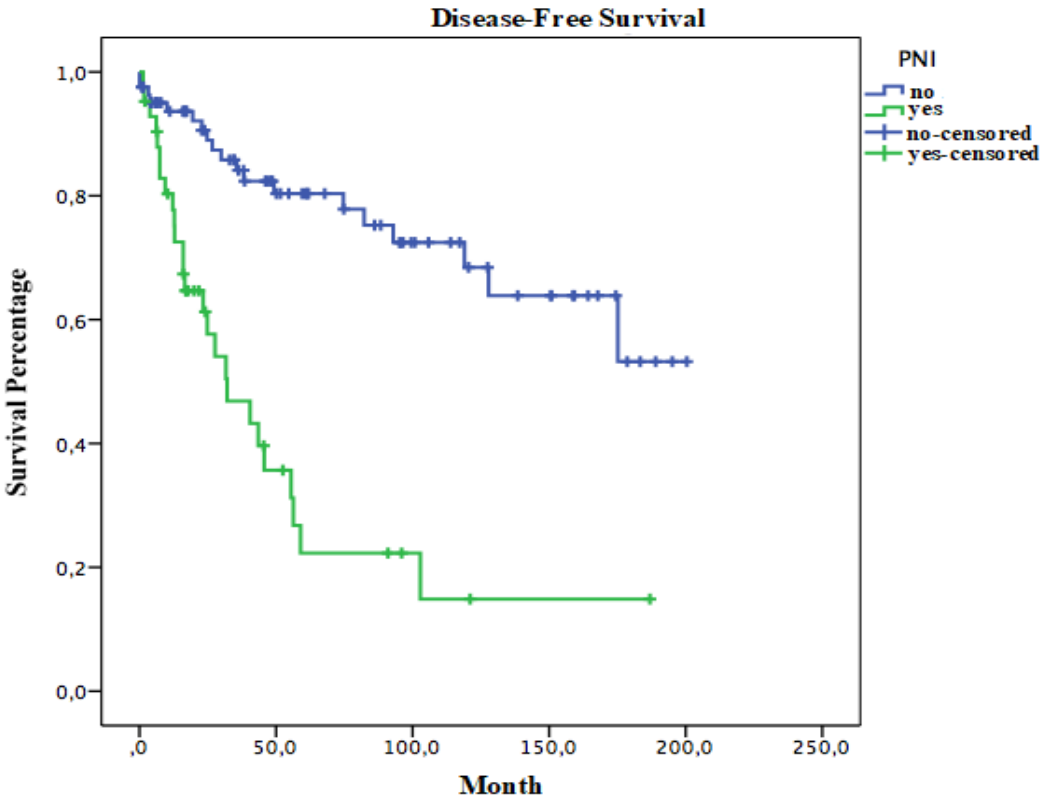

Figure 2. The Kaplan–Meier curve that shows the disease-free survival rates of patients by PNI status (Log rank test $p<0.05$)

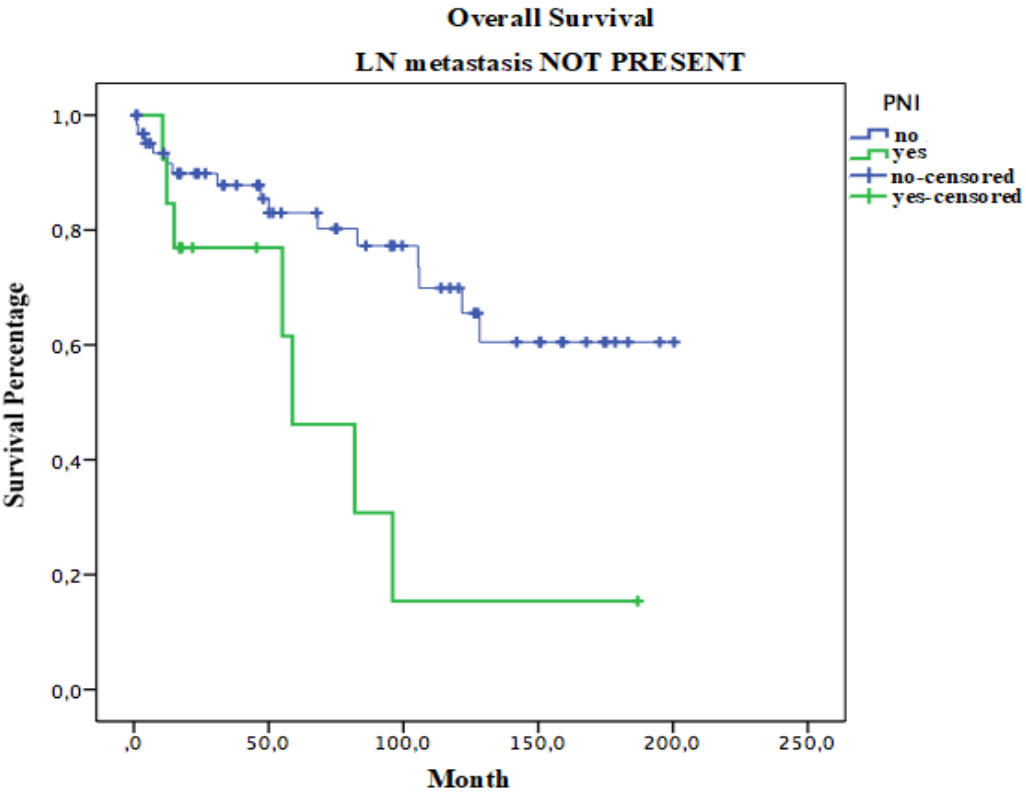

Figure 3. The Kaplan–Meier curve that shows the correlation between the presence of PNI and overall survival for the patients without LN metastasis (Log rank $p<0.01$)

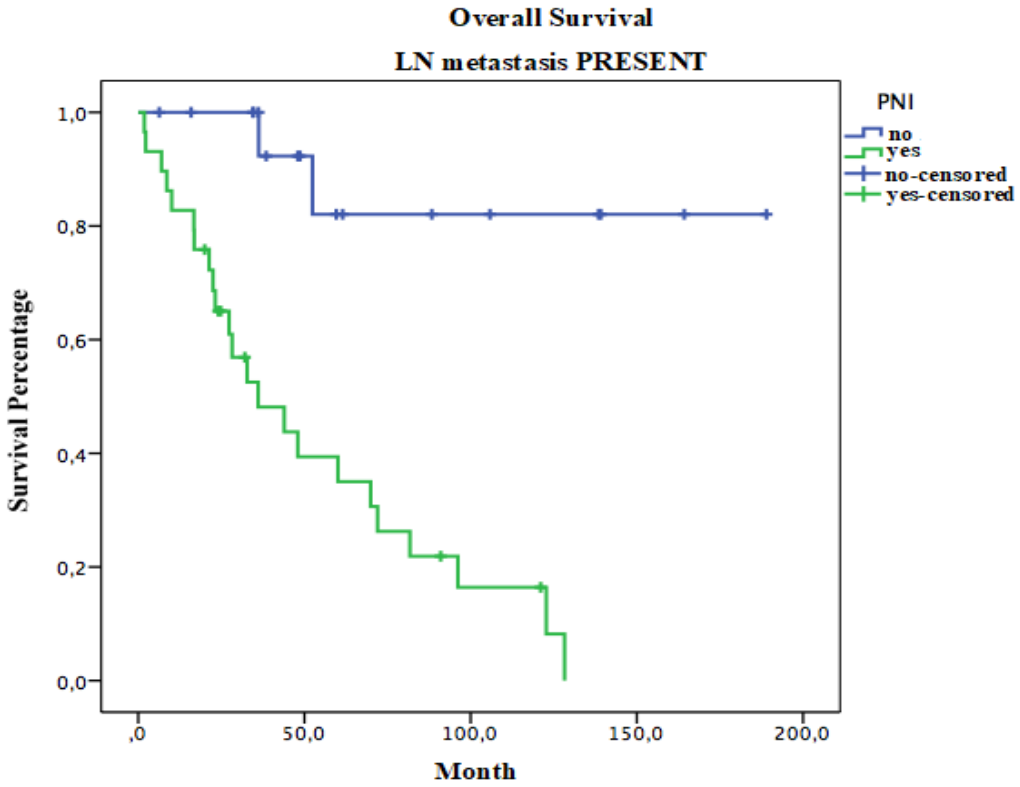

Figure 4. The Kaplan–Meier curve that shows the correlation between the presence of PNI and overall survival for the patients with LN metastasis (Log rank $p<0.01$)

Discussion

Our results documented that PNI was seen more frequently in deaths related to the disease. The average survival of patients with PNI was shorter than those without PNI. In the multivariate analysis in which other prognostic factors were also evaluated, the presence of PNI was found to be significantly associated with both overall survival and disease-free survival.

Rectal cancer is 1.5 times more prevalent in males than in females. Its incidence increases with age, and patients are diagnosed most often in the sixth and seventh decades [1, 2]. While female patients made up 33.9%, male patients accounted for 66.1% of our patient cohort, and the mean age of the patients was 62 years. Patient series in the literature revealed that the probability of a locally advanced stage tumor is higher, and vascular and neural invasion is more frequent on tumor margins in colorectal carcinoma cases diagnosed in patients aged under 60. It also revealed that young patients develop T4 and N2/3 tumors more often [7]. In contradiction to this data, the survival of young patients was significantly better than that of the older patients in our patient group.

PNI was defined for the first time as an indication of the spread of tumor to intracranial fossae in head and neck tumors [8]. There have been several recent studies on its prognostic importance in prostate cancer [9]. Indication of PNI in intrapancreatic tumors in pancreatic cancer indicates the spread of extrapancreatic tumor and invasion of extrapancreatic neural tissue [10]. Since colorectal cancers are the third most common cause of cancer-related deaths in developed countries, we aimed to conduct this study to investigate the effect of the presence of PNI on the prognosis of the disease. Rectal cancers are more likely to show PNI than colon cancers [5, 11]. A possible reason for this is that there are more rich nerve plexuses around the rectum than the colon. The higher rate of PNI associated with rectal cancers may explain why the prognosis of rectal cancers is worse than colon cancers at the same stage.

According to the AJCC cancer-staging manual (seventh edition), PNI is defined as a specific prognostic factor for colorectal cancer [12]. Nevertheless, some problems encountered in practical implementation of the definition of PNI await resolution. There is not a fully standardized criterion in the pathologic definition

of PNI in colorectal cancers in particular [13]. The level of aggrement among pathologists for the definition of PNI and reproducibility of the PNI diagnosis have not been studied yet.

According to the literature, the incidence rate of PNI in colorectal cancers is 10 to 35% [11]. In our series, it was 33.9% and it is coherent with the literature. The most significant reason for those different rates of incidence in the literature is attributable to the difference in the definition of PNI. While the presence of tumoral invasion around and within the neural tissue qualifies as PNI according to the definition made by Batsakis [14], Seefeld and Bargen require infiltration of the tumor into perineural and endoneural gaps before a diagnosis of PNI is reached [15]. According to recent studies, detection of extraneural tumoral invasion is also considered sufficient for the diagnosis of PNI [16]. Additionally, stricter definitions of PNI have also been made. Accordingly, it was suggested that diagnosis of PNI can be made only if tumoral cells are detected in the perineurium [17]. However, it was shown in the immunohistochemistry studies conducted with Glut1 that the perineurium cannot usually be seen by eosin staining, and when the tumors diagnosed with PNI using those criteria are re-examined, it was seen that tumoral invasion was actually outside the perineurium in a significant part of them [18]. Some researchers argue that PNI should be included in the staging system and reporting it in trichotomy is correlated with the prognosis. According to this view, it is emphasized that a correlation may be made with clinical prognosis when non-PNI cases are graded as Pn0, exclusively intramural PNI cases are graded as Pn1a, and extramural PNI cases are graded as Pn1b [19]. It is argued that this may also reduce the inter-observer variability in definition of PNI.

In our series, a significant difference in terms of prognosis was seen between patients with and without PNI, although there was not any significant difference between the cTNM stage and pT stage of the two groups. Although an association is expected between the presence of PNI and the size and aggressiveness of primary tumors, our series did not yield any statistical difference between the groups in this respect. The rate of LN metastasis was higher in PNI-positive cases. In addition, independently of LN mestastasis, detection of PNI in patients with and without LN metastasis was associated with poor prognosis. Moreover, both overall and disease-free survival rates of the patients who did not have PNI but had LN metastasis were better than those who had PNI but did not have LN metastasis. This indicates that presence of PNI is the most significant histopathological prognostic indicator of rectal cancer. Similarly, multivariate analyses in the literature showed that presence of PNI was a poor prognostic factor regardless of the stage of the disease [11].

Univariate analyses of previous studies established a correlation between the presence of PNI and an increase in the risk of locoregional recurrence risk, a decrease in five-year survival and an increase in the risk of metastatic disease [20-22]. Our study showed an increase in the risk of recurrence in the cases with PNI but recurrence patterns did not change based on the status of PNI.

As a result of our multivariate analysis, it was seen that the death risk of the PNI positive patients was 3.4 times higher than the PNI negative patients at the same stage. According to an 80-patient study conducted in Türkiye, in which factors that affected postoperative survival after curative surgery on rectal cancer were investigated, median survival was 26.12 months for the patients with PNI and 46.76 months for the patients without PNI [23].

As mentioned in the NCCN guidelines, presence of PNI should be determinant for the choice of adjuvant chemotherapy for stage 2 colorectal cancers in particular. An interesting outcome that we yielded in our study is that three-year survival rates of the patients without lymph node metastasis but with PNI was significantly lower than those who had lymph node metastasis but did not have PNI. This is an indication of a higher probability of recurrence and disease-related death for the patients with PNI who are at an early stage at the time of the operation. Therefore, one may think that patients who are in early stage but has PNI invasion may benefit from postoperative adjuvant treatment. However, no study has yet been conducted to clarify this. In this respect, Huh et al. [13] showed in their study that the presence of PNI in patients with early-stage colorectal carcinoma without lymph node metastasis indicated a poor

prognosis, which is in line with our findings.

A study conducted by the Japanese Society for Cancer of the Colon and Rectum (JSCCR) in recent years found a PNI rate of 17.7%, and established a correlation between PNI presence and the stages of T and N as well as tumor differentiation and LVSI [24]. It was shown in the multivariate analysis conducted under this polycentric study, where 2.485 patients were examined, that the presence of PNI is a poor prognostic factor for disease-free survival and overall survival. However, unlike in our study, immunohistochemical stains such as s100, synaptophysin and CD56 were used in the beginning to stain tumoral tissues in the aforementioned study.

In a study where patients with T1 and T2 colorectal cancer were examined, the presence of PNI in the early-stage disease was found to correlate with the lymph node metastasis [25]. Although the correlation of the presence of LVSI with lymph node metastasis in early-stage colorectal cancers is known, there are fewer studies in the literature on the importance of PNI in early-stage colorectal cancers.

In conclusion, according to the findings of our study, the presence of PNI is a prognostic factor independent of other poor prognostic factors, such as LVSI, LN metastasis or advanced stage of the disease, for both overall survival and disease-free survival.

Conflicts of interest: No conflict of interest was declared by the authors.

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Contributions of the authors to the article

U.S. and U.O. constructed and developed the theory, verified the analytical methods. U.O. and N.D. collected the patients' data. N.D. investigated the findings. U.O. performed the calculations, data analysis and created figures. U.S., N.D. and U.O. interpreted and discussed the results. U.O. provided critical feedback. U.O. wrote the manuscript with input from all authors. All authors discussed the results, reviewed and commented on the manuscript.

Research Article

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Relationship between cerebrovascular diseases and human leukocyte antigen subgroups

Serebrovasküler hastalıklar ile insan lökosit antijen alt grupları arasındaki ilişki

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Abstract

Purpose: Ischemic stroke is classified as large artery atherosclerosis, small vessel occlusion (lacunar infarcts), ischemic stroke due to other identified causes, and cryptogenic. The human leukocyte antigen (HLA) complex is a group of genes on chromosome six in humans responsible for encoding cell-surface proteins that regulate the immune system. In this study, HLA's roles in the pathophysiology of ischemic stroke, especially the lacunar subgroup, are investigated, and their potential mechanisms are presented.

Materials and methods: This study consisted of 49 patients with ischemic stroke and 50 healthy participants. HLA-A, HLA-B, HLA-C, HLA-DQB, and HLA-DRB subgroup genomes were assessed.

Results: A statistically significant difference in the presence of *HLA-A, HLA-B, HLA-C, HLA-DQB,* and *HLA-DRB* subgroups was found between the control and patient groups. The presence of HLA-A*02, HLA-A*30, *HLA-B*08, HLA-B*15,* and *HLA-DQB*06* genomes was higher in the patient group than in the control group ($p \leq 0.05$). Nevertheless, *HLA-DQB*03* and *HLA-DRB*11* genomes were found more in the control group than the patient group ($p \leq 0.05$)

Conclusion: The results of this study pioneered in scrutinizing HLA alleles in small vascular disease (SVD). *HLA-A*01, HLA-A*30, HLA-B*08, HLA-B*15, HLA-DQB*06, HLA-DQB*03* and *HLA-DRB*11* are associated with HLA alleles of stroke patients with small vessel occlusion. We attempted to provide objective evidence for whether HLA genomes could act as a discriminative factor between SVD patients and control groups, which might hold considerable promise for future therapies.

Key words: Stroke, major histocompatibility complex, genome, small vessel disease, ischemia.

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Öz

Amaç: İskemik inme, büyük arter aterosklerozu, küçük damar tıkanıklığı (laküner enfarktlar), diğer tanımlanmış nedenlere bağlı iskemik inme ve kriptojenik olarak sınıflandırılır. İnsan lökosit antijeni (HLA) kompleksi insanlarda bağışıklık sistemini düzenleyen hücre yüzeyi proteinlerini kodlamaktan sorumlu, altıncı kromozom üzerinde yer alan bir grup gendir. Bu araştırmada, HLA'nın iskemik inme patofizyolojisindeki özellikle laküner alt gruptaki rollerinin araştırılması ve olası mekanizmaların aydınlatması amaçlanmıştır.

Gereç ve yöntem: Bu çalışma iskemik inmeli 49 hasta ve 50 sağlıklı katılımcıdan oluşmaktadır. *HLA-A, HLA-B, HLA-C, HLA-DQB* ve *HLA-DRB* alt grup genomları değerlendirilmiştir.

Bulgular: Kontrol ve hasta gruplarında *HLA-A, HLA-B, HLA-C, HLA-DQB* ve *HLA-DRB* alt gruplarının varlığı arasında istatistiksel olarak anlamlı fark bulunmuştur. *HLA-A*02, HLA-A*30, HLA-B*08, HLA-B*15 ve HLA-DQB*06* genomları hasta grubunda daha fazla bulunmuştur ve istatistiksel olarak anlamlı fark vardır ($p \leq 0.05$). Bununla birlikte *HLA-DQB*03* ve *HLA-DRB*11* genomları kontrol grubunda daha fazla bulunmuştur ($p \leq 0.05$).

Sonuç: Bu araştırma, küçük damar hastalığında (KDH) HLA alellerini incelemede öncüdür. *HLA-A*01, HLA-A*30, HLA-B*08, HLA-B*15, HLA-DQB*06, HLA-DQB*03, HLA-DRB*11,* inme hastalarının HLA alelleri ile ilişkilidir. Küçük damar tıkanıklığı HLA genomlarının, KDH hastaları ve kontrol grupları arasında, gelecekteki tedaviler için umut vaat edebilecek, ayırt edici bir faktör olarak hareket edip edemeyeceğine dair nesnel kanıtlar sağlamaya çalıştık.

Anahtar kelimeler: Strok, major doku uygunluk kompleksi, genom, küçük damar hastalığı, iskemi.

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Introduction

Stroke is a significant health problem and the second most common worldwide mortality cause. It accounts for more than 50% of neurological diseases requiring hospital treatment. According to the 1993 TOAST (Trial of Org 10172 in Acute Stroke Treatment Study), ischemic stroke causes are classified as large artery atherosclerosis (LAA), SVD (lacunar strokes), ischemic stroke due to other determined causes, and cryptogenic ones. The most prevalent subtype is ischemic stroke, and the second is hemorrhagic [1]. A hospital-based, multicenter study investigating the general characteristics and risk factors of stroke patients in Türkiye found that ischemic stroke was 72% and hemorrhagic stroke was 28% [2].

Our study found a statistically significant difference between the control and patient groups in the presence of *HLA-A, HLA-B, HLA-C, HLA-DQB,* and *HLA-DRB* subgroups. *HLA-A*02, HLA-A*30, HLA-B*08, HLA-B*15*, and *HLA-DQB*06* genomes were found more in the patient group, and there was statistically significant difference. ($p \leq 0.05$) Nevertheless, *HLA-DQB*03* and *HLA-DRB*11* genomes were found more in the control group ($p \leq 0.05$). As examined in our study, the relationship between SVD and *HLA* alleles has never been investigated. Therefore, this research is a pioneer in its field.

Although there are some studies about stroke and its relationship with various parameters in the literature, a few studies have evaluated the relationship between ischemic stroke and *HLA* subgroups. To the best of our knowledge, no studies have determined the association between SVD and *HLA* subgroups.

Our study aims to determine any potential evidence for whether *HLA* alleles might act as a discriminative factor between SVD patients and healthy participants, which might pave the way to future therapeutic alternatives.

Materials and methods

This study has a randomized, controlled, cross-sectional clinical research design.

The Pamukkale University's Non-Interventional Clinical Research Ethics Committee approved this study's ethical suitability. All authors signed that they had complied with the Declaration of Helsinki.

Patients were evaluated in the Department of Neurology outpatient clinic at the Pamukkale University Hospital. Forty-nine patients aged 18-75 diagnosed with SVD according to the TOAST classification were selected. Patients had small vascular lesions detected on computerized cranial tomography (CT) or magnetic resonance imaging (MRI), large artery stenosis of less than 50% found by vascular imaging (carotid-vertebral doppler ultrasonography, MRI angiography, or CT angiography). On echocardiography, there was no thrombus, and 24-hour-holter electrocardiograph scans showed no paroxysmal atrial fibrillations. Fifty control group members were healthy people who did not have a stroke or any vascular disease risk factors. Demographic data were recorded from all participants. Risk factors and cranial imaging findings were also noted in the patient group.

Informed consent was obtained from all the participants. After obtaining informed consent, blood samples (5 mL) were drawn from the median cubital vein and transferred into purple-capped hemogram tubes containing ethylenediaminetetraacetic acid (EDTA). Deoxyribonucleic acid (DNA) isolation was performed with EZ 1 kit (Qiagen® Corp., California, USA) from peripheral whole blood samples taken into EDTA tubes. Sequence-specific oligonucleotide method was used. Extracted DNA was typed *HLA-A, -B, -C, DRB*, and *DQB* alleles using Mia For a Flex software with next-generation sequencing (NGS) reagents provided by Immucor® (Mia Fora NGS *HLA* Typing Kit, NJ, USA).

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS)® version 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0 Armonk, NY, USA). Continuous variables were stated as mean ± standard deviation (SD) and median (minimum and maximum values), and categorical variables were expressed as numbers and percentages. The suitability of data for normal distribution was examined with the Shapiro-Wilk and Kolmogorov-Smirnov tests. Differences between categorical variables were

analyzed with Pearson's Precise chi-square test. In all analyses, $p<0.05$ was considered statistically significant.

Results

The patient group consisted of 49 people. In patients with SVD, 21 (42.9%) women and 28 (57.1%) men are present. There was no statistically significant difference between the patient and control groups regarding mean age and gender distribution. The patient group's mean age was 57.67±11.14, and the median was 60. In both groups, there were 35 participants with a history of cerebrovascular disease (CVD), 30 people with hypertension (HT), 25 people with hyperlipidemia (HPL), and 38 participants with a sedentary lifestyle.

The *HLA-A*02* genome was found in 22 different alleles in the patient group. In comparison, there were 11 different alleles in the control group, and the difference was statistically significant ($p=0.024$). The *HLA-A*30* genome was found in seven different alleles in the patient group and one in the control group, and the difference was statistically significant ($p=0.030$). The *HLA-B*08* genome was in nine different alleles in the patient group, while there were two in the control group, and a statistically significant difference was found ($p=0.027$).

The *HLA-B*15* genome was found in 10 different alleles in the patient group and two in the control group, with a statistically significant difference ($p=0.015$). A statistically insignificant difference was found in the *HLA-C* genome between the two groups.

The *HLA-DQB*03* genome was found in 30 different alleles in the patient group while in 49 different alleles in the control group, and the difference was statistically significant ($p=0.006$).

The *HLA-DQB*06* genome was found in 21 different alleles in the patient group and 11 in the control group. The difference was statistically significant ($p=0.035$). There were 13 different alleles of the *HLA-DRB*11* genomes in the patient group, while there were 26 different alleles in the healthy control group. The difference was statistically significant ($p=0.019$).

While the *HLA-B*08* genome was found in nine different alleles in the patient group, there were two in the control group, and the difference was statistically significant ($p=0.027$). In the patient group, there were ten different alleles of the *HLA-B*15* genome. In the control group, there were two different alleles. The difference was statistically significant ($p=0.015$). The *HLA-DRB*11* genomes were found in 13 different alleles in the patient group, while it was in 26 different alleles in the control group. There was a statistically significant difference ($p=0.019$).

The *HLA* genomes, which had a statistically significant difference between the groups, are demonstrated in Table 1.

Table 1. Comparison of *HLA genome* in patient and control groups

HLA Genome [+]	**Available (%) / Non-available (%)**		**All Groups**	***p*-Value** [++]
	Patient Group	**Control Group**		
HLA-A*02	22 (23.5)/75 (76.5)	11 (13.0)/87 (87.0)	33	0.024
HLA-A*30	7 (7.1)/91 (92.9)	1 (1.0)/99 (99.0)	8	0.030
HLA-B*08	9 (9.2)/89 (90.8)	2 (2.0)/98 (98.0)	11	0.027
HLA-B*15	10 (10.2)/88 (89.8)	2 (2.0)/98 (98.0)	12	0.015
HLA-DQB*03	30 (30.6)/68 (69.4)	49 (49.0)/51 (51.0)	79	0.006
HLA-DQA*06	21 (21.4)/77 (78.6)	11 (11.0)/89 (89.0)	32	0.035

[+]*HLA: Human leukocyte antigen*; [++]Pearson's chi-square test in which, $p<0.050$ was considered statistically significant

Discussion

Cerebrovascular diseases, or strokes, are neuronal dysfunctions caused by the rupture or obstruction of one of the arteries feeding the contralateral hemisphere of the brain (atherosclerosis, thrombosis, etc.) [3]. Pathological changes in blood vessels to the brain, trauma or some cerebrovascular diseases may cause this neurological picture. Although it can occur at any age, it is rare to see before age 40 [4]. Inflammatory mechanisms are essential for forming atherosclerosis in the pathogenesis of CVD [5]. CVD is one of the most critical health

problems that still cause health and workforce loss worldwide [6]. 80% of cerebrovascular diseases are caused by ischemic and 20% by hemorrhagic causes [7]. According to the 1993 TOAST, ischemic stroke causes are classified as large artery atherosclerosis (LAA), cardiac causes, SVD (lacunar), ischemic stroke due to other determined causes, and cryptogenic ones.

HT is the primary risk factor for stroke and is thought to accelerate the atherosclerotic process and the risk of stroke increases with increasing systolic and diastolic blood pressure values [8]. The most common known risk factors for ischemic stroke include HT, diabetes, and high cholesterol [9-11]. High blood pressure, high cholesterol, carotid stenosis, and atrial fibrillation are conclusively shown in randomized clinical trials to be causally related to ischemic stroke, and their treatment reduces the incidence of stroke [11, 12].

This research evaluated the relationship between *HLA* gene polymorphism and patients with SVD. According to the literature, the age of the population in this study was lower [9]. Since the *HLA* gene assets were examined, we assumed that the genetic presence would not pose a problem for the study when it was considered not age-related. The patient group included 28 (57.1%) men and 21 (42.9%) women, and in terms of gender, the female-male ratio was compatible with the literature [9, 13].

The major histocompatibility complex (MHC) locus, known as the human leukocyte antigen locus, is on the short arm of chromosome 6. Molecules encoded by this region participate in antigen presentation, inflammation regulation, the complement system, and immune responses. Therefore, *MHC* is essential in immune-mediated, autoimmune, and infectious diseases [14]. *MHC* plays a role in some neurological disorders [15-19]. The *MHC* genes are subdivided into five regions from the telomeric to the centromeric end: the extended class I, class I, class III, class II, and the extended class II regions. The class I region consists of the three classic *HLA* gene loci: *HLA-A, HLA-B,* and *HLA-C*; three non-classic *HLA-E, HLA-F*, and *HLA-G* gene loci [14]. The class II region comprises the classic gene loci *HLA-DP, HLA-DQ, HLA-DR*, and the non-classic *HLA-DO* and *HLA-DM* loci. The class III region includes genes with roles in inflammation via complement cascades and cytokine production, like tumour necrosis factor (TNF) [14]. So, *MHC I* and *II* molecules supervise T-cells and are crucial for instructing the cellular adaptive immune responses.

Many studies have investigated the *HLA* class I region in CVD. The majority have reported insignificant results for *HLA-A* alleles. For example, some researchers examining Behcet's disease that might cause SVD have detected no significant *HLA-A* allele [20, 21]. Our study showed no specific allele in the *HLA-A*01, HLA-A*26*, or *HLA-A*33* genomes.

Kang et al. [22] revealed that the *HLA-A* alleles *A*02:07, A*26:01*, and *A*30:04* might be CVD susceptibility alleles *HLA-A*33:03* can be a protective agent in the Korean population. They also found that *A*02:07* was associated with skin lesions and arthritis, *A*26:01* with uveitis, and *A*30:04* with vascular lesions, genital ulcers, and positive paternity testing independent of *HLA-B*51. HLA-A*02:07* and *A*26:01* were confirmed to be CVD susceptibility alleles. However, *HLA-A*33:03* was associated with a reduced risk of CVD. In our research, the *HLA-A*02* allele was related to a higher risk of SVD (p=0.024), similar to the literature.

This study found no statistically significant difference between the healthy group and those with small vessel disease in the presence of *HLA-A*01, HLA-A*26 and HLA-A*33* alleles. Hence, this could be due to the small number of participants or might be explained by the absence of a causal relationship, especially in our population. There was only one patient with *HLA-A*33:03* alleles in both groups. Although there was one patient with *HLA-A*33:03* alleles, there were four more patients with *HLA-A*33:01* alleles.

The *HLA-A*30* allele was higher in the patient group, indicating that this allele might be associated with SVD. Comparing our results with other studies might reveal a striking genetic difference. On the other hand, patients with *HLA-A*02:07* alleles should be examined further in terms of arthritis [22].

The prevalence of *HLA-B*15* in the Moroccan population was similar to that observed in other

people in North Africa or Southern Europe. In these populations, the *B*15* genome was twice as abundant in controls as in controls in the same order as in *B*51*. Approximately 56% of patients were shown to express *B*51* or *B*15*, compared to 27% of healthy controls. It was stated that *HLA-B*15* was expressed more frequently in female patients [1, 20]. Our research demonstrated that the *HLA-B*15* genome was found in 10 different alleles in the patient group, and there were two different alleles in the healthy group (p=0.015). This result was undoubtedly compatible with the literature.

In a study on the association of *HLA-B*08* alleles with cerebrovascular diseases, the results of the Caucasian population were compared, and significance was found in small and local vascular disease with stroke aetiology with the control group [23]. In our research, there was a similar result (p=0.027). In another study in the Iranian population, no significant relationship was found between *HLA-B*08 and HLA-B*15* genomes [24]. In conclusion, *HLA-B*08* and *HLA-B*15* in homozygous *HLA-B* patient carriers as additional alleles detected in our study appeared to represent an increased risk for SVD.

In this research, the presence of *HLA-DQB*03, HLA-DQB*04*, and *DQB*06* in control and patient groups was found statistically significant (p<0.05). Interestingly, the *DRB* and *DQB* alleles reveal a stronger association between their observed profile in ischemic stroke and disease expression than the disease itself or individual clinical symptoms. *HLA-DQB*04:02* alleles did not show a statistically significant difference in the relationship between patients with SVD and the control group. However, the frequency of the *DQB* allele was more intense in the patient group. In the *HLA-DQB*06* areas, the patient group was in the majority, with 21 patients, which was statistically significant (p=0.035). The significant difference in these *HLA-DQB*06* alleles in our patient group was associated with SVD.

A significant *HLA-DQB* gene polymorphism, which might be protective, was in *DQB*03* alleles. This gene, found in 49% of the control group, was in around 30.6% of the patient group and could have protective effects based on these results.

A recent high-resolution genotyping study from China revealed higher haplotypes of allele *HLA-DRB*11* and haplotype *HLA-DRB*11:06* and *DQB*03:01* in patients with ischemic stroke [25]. In our research, the *HLA-DRB*11* genomes, even if not of this haplotype, revealed a different result from the previously mentioned study. In this research, *HLA-DRB*11* genomes were found in 13 different alleles in the patient group while in 26 different alleles in the control group (p=0.019), which suggested that *HLA-DRB*11* could have protective effects on SVD.

The significant limitation of this study was that our patient group consisted of only SVD patients, which means that the other types of stroke could not be evaluated. Only *HLA-A, HLA-B, HLA-C, HLA-DQB*, and *HLA-DRB* subgroup genomes were assessed. In the future, all *HLA* subgroup genomes should be addressed in more extensive and clinically different stroke patients.

In conclusion, this research endeavoured to find out whether there was a causal relationship between *HLA-A, HLA-B, HLA-C, HLA-DQB*, and *HLA-DRB* subgroup genomes with small vascular disease. We assumed that *HLA-A*02, HLA-A*30, HLA-B*08, HLA-B*15, HLA-DQB*03, HLA-DQB*06*, and *HLA-DRB*11* were associated with *HLA* alleles of SVD patients. These findings could be promising for developing new therapeutic alternatives. In addition, *HLA* genomes might even be used as a biomarker.

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Authors' contributions to the article

E.T. and U.Ç. constructed the main idea and hypothesis of the study.

E.T., U.Ç., and E.M. developed the theory and arranged the material and method section.

E.T. and U.Ç. evaluated the data in the Results section.

The discussion section of the article was written by E.T. and U.Ç.

E.T., U.Ç., and E.M. reviewed, corrected and approved.

In addition, all authors discussed the entire study and approved the final version.

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The relationship between problematic internet use of adolescents and their level of satisfaction with family life

Ergenlerde problemli internet kullanımı ve aile yaşam doyumu düzeyleri arasındaki ilişki

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Abstract

Purpose: This study aimed to determine the relationship between the level of problematic internet use of adolescents and their level of satisfaction with family life.

Material and method: This cross-sectional descriptive study included 508 volunteer students who were attending high schools in Yozgat city centre in the 2021-2022 academic year. The research data were collected between 27.12.2021-22.04.2022, using a Personal Information Form, the Problematic Internet Use Scale, and the Family Life Satisfaction Scale. Questionnaires created through Google Forms were sent to students' smartphones. In the data analysis, the t-test, One-Way Analysis of Variance (ANOVA), correlation analysis and linear regression analysis were used.

Results: The results of the analysis revealed a significant negative relationship between the problematic internet use of adolescents and their level of satisfaction with family life (r:-0.262, p<0.001). An increase in family life satisfaction was determined to reduce the level of problematic internet use (β1:-0.209, p<0.001). Family income and academic achievement were found to be predictors of problematic internet use (p<0.05).

Conclusion: From the results of the study, it was concluded that problematic internet use behavior increased in adolescents with low family life satisfaction. In this context, it is recommended to conduct preventive, protective, and educational studies that emphasize the importance of the family in the development of adolescents as healthy internet users, and to include the use of different methods in the follow-up studies, including other possible predictors.

Key words: Problematic behavior, internet use, family, life, satisfaction.

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Öz

Amaç: Bu araştırmada ergenlerin problemli internet kullanım düzeyleri ile aile yaşam doyumu düzeyleri arasındaki ilişkinin belirlenmesi amaçlanmıştır.

Gereç ve yöntem: Araştırma, 2021-2022 Eğitim-Öğretim yılında Yozgat il merkezinde bulunan liselerde öğrenim gören öğrencilerden gönüllü olan 508 öğrencinin katıldığı kesitsel desende tanımlayıcı bir araştırmadır. Araştırma verileri, 27.12.2021-22.04.2022 tarihleri arasında toplanmıştır. Veriler, Kişisel Bilgi Formu, Problemli İnternet Kullanımı Ölçeği, Aile Yaşam Doyumu Ölçeği aracılığıyla toplanmıştır. Veriler, öğrencilerin akıllı telefonlarına gönderilen Google Form anketleri aracılığıyla elde edilmiştir. Verilerin analizinde t testi, Tek Yönlü Varyans Analizi (ANOVA), korelasyon analizi ve lineer regresyon analizi tekniğinden yararlanılmıştır.

Bulgular: Yapılan analiz sonucunda ergenlerin problemli internet kullanımı ile aile yaşam doyumu düzeyleri arasında negatif yönde anlamlı bir ilişki olduğu (r:-0,262, p<0,001) ve aile yaşam doyumunun artmasının problemli internet kullanım düzeyini azalttığı saptanmıştır (β1:-0,209, p<0,001). Ayrıca aile geliri ve okul başarısı düzeylerinin problemli internet kullanımının birer yordayıcısı olduğu saptanmıştır (p<0,05).

Sonuç: Çalışmanın sonucunda aile yaşam doyumu düşük olan bireylerde problemli internet kullanımı davranışının arttığı sonucuna ulaşılmıştır. Bu kapsamda, ergenlerin sağlıklı kullanıcılar olarak gelişmelerinde ailenin öneminin vurgulandığı önleyici, koruyucu ve eğitici çalışmaların yapılması ve takip edecek çalışmalarda, başka olası yordayıcıları da içerecek şekilde ve farklı yöntemlerin kullanımına yer verilmesi önerilmektedir.

Anahtar kelimeler: Problemli davranış, internet kullanımı, aile, yaşam, doyum.

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Introduction

The internet is one of the most important and effective tools that science has added to the modern world. Although the increasingly frequent use of the internet can have positive results, it has also raised issues of addiction/ problematic use [1]. Researchers have used different terms such as internet addiction [1], internet addiction disorder [2], pathological internet use [3] and problematic internet use [4, 5] to define the negative effects of the internet, and the concept of problematic internet use has recently become more important. Problematic internet use, refers to a situation where the duration of internet use is more than usual, customary, or planned [5]. This may lead to a decrease in the sleep quality of the individual, a decrease in productivity in daily life, and a decrease in the time spent in a social environment such as with family and friends [6]. Adolescents are prone to problematic internet use behavior due to the characteristics of their developmental period [7]. Previous studies have revealed that adolescents particularly prefer being online on the Internet to many other life events in which they have previously participated and that have facilitated their socialization [8-10].

The fact that there are various disagreements about the diagnostic criteria of problematic internet use explains the scarcity of comprehensive epidemiological studies on this topic. The results of several large-scale studies on this subject have indicated that the rates of problematic internet use among European adolescents range from 7% to 18.5% [11, 12] while these rates are between 18% and 26.5% in Asian adolescents [13, 14]. The results of studies conducted in Türkiye [16, 17] have revealed that this rate varies between 16% and 21%. Furthermore, according to the results of the "Research on the Children's Use of Information Technologies" conducted in Türkiye in 2021, the rate of the children aged between 6-15 years using the Internet was 50.8% in 2013, and this rate increased to 82.7% in 2021. When the rate of using the Internet is examined on the basis of gender, it was determined that the rate for boys using the Internet was 53.7% in 2013, which increased to 83.9% in 2021, and for girls, the rate was 47.8% in 2013, which then increased to 81.5% in 2021 [17]. Therefore, it would not be wrong to assume that use of the internet, the main purpose of which is to provide faster, safe and cheaper access to information, is moving away from this purpose particularly with regard to young people and uncontrolled use causes addiction.

The rapid spread of problematic internet use and the fact that it causes serious negativities in the lives of adolescents [18, 19] have maintained interest on the subject and led academicians to analyze various variables that may be relevant to the subject. One of these variables is the level of family life satisfaction. Family life satisfaction includes the perception of certain characteristics of family members and family life together with thoughts about the interaction of family members with each other. Each family member may experience different levels of family life satisfaction. Therefore, this concept should be considered an individual characteristic rather than a broader concept representing the family [20]. Family life satisfaction, which is an important sub-component of life satisfaction, is related to one's mental health. In this study it was aimed to raise awareness of the potential risks of problematic internet use and family relationships together with the measures to be taken in this regard. This study can be considered of value as to the best of our knowledge there is no previous study in which the level of family life satisfaction and problematic internet use of adolescents have been discussed together, and it is therefore thought that the results of the study will be of guidance for future studies.

The aim of this study was to evaluate the relationship between levels of problematic internet use and family life satisfaction of high school students in a city in Türkiye.

Materials and methods

Type of research: The research was conducted in a cross-sectional descriptive design.

Participants: The study population comprised volunteer students attending high schools in Yozgat city centre in the 2021-2022 academic year. The sample consisted of students from a regular high school, a science high school, a vocational and technical high school, and an Anatolian Imam Hatip (religious) high school. The convenience sampling method as one of the non-probability sampling methods was used in the study. Data collection forms were sent to 1733 students, 508 of whom consented

to participate in the study (29.31%). The power of the sample was evaluated using G *Power 3.1 statistical software at the end of the post hoc analysis performed on the results of the study. Considering the results derived in the correlation analysis (r:0.262) between the mean scores of the Problematic Internet Use Scale-Adolescent and The Family Life Satisfaction Scale, the power of the study with an effect size determined as 0.50 was calculated as 100%.

Inclusion Criteria:

-Being able to read and write in Turkish

- Owning a smart phone and using the internet

Data collection: The research data were collected between 27.12.2021-22.04.2022, using a Demographic Questionnaire, the Problematic Internet Use Scale-Adolescent (PIUS-A) and the Family Life Satisfaction Scale (FLSS). Questionnaires prepared using Google Forms were delivered to students' smartphones. The "Informed Consent" form was sent to the participants before the study was conducted, then those who agreed to participate in the research completed and submitted the other data collection forms.

Demographic questionnaire: This 13-item questionnaire was developed by the researchers based on previous literature [1-7, 21-23] to determine the demographic characteristics of the participants, including school year, gender, age, number of siblings, perceived family income, perceived parental attitudes, parents' educational background, parents' working status, parental living together and perceived academic achievement.

Problematic Internet Use Scale-Adolescent (PIUS-A): The validity and reliability of the PIUS-A for a sample of high school students were confirmed by Ceyhan and Ceyhan [7]. The scale consists of 27 items in 3 sub-dimensions of "negative consequences of using the internet", "social benefits/social comfort" and "overuse". The scale addresses 9th- 12th grade students. Each item is scored using a 5-point Likert scale, with items 7 and 10 reverse coded. The points of the items provide a total score in the range of 27 and 135 points, with higher scores indicating a higher level of problematic internet use. This therefore, indicates that adolescents are more likely to exhibit problematic internet use behavior and that they may show a tendency towards internet addiction. The Cronbach-alpha internal consistency coefficient of the original scale was found to be 0.93 [7]. In this study, the Cronbach-alpha internal consistency coefficient was 0.93.

Family Life Satisfaction Scale (FLSS): The FLSS, developed in 2000 by Barraca et al. [20], was adapted into Turkish by Tasdelen Karckay [21]. The scale consists of 27 items in two sections of bipolar and unipolar. The respondent is instructed to complete the sentence "When I am at home, with my family, I mostly feel..." by selecting one of six choices from a range of bipolar adjectives (e.g., end points on one range are happy and unhappy). The total score is in the range of 27 to 135, with higher scores indicating a higher level of satisfaction with family life [21]. The Cronbach-alpha internal consistency coefficient was found to be 0.83 in this study.

Data analysis: Data obtained in the study were analysed statistically using SPSS vn. 23.0 software. Frequency distribution for categorical variables and descriptive statistics for numerical variables were used to analyze the research data. Differences between two groups were determined using the Independent Samples t-test and differences between more than two groups with One-way Analysis of Variance (ANOVA). In line with the results of ANOVA, the Levene test was performed to determine the homogeneity of variance, then the group or groups causing the difference was determined with the "multiple comparison test" (Bonferroni or Tamhane's T2). The difference between groups of homogeneously distributed variables was analyzed with Bonferroni and the difference between group of variables that did not provide homogeneity of variance was analyzed with the Tamhane T2 test. Pearson correlation analysis was used to examine the relationship between numerical measurements, then linear regression analysis was applied to examine the effects on the measurements. The Cronbach alpha value was used to evaluate the reliability of the scale. A value of $p<0.05$ was accepted as statistically significant.

Results

The descriptive characteristics of the adolescents are shown in Table 1. The mean age of the participants was 16.04±1.06 years, 51.2% were female, 92.7% of the participants' parents were living together, 96.7% of the participants' parents were alive, 25.0% of the participants' mothers were working, 88.8% of the participants' fathers were working, 58.1% thought that their income was equal to their expenses, 29.3% thought that their mother was highly tolerant, 24.0% that their father was highly tolerant, and 44.7% expressed their academic achievement as moderate.

Table 1. Descriptive characteristics of adolescents

	Number	%
Gender		
Female	260	51.2
Male	248	48.8
Grade		
9. Grade	123	24.2
10. Grade	168	33.1
11. Grade	114	22.4
12. Grade	103	20.3
Age Mean ± SD (min-max)	16.04±1.06 (12-20)	
Parents' Living Together		
Together	471	92.7
Apart	37	7.3
Parents' Life Status		
Both are alive	491	96.7
Mother is alive, father is dead	12	2.4
Father is alive, mother is dead	4	0.8
Both are dead	1	0.2
Mother's Working Status		
Working	127	25.0
Not Working	381	75.0
Father's Working Status		
Working	451	88.8
Not Working	57	11.2
Mother's Educational Background		
Primary School	197	38.8
Secondary School	86	16.9
High School	121	23.8
University and higher	104	20.5
Father's Educational Background		
Primary School	93	18.3
Secondary School	94	18.5
High School	149	29.3
University and higher	172	33.9
Mother's Child-rearing Attitudes		
Authoritarian	99	19.5
Democratic	96	18.9
Highly tolerant	149	29.3
Highly protective	85	16.7
Ignorant	24	4.7
Perfectionist	55	10.8
Father's Child-rearing Attitudes		
Authoritarian	107	21.1
Democratic	105	20.7
Highly tolerant	122	24.0
Highly protective	56	11.0
Ignorant	65	12.8
Perfectionist	53	10.4

Table 1. Descriptive characteristics of adolescents (continued)

	Number	%
Economic Status		
Income less than expenses	87	17.1
Income equals expenses	295	58.1
Income above the expenses	126	24.8
Number of Siblings		
1	21	4.1
2	161	31.7
3 and more	326	64.2
Academic Achievement		
Poor	79	15.6
Moderate	227	44.7
Good	171	33.7
Very Good	31	6.1

The analysis of the mean scores of the problematic internet use scale based on academic achievement revealed a significant difference between PIUS-A mean score (p<0.001). The total scale mean score of participants with poor academic achievement were found to be higher (Table 2).

When the mean scores of the problematic internet use scale according to the economic situation were compared, it was defined that there was a significant difference between PIUS-A mean score (p<0.001). The average score of those who expressed their economic status as lower than income and expenditure was found to be the highest (Table 2).

Table 2. Mean PIUS-A and FLSS scores with regard to personal characteristics of adolescents

CHARACTERISTICS	PIUS-A	FLSS
Total	61.35±23.18	96.62±19.17
Gender		
Female	62.23±22.84	95.36±19.75
Male	60.42±23.54	97.94±18.49
p value	0.380	0.130
Grade		
9.	61.94±22.51	97.33±18.97
10.	62.96±23.00	96.19±18.32
11.	60.33±24.43	95.60±20.52
12.	59.15±22.95	97.61±19.42
p value	0.566	0.889
Parents' Living Together		
Together	61.16±22.92	97.17±19.16
Apart	63.70±26.45	89.62±18.17
p value	0.576	0.021**
Mother's Working Status		
Working	59.95±20.66	96.33±20.46
Not Working	61.82±23.97	96.71±18.75
p value	0.389	0.847
Father's Working Status		
Working	61.00±22.98	97.28±19.04
Not Working	64.14±24.77	91.42±19.55
p value	0.333	0.031**

Table 2. Mean PIUS-A and FLSS scores with regard to personal characteristics of adolescents (continued)

CHARACTERISTICS	PIUS-A	FLSS
Economic Status		
Income less than expenses	69.36±25.64[1]	92.59±19.81
Income equals expenses	61.28±23.46[2]	95.97±19.44
Income above the expenses	55.99±18.93[3]	100.92±17.34
p value	<0.001* 1>2>3	0.005** 3>1-2
Academic Achievement		
Bad	69.36±25.64[1]	89.78±21.81
Moderate	61.28±23.46[2]	95.33±17.58
Good	55.99±18.93[3]	100.74±18.91
p value	<0.001 1>2>3	<0.001* 3>1-2
Mother's Educational Background		
Primary School	63.02±24.48	95.95±18.69
Secondary School	62.11±26.15	96.02±18.72
High School	62.24±23.04	95.32±21.16
University and higher	56.52±17.08	99.89±17.87
p value	0.122	0.273
Father's Educational Background		
Primary School	62.81±23.40[1]	95.12±17.56[1]
Secondary School	67.40±26.86[2]	92.93±19.26[2]
High School	59.01±21.90[3]	100.77±18.99[3]
University	59.28±21.42[4]	95.85±19.6[4]
p value	0.021	0.010*
Father's Child-rearing Attitudes		
Authoritarian	61.69±21.53	91.42±18.68[1]
Democratic	60.18±21.70	101.94±14.60[2]
Highly tolerant	59.04±23.11	101.91±17.83[3]
Highly protective	57.33±23.08	99.19±17.11[4]
Ignorant	69.15±25.41	82.30±21.54[5]
Perfectionist	63.00±25.28	99.22±19.40[6]
p value	0.054	<0.001* 2,3,4,6>5
Mother's Child-rearing Attitudes		
Authoritarian	62.80±22.65	89.04±19.101
Democratic	59.52±20.01	100.69±14.69
Highly tolerant	60.00±23.67	104.12±17.79
Highly protective	64.14±24.95	94.50±17.65
Ignorant	65.83±29.98	76.04±19.64
Perfectionist	59.34±21.96	95.10±20.15
p value	0.546	<0.001*
Number of Siblings		
1	62.23±22.50	95.76±12.86
2	62.47±22.95	97.22±21.74
3 and more	60.74±23.38	96.38±18.17
p value	0.731	0.888

F1: One-way ANOVA test $p<0.05$*, Tamhane's T2**, Bonferroni, t2: Independent sample t-test**, $p<0.05$

A significant difference was found between the total score average of PIUS-A mean score according to the father's educational status of the adolescents. It was determined that the average score of those whose father's education level was secondary school was significantly higher than the others (p<0.021) (Table 2).

There was no significant (p>0.05) difference between the FLSS score averages according to age, gender, grade level, number of siblings, educational status of the mother, and employment status of the mother (Table2).

A significant difference was determined between the mean scores of the FLSS according to their parents' living together, father's working status, father's education level, family's economic status, academic success and parental attitudes (p<0.05) (Table 2).

The relationship between PIUS-A and FLSS is shown in Table 3. A low-level negative significant correlation was found between the PIUS-A mean score and the FLSS mean score (r:-0.262, p<0.001). A very low and significant negative correlation was found between FLSS and negative consequences of using the internet (r:-0.027, p<0.001) and overuse (r:-0.094, p:0.034*)*. A low, negative and significant relationship was determined between FLSS and the social benefit sub-dimension (r:0.255, p<0.001).

Table 3. The relationship between adolescents' mean scores in Problematic Internet Use Scale-Adolescent (PIUS-A) and The Satisfaction with Family Life (FLSS) scale

Scales	**FLSS**	
PIUS-A	*r:* -0.262	p<0.001
Negative Consequences of Using the Internet	r: -0.027	p<0.001
Overuse	r: -0.094	p:0.034
Social Benefits	r: -0.255	p<0.001

r: Pearson correlation coefficient, *p<0.001

The independent variables affecting the problematic internet use score are shown in Table 4. The effect of the variables on the problematic internet use score was examined with linear regression analysis and the regression model created was found to be statistically significant (F:8.401, p<0.001). The enter method was used as the method in the regression model created and was seen to explain 10.5% of the independent variables and the dependent variable. According to the standardized regression coefficient, the FLSS score (β1:-0.209, p<0.001) appeared to be a predictor of problematic internet use. In addition, it was determined that the PIUS-A score of those with good economic status was 7.840 units lower (p:0.019) than that of those with poor economic status, and the PIUS-A score of those with good school performance was 10.426 units lower than that of those with poor economic status (p:0.001). The father's education level was not determined to be an important predictor of PIUS-A (p>0.05).

Discussion

The aim of this study was to determine the relationship between the problematic internet use levels of adolescents and their family life satisfaction levels. The results of the analysis revealed a significant negative relationship between the problematic internet use of adolescents and their level of satisfaction with family life. In the literature review, no previous study could be found which examined the relationship between family life satisfaction and problematic internet use. However, family life satisfaction is known to be associated with concepts such as a positive relationship between family members, being happy in the family environment, harmony between family members and life satisfaction [22]. The results of other studies examining the relationship between life satisfaction and problematic internet use have also revealed that life satisfaction decreases as problematic internet use behavior increases. In a study conducted in adolescents whose

Table 4. Examining the effect of independent variables affecting the PIUS-A score by linear regression analysis

	β_0 (%95 CI)	S. Error	β_1	t	*p*
Constant	98.395 (87.011- 109.779)	5.794		16.981	<0.001
FLSS	-0.253(-0.357- -0.149)	0.053	-0.209	-4.786	<0.001
Economic Status (Income less than expenses)			Reference		
Income equals expenses	-5.537 (-10.946- -0.128)	2.753	-0.118	-2.011	0.045
Income above the expenses	-7.840(-14.379- -1.301)	3.328	-0.146	-2.356	0.019
Academic Achievement (Bad)			Reference		
Moderate	-7.267 (-13.011- -1.523)	2.924	-0.156	-2.486	0.013
Good	-10.426(-16.347- -4.505)	3.013	-0.220	-3.460	0.001
Father's Educational Background (Primary school)			Reference		
Secondary School	4.322(-1.993-10.638)	3.214	0.072	1.345	0.179
High School	-1.404(-7.169- 4.362)	2.935	-0.028	-0.478	0.633
University and upper	-1.314(-7.109- 4.480)	2.949	-0.027	-0.446	0.656

F=8.401, *p*<0.001, R^2=11.9%, Adjusted R^2=10.5%, β_0: Unstandardized Coefficients, β_1: Standardized Coefficients

parents were divorced, Van Dijk et al. [23] found that problematic internet use is more common particularly among adolescents who stated that they did not feel close to their mothers. In another study of 466 Brazilian adolescents, Andrade et al. [24] emphasized that family conflicts and child-parent conflicts cause unhealthy behaviors in children such as problematic internet use and that families should be involved in studies that aim to limit internet use. Say and Durak Batigun [16] also reported that an adolescent's negative relationship with their parents has the effect of increasing problematic internet use. It is an undeniable fact that the family has a great influence on the development of the child. In a family environment where family relationships are inadequate, the development of the child is hindered. The child may think that his own thoughts and feelings are not important and may seek other relationships. Consequently, a child who is not satisfied with family life can take refuge on the internet and the needs that cannot be met with family relationships are tried to be met by turning to the internet (smartphone, tablet, computer, etc.).

The problematic internet use levels of adolescents were examined according to their individual characteristics. Accordingly, a significant difference was determined between the variables of family income, academic achievement, father's educational background and father's child-rearing attitudes and problematic internet use, although as a result of the regression analysis, it was determined that the educational status of the father was not an important predictor of problematic internet use.The adolescents who thought that their family income was more than their expenses were determined to have lower levels of problematic internet use. In previous studies [25-28] it has been observed that individuals with better economic status have higher levels of problematic internet use. It is thought that the economic situation improves in parallel with the education level of the father, and therefore fathers with a better level of education show a more conscious approach to their children's internet use. In addition, the level of problematic internet use was determined to be lower among the adolescents who expressed their perceived academic achievement as good compared to those who expressed their perceived academic achievement as moderate or poor. In various studies, both in the national and international literature [29, 30], the results are in parallel with the current study results. In studies of adolescents studying in high school by Sabaz and Bilgin [29], and Yavuz [27], and in a study of university students by Buzzai et al. [30], it was determined that individuals with high levels of problematic internet use exhibit poorer academic achievement. The results of those studies showed that individuals with a problematic internet use disorder face some problems such as not using time effectively, not allocating appropriate time to study, deterioration in social interaction and deterioration in interpersonal relations with friends. Therefore, it seems inevitable that the negative consequences brought about by problematic internet use will adversely affect academic achievement.

Limitations of the study

The main limitations of this study were that the research data could not be collected face-to-face due to the COVID-19 pandemic, that the responses were limited to the answers given by the adolescents and that the results of the study could only be generalized to the group participating in the study.

Since the data of the study was collected on the internet, only adolescents who could access the internet and had a smart phone could participate in the study, which may have affected the level of problematic internet use.

The use of a non-probability method in determining the sample and the fact that the number of participants was much lower than the population also constituted an important limitation.

Finally, as this was a cross-sectional study design, the direction of causation could not be determined and reverse causation cannot be excluded.

In conclusion, from the results of this study, it can be concluded that problematic internet use is higher in children with low family life satisfaction. The study can be considered of value as there is no other study in the literature which has examined the level of family life satisfaction and problematic internet use together. This study can be of guidance for further studies to be conducted and will contribute to the relevant literature.

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Authors' contributions to the article

A.N.K. have constructed/constructed the main idea and hypothesis of the study E.C. and A.N.K. they developed the theory and arranged/edited the material and method section. E.C. and A.N.K. have done the evaluation of the data in the Results section. Discussion section of the article written by E.C. and A.N.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Machine learning model to identify prognostic factors in glioblastoma: a SEER-based analysis

Glioblastomda prognostik faktörleri tanımlamak için makine öğrenmesi modeli: SEER tabanlı analiz

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Abstract

Purpose: Analyzing and interpreting large amounts of complex health care data are becoming more insufficient by traditional statistical approaches. However, analyzing Big Data (BD) by machine learning (ML) supports the storage, classification of patient information. Therefore, improves disease identification, treatment evaluation, surgical planning, and outcome prediction. The current study aims to create a competing risk model to identify prognostic factors in glioblastoma (GB).

Materials and methods: The study included 31663 patients diagnosed with GB between 2007 and 2018. The data in the study were taken from the Surveillance, Epidemiology, and End Results (SEER) database. Overall survivals (OS), age, race, gender, primary site, laterality, surgery and tumor size at the time of diagnosis, vital status, and follow-up time (months) were selected for the analyzes.

Results: The median OS of the patients was found to be 9.00±0.09 months. In addition, all variables in the table were statistically significant risk factors for survival except gender. Therefore, surgery, age, laterality, primary site, tumor size, race, gender variables were used as independent risk factors, and vital status was used as a dependent variable for ML analysis. Looking at the ML results, hybrid model gave the best results according to Accuracy, F-measure, and MCC performance criteria. According to hybrid model, which has the best performance, the diagnosis of alive/dead in 84 and 74 out of 100 patients can be interpreted as correct for 1- and 2-year, respectively.

Conclusions: The model created by ML was 84.9% and 74.1% successful in predicting 1- and 2-year survival in GB patients, respectively. Recognition of the fundamental ideas will allow neurosurgeons to understand BD and help evaluate the extraordinary amount of data within the associated healthcare field.

Key words: Machine learning, big data, glioblastoma, SEER.

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Öz

Amaç: Büyük miktarlardaki karmaşık sağlık hizmeti verilerinin analiz edilmesi ve yorumlanmasında geleneksel istatistiksel yaklaşımlar giderek yetersiz kalmaktadır. Bununla birlikte, Büyük Verinin makine öğrenmesi ile analiz edilmesi, hasta bilgilerinin depolanmasını, sınıflandırılmasını destekler. Bu nedenle hastalık tanımlamasını, tedavi değerlendirmesini, cerrahi planlamayı ve sonuç tahminini geliştirir. Mevcut çalışma, glioblastomda (GB) prognostik faktörleri tanımlamak için bir risk modeli oluşturmayı amaçlamaktadır.

Gereç ve yöntem: Çalışmaya 2007-2018 yılları arasında GB tanısı konan 31663 hasta dahil edilmiştir. Çalışmadaki veriler Surveillance, Epidemiology, and End Results (SEER) veri tabanından alınmıştır. Analizler için genel sağ kalımlar, yaş, ırk, cinsiyet, primer bölge, lateralite, cerrahi ve tanı anındaki tümör boyutu, vital durum ve takip süresi (ay) seçildi.

Bulgular: Hastaların ortanca sağ kalımı 9,00±0,09 ay olarak bulundu. Ayrıca tablodaki tüm değişkenler cinsiyet dışında sağ kalım için istatistiksel olarak anlamlı risk faktörleriydi. Bu nedenle, makine öğrenmesi analizi için bağımsız risk faktörleri olarak cerrahi, yaş, lateralite, primer bölge, tümör boyutu, ırk, cinsiyet değişkenleri ve vital durum bağımlı değişken olarak kullanıldı. Makine öğrenmesi sonuçlarına bakıldığında, doğruluk, F-ölçümü ve MCC performans kriterlerine göre Hibrit Model en iyi sonuçları vermiştir. En iyi performansa sahip olan hibrit modele göre 100 hastanın 84'ünde canlı/ölü tanısı sırasıyla 1 ve 2 yıl için doğru olarak yorumlanabilmektedir.

Sonuç: Makine öğrenmesi ile oluşturulan model GB hastalarında 1 ve 2 yıllık sağ kalımı öngörmede sırasıyla %84,9 ve %74,1 başarılıydı. Temel fikirlerin tanınması, beyin cerrahlarının Büyük Veriyi anlamalarına ve ilgili sağlık hizmetleri alanındaki olağanüstü miktarda veriyi değerlendirmelerine yardımcı olacaktır.

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Introduction

Science and industry have an extraordinary data production in our age. Traditional statistical approaches are not sufficient in the analysis and interpretation of Big Data (BD). Machine learning (ML) and artificial intelligence methods have become essential in the perception of these data [1, 2]. The BD analysis supports the storage, classification, and analysis of patient information in the healthcare field and improves disease identification, treatment evaluation, surgical planning, and outcome prediction [3]. Hidden patterns in large datasets can be revealed by BD analysis [4].

In adults, the most common primary malign brain tumor is glioblastoma (GB) [5]. Surgical resection, adjuvant external beam radiation therapy, plus concurrent and adjuvant temozolomide is the standard management of newly diagnosed high-grade gliomas (HGG) [6, 7]. The median survival in patients with this protocol was 14.6 months [7], and 5-year survival is 5% despite aggressive therapies [8-10]. The independent prognostic factors for progression-free survival (PFS) and overall survival (OS) are age, preoperative performance status, and tumor size [11]. MGMT promoter methylation was added to these factors in a recent systematic review [12].

This study extracted 31663 patients with histologically confirmed GB from Surveillance, Epidemiology and End Results (SEER) database. This study aims to create a competing risk model to identify prognostic factors in GB.

Material and methods

Study design

The study included 31663 patients diagnosed with GB between 2007 and 2018, and all patient data were analyzed for the study. January 2007 was chosen as the starting point for the study, and December 2018 was selected as the end date of the study. The data in the study were taken from the SEER database. These data, published by the National Cancer Center Institute, are a compilation of databases of 18 SEER cancer registries in the USA. The SEER program is used to summarize data from patients' medical records. It is estimated that more than 95% of all cancer cases are detected and included in this database in areas under surveillance [13]. The duration of follow-up is calculated in months using the date of diagnosis and whichever occurs first, 1) date of death, 2) date last known to be alive, 3) December 2018 (the follow-up cutoff date used in our analysis). Since all patient data were obtained with the permission of SEER without including personal patient information, there is no need to get ethical committee approval from any committee within the scope of this research.

The main hypothesis in the study was OS in years (censored observations), defined from the date of diagnosis to the date of death or, for living patients, the last control date. In addition to survival, other variables selected for the analyzes were age, race, gender, primary site, laterality (unilateral/bilateral), surgery and tumor size at the time of diagnosis, vital status, and follow-up time (months). Surgical methods, radiotherapy, and chemotherapy techniques were not included in the study because of missing data.

In this study, in addition to the classical ML methods, we created a hybrid model consisting of a combination of existing methods. Such hybrid models have been preferred more in recent years, as they are a combination of ML methods and use the most substantial aspects of these methods. For 2-year survival prediction model, we used J48, Multilayer Perceptron and Naïve Bayes to create a hybrid model. For 1-year survival prediction model, we used J48, Multilayer Perceptron and Logistic Regression to create a hybrid model.

Structure of hybrid model

For the hybrid model, first the five data mining methods with the best performance are selected. The methods chosen as the second stage are ranked from the method with the best performance to the method with the worst performance. In the next stage, the method

with the best performance is the first chosen method for the hybrid model. The remaining four methods are added to the first method to form a group of double, triple and quadruple methods, respectively. The performance criteria of these groups are calculated one by one and a hybrid model is created based on the group that gives the best results. All of these stages were checked in the background automatically by hybrid model software previously written.

Statistical analysis

SPSS 11.5 and Weka 3.7 programs were used in the analysis of the data. Mean±standard deviation and median (minimum-maximum) were used as descriptors for quantitative variables, and the number of patients (percentage) for qualitative variables. Survival analyzes on qualitative variables were performed using the Kaplan-Meier method, and significant differences between groups were determined using the log-rank test. The statistical significance level was taken as 0.05.

Classification methods of Logistic Regression [14], Naive Bayes [15], Multilayer Perceptron [16], Bagging [17], and J48 [18] were used in the WEKA program. The data set was evaluated using the 10-fold Cross-Validation test option. Accuracy, F-Measure, Matthews correlation coefficient (MCC), Precision-Recall Curve (PRC Area), and Receiver Operating Characteristic (ROC) Area were used as data mining performance criteria.

Results

General descriptors of the variables in the data set are given in Table 1. According to descriptors, 1.1% of the patients were younger than 19 years old or equal, 7.0% were in the 20-44 age range, 42.3% were in the 45-64 age range, and 49.6% were 65 years old or older. While 88.8% of the patients were White, 5.8% were Black, and 5.3% were from other races. In addition, the male-female ratio was 58.4%/41.6%. The table shows the primary site, laterality, and surgery information of the patients. Tumor sizes of the patients are also grouped, and the patients' vital status and follow-up periods are given (Table 1).

Table 2 shows the survival analysis results of the patients. The median OS of the patients was found to be 9.00±0.09 months. In addition, all variables in the table were statistically significant risk factors for survival except gender. Median life expectancy was found to be 16.00±0.93 months for those younger than or equal to 19 years of age, 22.00±0.58 months for 20-44 years old, 14.00±0.14 months for 45-64 years old, and 5.00±0.07 months for over 65 years old. When evaluated in terms of race, the median life expectancy was 9.00±0.10 months for the White race, and 10.00±0.39 months and 12.00±0.47 months for the Black and other races, respectively. In the study, the median life expectancy of women was equal to that of men.

When survival is evaluated in primary site types, the lowest median survival time is found in the group classified as ventricle, cerebellum, and overlapping brain lesion, followed by the brain stem, parietal, frontal, occipital, and temporal lobes, respectively. Survival statistics for laterality, tumor size, and surgery are also given in Table 2.

Gain Ratio Attribute Evaluation and Information Gain Attribute Evaluation attribute selection methods in WEKA were used. Using these methods, the importance of the variables and the values added to the data set were examined for last 2-year (2017-2018). A total of 8 variables (7 independent variables and one dependent variable) were used from the data set. These variables are surgery, age, laterality, primary site, tumor size, race, gender, and vital status. Percentages of variable importance according to the dependent variable vital status were given in Figure 1A. For 1-year data set, a total of 8 variables (7 independent variables and 1 dependent variable) used. These variables are surgery, age, laterality, primary site, tumor size, race, gender and vital status. Percentages of variable importance according to dependent variable vital status was given in Figure 1B.

The performance criteria of ML Methods for the 2-year survival prediction model are given in Table 3. Looking at the ML results, the hybrid model gave the best results according to Accuracy, F-measure, and MCC performance criteria, which are the most accepted criteria in the literature. Considering these three performance criteria, the hybrid model is followed by J48, Naïve Bayes, Logistic Regression, Bagging, and Multilayer Perceptron, respectively. According to the hybrid model, which has the best performance, the diagnosis of alive/dead

Table 1. Description of the variables in the data for patients with glioblastoma

Variables		
Age, n (%)	≤19 years	343 (1.1)
	20-44 years	2208 (7.0)
	45-64 years	13403 (42.3)
	≥65 years	15709 (49.6)
Race, n (%)	White	28127 (88.8)
	Black	1849 (5.8)
	Other	1687 (5.3)
Gender, n (%)	Male	18479 (58.4)
	Female	13184 (41.6)
Primary Site, n (%)	Frontal Lobe	10113 (31.9)
	Temporal Lobe	8936 (28.2)
	Parietal Lobe	5490 (17.3)
	Occipital Lobe	1461 (4.6)
	Ventricle	154 (0.5)
	Cerebellum	273 (0.9)
	Brain Stem	201 (0.6)
	Overlapping Lesion of Brain	5696 (19.8)
Laterality, n (%)	Unilateral	31023 (98.0)
	Bilateral	640 (2.0)
Surgery, n (%)	Not Performed	6414 (20.3)
	Performed	25249 (79.7)
Tumor Size, n (%)	Less than 1 cm	170 (0.6)
	Between 1 cm and 2 cm	1291 (4.7)
	Between 2 cm and 3 cm	3329 (12.2)
	Between 3 cm and 4 cm	5117 (18.8)
	Between 4 cm and 5 cm	7336 (27.0)
	Greater than 5 cm	9976 (36.7)
Follow-up Time (months)	Mean±SD	13.21±17.14
	Median (Min.-Max.)	8.00 (0.00-143.00)
Vital Status, n (%)	Alive	4409 (13.9)
	Dead	27254 (86.1)

SD: Standard Deviation, Min: Minimum, Max: Maximum

Table 2. Kaplan-Meier results (SE: Standard error) of the study

Variables		Survival					*p* value
		1 year (%)	3 year (%)	5 year (%)	Survival Time		
					Mean±SE	Median±SE	
Overall		40.5	10.2	5.2	17.03±0.17	9.00±0.09	-
Age	≤19 years	56.9	22.8	14.7	33.99±2.75	16.00±0.93	<0.001
	20-44 years	72.7	32.6	20.2	39.50±1.11	22.00±0.58	
	45-64 years	53.5	13.0	6.5	21.03±0.27	14.00±0.14	
	≥65 years	24.4	4.5	1.8	10.09±0.14	5.00±0.07	
Race	White	39.8	9.9	5.1	16.76±0.18	9.00±0.10	<0.001
	Black	42.9	11.9	6.2	18.26±0.71	10.00±0.39	
	Other	48.9	14.6	6.8	19.96±0.76	12.00±0.47	
Gender	Male	40.8	9.8	4.7	16.60±0.21	10.00±0.12	0.544
	Female	42.0	10.8	5.9	17.64±0.28	10.00±0.15	
Primary Site	Frontal Lobe	39.9	11.3	5.9	17.87±0.32	9.00±0.16	<0.001
	Temporal Lobe	45.4	10.6	5.0	17.69±0.30	11.00±0.17	
	Parietal Lobe	40.7	9.7	5.1	17.01±0.40	9.00±0.22	
	Occipital Lobe	43.2	9.9	5.0	16.92±0.70	10.00±0.40	
	Ventricle	34.5	11.7	6.1	18.20±2.74	6.00±1.05	
	Cerebellum	37.8	10.3	5.4	16.52±1.79	6.00±0.78	
	Brain Stem	35.7	10.3	6.7	16.60±2.01	8.00±0.84	
	Overlapping Lesion of Brain	32.4	8.2	4.2	14.06±0.37	6.00±0.20	
Laterality	Unilateral	40.8	10.3	5.2	17.11±0.17	9.00±0.09	<0.001
	Bilateral	26.1	7.9	4.2	12.74±1.03	5.00±0.43	
Tumor Size	Less than 1 cm	50.2	15.3	6.6	19.85±2.35	12.00±0.97	<0.001
	Between 1 cm and 2 cm	48.7	14.8	6.3	19.11±0.83	12.00±0.41	
	Between 2 cm and 3 cm	46.4	12.3	5.4	18.85±0.52	11.00±0.30	
	Between 3 cm and 4 cm	42.1	10.3	5.3	17.52±0.42	10.00±0.22	
	Between 4 cm and 5 cm	41.9	9.8	5.0	17.06±0.33	10.00±0.20	
	Greater than 5 cm	36.5	9.6	4.7	16.10±0.30	8.00±0.15	
Surgery	Not Performed	14.4	3.0	1.3	7.16±0.21	3.00±0.05	<0.001
	Performed	47.0	12.1	6.2	19.53±0.20	11.00±0.10	

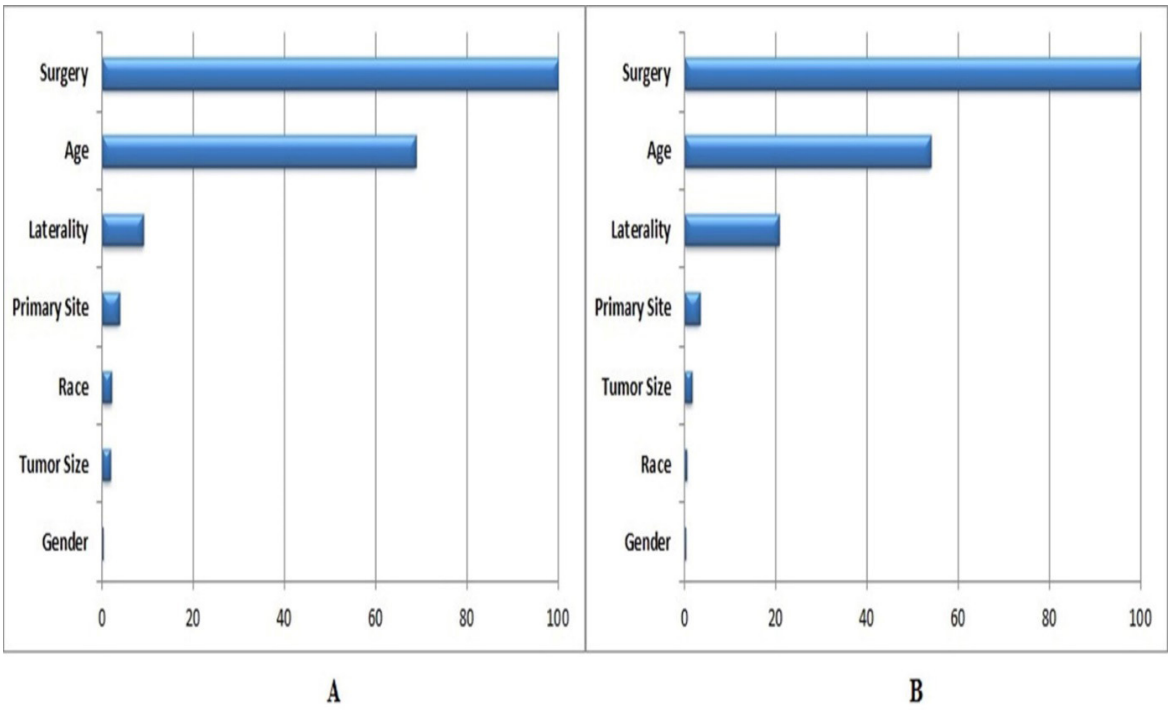

Figure 1. Variable importance according to vital status variable

Table 3. Performance results of Machine Learning methods for 2-year survival

Methods		Performance Criteria				
		Accuracy	F-measure	MCC	PRC Area	ROC Area
Logistic Regression	**Alive**	0.589	0.613	0.272	0.648	0.681
	Dead	0.682	0.657	0.272	0.688	0.681
	Overall	0.636	0.636	0.272	0.668	0.681
Naive Bayes	**Alive**	0.591	0.614	0.272	0.648	0.682
	Dead	0.680	0.657	0.272	0.689	0.682
	Overall	0.637	0.636	0.272	0.669	0.682
Multilayer Perceptron	**Alive**	0.648	0.618	0.218	0.622	0.653
	Dead	0.570	0.598	0.218	0.660	0.653
	Overall	0.608	0.608	0.218	0.641	0.653
Bagging	**Alive**	0.601	0.611	0.250	0.639	0.668
	Dead	0.649	0.639	0.250	0.676	0.668
	Overall	0.626	0.625	0.250	0.658	0.668
J48	**Alive**	0.568	0.607	0.279	0.629	0.664
	Dead	0.708	0.668	0.279	0.647	0.664
	Overall	0.640	0.638	0.279	0.638	0.664
Hybrid Model	**Alive**	0.698	0.725	0.481	0.714	0.764
	Dead	0.781	0.755	0.481	0.793	0.764
	Overall	0.741	0.740	0.481	0.754	0.764

MCC: Matthews correlation coefficient, PRC: Precision Recall Curve, ROC: Receiver Operating Characteristic

in 74 out of 100 patients can be interpreted as correct. As another explanation, when a patient is diagnosed as alive/dead with the hybrid model method, the accuracy rate of this diagnosis is 74.1%.

The performance criteria of ML methods for the 1-year survival prediction model are given in Table 4. Looking at the ML results, the hybrid model gave best results according to Accuracy, F-measure and MCC performance criteria, which are the most accepted performance criteria in the literature. Considering these three performance criteria, the hybrid model is followed by J48, Naïve Bayes, Logistic Regression, Bagging and Multilayer Perceptron, respectively. According to the hybrid model which has the best performance, the diagnosis of alive/dead in 85 out of 100 patients can be interpreted as correct. As another explanation, when a patient is diagnosed as alive/dead with the hybrid model method, the accuracy rate of this diagnosis is 84.9%.

Table 4. Performance results of Machine Learning methods for 1-year survival

Methods		Performance Criteria				
		Accuracy	F-measure	MCC	PRC Area	ROC Area
Logistic Regression	**Alive**	0.927	0.816	0.297	0.814	0.704
	Dead	0.295	0.409	0.297	0.548	0.704
	Overall	0.719	0.682	0.297	0.726	0.704
Naive Bayes	**Alive**	0.918	0.814	0.297	0.815	0.704
	Dead	0.312	0.422	0.297	0.543	0.704
	Overall	0.718	0.685	0.297	0.725	0.704
Multilayer Perceptron	**Alive**	0.877	0.796	0.257	0.776	0.665
	Dead	0.340	0.427	0.257	0.506	0.665
	Overall	0.700	0.675	0.257	0.687	0.665
Bagging	**Alive**	0.914	0.812	0.292	0.810	0.704
	Dead	0.313	0.421	0.292	0.540	0.704
	Overall	0.716	0.683	0.292	0.721	0.704
J48	**Alive**	0.938	0.818	0.301	0.722	0.609
	Dead	0.281	0.399	0.301	0.468	0.609
	Overall	0.721	0.680	0.301	0.638	0.609
Hybrid Model	**Alive**	0.941	0.893	0.647	0.958	0.856
	Dead	0.661	0.742	0.647	0.698	0.856
	Overall	0.849	0.843	0.647	0.872	0.856

MCC: Matthews correlation coefficient, PRC: Precision Recall Curve, ROC: Receiver Operating Characteristic

Discussion

Many studies [19-28] investigate prognosis and survival in GBs using the SEER database. The main difference of our study is that it processes data created following the last two World Health Organisation (WHO) classifications and creates a high-performance model that predicts 1- and 2-year survival using ML.

The overall median survival of our study was 9.00±0.09 months. It is quite a short time compared to the literature, but the main reason is that 49.6% of the patient group in our study was 65 years and older. Less than 20% of elderly GB patients survive up to 1 year, with median survival between 5 and 9 months [28, 29]. Survival may differ according to race and ethnicity in patients diagnosed with GB [30]. The incidence of GB was higher in the White population than others in our study, and it is consistent with previous publications [7, 31-34]. Survival in the White race was lower than in the other races, as in the analysis by Ostrom et al. [32] Although some publications are stating that survival is higher in the female gender [7, 19, 31], no significant relationship was found between gender and survival in our study.

There is no consensus on whether tumor location is a prognostic factor. In a recent study

[35], GBs' survival in the central core (basal ganglia, corpus callosum) and left temporal lobe pole was less than six months. The survival of the dorsomedial right temporal lobe GBs was more than 24 months. In our study, the temporal lobe tumors' survival was the highest, but no comparison was made in the right or left hemispheres. The prognosis of ventricular [36-38], brainstem [39], and bilateral hemispheric [40] HGGS are poor, and the results of our study are similar. Although some authors state that cerebellar GBs are worse, comparable, or better than supratentorial ones [41-45], cerebellar GBs had significantly improved lower survival in our study.

Liu et al. [22] stated that tumor size over 5.4 cm in the SEER database between 2007 and 2016 in patients over 65 years of age is an independent risk factor for GB-related deaths. The larger the FLAIR-T2 hyperintensity volume correlates with, the worse OS and PFS prediction [46]. In our study, the survival of tumors larger than 5 cm was the shortest.

Despite the existence of different treatment modalities, the management of GBs remains a challenge [47]. Although there is no consensus on the limits of surgery in the literature [47, 48] when the maximal surgical resection of abnormal tissue (including FLAIR signal) is safe, it optimizes the patient survival [49]. In our study, the survival of patients who underwent surgical resection was significantly higher.

Various survival predicting models created with the ML method has been published [50-55], and a recent systematic review reported that the accuracy of these studies was in the range of 0.66-0.98 [55]. The success of our model to predict 1- and 2-year survival was 0.849 and 0.741, respectively.

Limitations

There are some limitations to this study. There are many subclassifications for each variable when creating data stored in online databases. The authors who process the data can combine or narrow these subsets to the extent they choose for the years they will evaluate. For this reason, different results can be obtained using the same database. The clusters we created in our study are a similar limitation.

Age, race, gender, tumor site/laterality/size, and surgical resection are independent survival risk factors in the analysis performed on 31633 patients between 2007-2018 in the SEER database. The model created by ML was 84.9% and 74.1% successful in predicting 1- and 2-year survival in GB patients, respectively. Recognition of the fundamental ideas will allow neurosurgeons to understand BD and help assimilate and evaluate the extraordinary amount of data within the associated healthcare field.

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

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Authors' contributions to the article

B.B. and F.Y. have constructed the main idea and hypothesis of the study. They developed the theory and arranged/edited the material and method section. E.E. have done the evaluation of the data in the Results section. Discussion section of the article written by E.E. and F.Y., B.B, U.A.D. and E.E. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Comparison of graft bypass surgery and endovascular interventional techniques in iliofemoral and above knee femoropopliteal arterial occlusion

İliofemoral ve femoropopliteal arterlerin tıkanıklığında endovasküler girişimler ile greft bypass cerrahisi tekniklerinin karşılaştırılması

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Abstract

Purpose: Peripheral vascular disease is associated with significant morbidity and mortality. In this study, we made a comparison between surgical bypass grafting and endovascular stenting in iliofemoral and above-knee femoropopliteal arterial occlusion to find out the advantages and disadvantages of each treatment method.

Matarials and methods: This is a retrospective study, where 60 patients had undergone endovascular and surgical interventions due to the occlusion of the iliofemoral and above-knee femoropopliteal arteries between January 2015 and December 2020. Patients were divided into two groups according to their treatment method. Group 1 contained 33 patients and was operated on with surgical bypass grafting, while group 2 contained 27 with endovascular intervention. Patients' morbidities, imaging methods, localization of occlusion, type of grafts and endovascular procedure, vascular patency, length of hospital stay, blood transfusion, revision, and complications were evaluated and analyzed.

Results: The femoral artery occlusion (72.7%) was in the majority in Group 1, while the iliac artery (66.7%) was in Group 2. There were three patients in Group 1 and 14 patients in Group 2 who had balloon administration. Regarding vascular patency, the grafts were occluded in 10 (45.5%) patients in Group 1, while stents were occluded in 12 patients (54.5%) in Group 2. Blood transfusion was performed in fifteen patients in Group 2 and six patients in Group 2.

Conclusion: The graft patency rate was higher in Group 1, and the re-revascularization rate of surgical treatment was lower than endovascular treatment. The highest graft patency rate was seen in the Dacron grafts then saphenous veins. Group 1 patients received more blood transfusions and extended hospital stayment periods than Group 2 patients.

Key words: Surgical bypass grafting, endovascular stenting, artery occlusion, vascular patency.

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Öz

Amaç: Periferik damar hastalığı önemli morbidite ve mortalite ile ilişkilidir. Bu çalışmada iliofemoral ve diz üstü femoropopliteal arter tıkanıklığında cerrahi baypas greftleme ile endovasküler stentleme arasında bir karşılaştırma yaparak her bir tedavi yönteminin avantaj ve dezavantajlarını ortaya çıkarmaya çalıştık.

Gereç ve yöntem: Ocak 2015 ile Aralık 2020 arasında 60 hasta bu çalışmaya retrospektif olarak dahil edildi. Hastalar tedavi yöntemlerine göre iki gruba ayrıldı. Grup 1 33 hastadan oluşuyordu ve cerrahi baypas greftleme ile ameliyat edildi, grup 2 ise endovasküler girişim ile 27 hastadan oluşuyordu. Hastaların morbiditeleri, görüntüleme yöntemleri, tıkanıklığın lokalizasyonu, greft tipi ve endovasküler işlem, damar açıklığı, hastanede kalış süresi, kan transfüzyonu, revizyon ve komplikasyonlar değerlendirildi ve analiz edildi.

Bulgular: Grup 1'de femoral arter tıkanıklığı (%72,7) çoğunluktayken, Grup 2'de iliak arter (%66,7) idi. Grup 1'de üç hasta, Grup 2'de balon uygulanan on dört hasta vardı. Vasküler açıklık açısından Grup 1'de 10 (%45,5) hastada greftler, Grup 2'de ise 12 (%54,5) hastada stent tıkandı. Grup 1'de on beş hastaya, Grup 2'de altı hastaya kan transfüzyonu yapıldı.

Sonuç: Grup 1'de greft açıklık oranı daha yüksek, cerrahi tedavinin yeniden revaskülarizasyon oranı endovasküler tedaviye göre daha düşüktü. En yüksek greft açıklık oranı, safen venlerden sonra Dakron greftlerde görüldü. Grup 1 hastalara Grup 2 hastalarından daha fazla kan transfüzyonu yapıldı ve hastanede kalış süreleri uzatıldı.

Anahtar kelimeler: Cerrahi bypass greftleme, endovasküler stentleme, arter tıkanıklığı, vasküler açıklık.

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Introduction

Peripheral arterial disease (PAD) is a chronic, lifestyle-limiting disease that results from arterial pathologies developing in the lower, upper extremities, intra-abdominal, carotid, and intracerebral branches. The prevalence of PAD, which usually develops based on atherosclerosis, increases with age. The prevalence increases up to 29% after 75, and just about 6% in the population aged 65-70. The risk of PAD increases in post-menopausal women due to the decreased protective estrogen hormone in elder females [1].

Most patients with PAD have no obvious symptoms. However, the prevalence of asymptomatic peripheral arterial disease is between 3-10%, and this rate rises to 15-20% at the age of 70 and above (35). In asymptomatic PAD's, death is 4%, and the risk of developing claudication in the future is 7-15% [2, 3].

Treatment options are conservative, medical, and revascularization. Revascularization options are surgical, endovascular, or hybrid procedures that apply both methods [4]. With the developing technology in recent years, the choice of endovascular treatment is rapidly increasing. Mortality and morbidity rates are lower than surgery, and many centers prefer endovascular intervention in the first place and resort to surgical treatment in case of failure. Anatomically, the size and location of the lesion, comorbidities, and experience effectively select endovascular or surgical treatment.

According to the stent types, the most commonly used stents are self-expandable nitinol stents and balloon-expandable stainless steel stents. Some studies have shown that self-expandable stents work better in the long run, but this theory is still unclear [5]. In isolated short and proximal lesions, stenting should be preferred in the first place. The length of the stenosis in the iliac artery should be less than 5 cm. The procedure's success rate has been reported as 90%, with patency rates of 80% for one year and 60% for five years. However, especially in the infrapopliteal region, endovascular treatment's success and patency rates are low. Therefore, it is not recommended except for particular indications [5].

In iliofemoral lesions, endovascular treatment to the femoral artery and surgical treatment to the iliac artery can be applied simultaneously [6]. Aorto-biiliac or aorto-bifemoral bypass surgery is usually performed for aortoiliac lesions. The surgical strategy may vary depending on the localization of the lesion, technical possibilities, and the patient's condition. Aortofemoral or aortoiliac bypass is more successful than other bypasses and has lower complication rates. Ten-year primary patency rates for aortobifemoral bypass range from 80% to 90% [7].

In the guideline published by the ESC in 2017, it was reported with the TASC II Class I indicated that the primary preference was endovascular treatment for isolated occlusive lesions at the aortoiliac level below 5 cm, surgical treatment should be considered for longer occlusive lesions [8]. Hybrid Procedures Treatments in which surgical treatment is used together with endovascular techniques are hybrid treatment. Hybrid procedures provide patients with multivessel disease the opportunity for simultaneous endovascular intervention and surgical reconstruction. Historically, hybrid therapy for the vascular disease was first reported in 1973 as a combination of iliac angioplasty and femoro-femoral bypass [9].

In this study, we aimed to study and analyze our center's results in managing iliofemoral and above knee femoropopliteal arterial occlusion. But, deciding which surgical technique is better was always the question. So, we collected the data of all the patients treated for such vascular pathology, and we included only the patients with whom we could connect.

Matherials and methods

This is a retrospective study, where 60 patients had undergone endovascular and surgical interventions due to the occlusion of the iliofemoral and above-knee femoropopliteal arteries between January 2015 and December 2020. In addition, mortality and morbidity were re-evaluated by calling the patients by phone. Ethics Committee Approval was taken, and all the patients we connected with signed the consent form.

Inclusion criteria for the patients included; Patients who previously underwent endovascular or surgical intervention or both of them for occlusion or stenosis of the iliac or above-knee femoropopliteal arteries. Only the patients who had ischemic pain, ischemic

ulcers, risk of losing extremity and whose life quality was affected negatively by this disease underwent operation (TASCII C or D).

Thirty-seven patients who could not cooperate or did not want or could not come to the control were not included in the study. A total of 60 patients were examined in two groups; Group 1: Patients who underwent surgical grafting (n=33), and group 2; Patients who underwent endovascular treatment with stent implantation (n=27). The decision on the type of treatment depended on several factors. The patient's general condition and age, the type of the lesion, the extension of the primary lesion, the kidney function tests of the patient, and the surgeon's preference with the result of the doctor-patient discussion were the main factors brought into attention while deciding on treatment method.

All patients' surgical notes pre-and post-procedure records were reviewed. In addition, the type of graft used in the surgery, the presence of stent/balloon, revision status, the level of the lesion, and the degree of obstruction were evaluated.

Follow-up period, mortality/morbidity, and patency of the vessel undergoing the vascular procedure were recorded as a result of the noninvasive evaluation (doppler, contrast-enhanced CT angiography, or contrast-enhanced MRI) and invasive (angiography) examinations.

Smoking and alcohol history, diabetes Mellitus (DM), hypertension (HT), hyperlipidemia (HL), history of coronary artery disease (CAD) and pre-op hemoglobin (HGB), pre-op White blood cell (WBC), pre-and post-op creatinine (CR) were evaluated too. In addition, medication, follow-up period, and length of hospital stay were recorded and analyzed.

Surgical Technique: Open surgical grafting (Group 1): After painting and draping, the femoral region is opened longitudinally, and gentle dissection is done to reach common femoral, superficial, and deep femoral arteries, which are wholly freed and rounded by vessel loops. A lower quadrant incision is made in an oblique shape of about 12-15 cm. Attention is taken not to enter the peritoneum, and extraperitoneal exposure to the iliac artery is achieved with the aid of retractors.

Before heparinization, graft tunneling is achieved by gentle finger dissection from the retroperitoneum to the femoral artery. The graft is passed through the tunnel under the inguinal ligament. Then heparin is given at the dose of 1mg/kg to keep activating clotting time (ACT) up to 250-350 seconds. First, proximal anastomosis (end-to-side) is achieved usually by using 4/0 polypropylene sutures. Then the distal anastomosis (end-to-side) is done by 5/0 polypropylene after de-airing the graft. Finally, hemostasis control is done, and a hemovac drain is inserted. Then, the incision is closed in the traditional fashion.

Endovascular balloon and stenting technique (Group 2): All our endovascular procedures are performed in the angiography department. After painting and draping, arteriography is performed via an ipsilateral or contralateral femoral approach. A 7F or 8F sheath is placed, and an angiogram is achieved using a contrast medium. After giving heparin at the dose of 1mg/kg to achieve ACT up to 250-350 seconds, a balloon-expandable stent is applied preferentially for focal lesions according to the lesion site, length, and nature. A control angiogram is taken before ending the procedure.

The anticoagulation protocol for patients in Group 1 and 2: In group 1: We gave low molecular Heparin anticoagulant ± antiplatelets (acetylsalicylic acid (ASA)) according to the patient's comorbidities and blood tests (mainly CBC). Pre-discharge, we shifted low molecular Heparin to oral anticoagulants (such as rivaroxaban, dabigatran, or apixaban)± ASA for two months at least. Then according to the patient's risk factors, we continued on those drugs or kept only ASA.

In group 2: Immediately after the procedure, we started clopidogrel ± ASA for at least six months. Then, according to the patient's risk factors and comorbidities, we continue those drugs or just ASA.

Statistical analysis

IBM SPSS Statistics 25 Windows Multilingual Assembly Editions program was used for analysis. Continuous variables are given as mean ± standard deviation and categorical variables as numbers and percentages. One-way analysis of variance compared independent group differences when

parametric test assumptions are met; When parametric test assumptions were not met, Kruskal Wallis Analysis of Variance was used to compare independent group differences. Repeated Measures Analysis of Variance was used for independent group comparisons when parametric test assumptions are provided; Friedman test was used when parametric test assumptions were not met. The differences between the categorical variables were analyzed by Chi-square analysis. In addition, the relationships between continuous variables were analyzed with Spearman or Pearson correlation analyzes.

This study was approved by Pamukkale University Clinical Research Ethics Committee.

Results

The mean age of the patients was 62.85 in the patient group who underwent a surgical procedure (Group 1) and 61.63 years in the patient group who underwent stenting with the endovascular procedure (Group 2). There was no statistically significant difference between the groups regarding age difference (p=0.663). When the genders of the patients were examined, 28 males and five females were in Group 1, while there were 24 men and three women in Group 2. Men were the majority in both groups, but statistically, there was no significant difference (p=0.108). On admission, 17 patients (51.5%) presented with acute status in Group 2, and 16 patients (48.5%) had chronic disease history. In Group 2, 15 (55.6%) were acute, while 12 (44.4%) were chronic. Statistically, there was no significant difference (p=0.480). When the operation site was examined, the femoral artery (72.7%) was in the majority in Group 1, while the iliac artery (66.7%) was in Group 2. In Group 1, 7 (100%) of 7 patients had iliac bypass grafts, and 17 (70%) of 24 patients whose femoral localization was involved and grafted were found to be open. In Group 2, the grafts were open in 6 (33%) of 18 patients with iliac artery stents and 1 (33%) of the three patients whose femoral localization was involved and stented.

While hypertension (HT) was nine patients in Group 2 (33.3%), it was 22 patients (68.8%) in Group 1, and there was a statistically significant difference (p=0.007). However, no statistically significant difference was found between the groups in terms of Diabetes Mellitus (DM), hyperlipidemia (HL), coronary artery disease (CAD), COPD, smoking history, and alcohol history (p<0.050) (Table 1).

Imaging methods and treatment procedures are given in Table 2. Accordingly, while the patients who underwent stent were three patients (9.1%) in group 1, they were 27 (100%) in group 2, a statistically significant difference was found between the groups (p=0.000).

There were three (9.1%) patients in group 1 and 14 (51.9%) patients in group 2 who had balloon administration, with a statistically significant difference between groups (p=0.000). When the vascular patency of the patients was examined, the grafts were occluded in 10 (45.5%) patients in group 1, while Stents were occluded in 12 patients (54.5%) in Group 2. There was no statistically significant difference between the groups (p=0.194).

Surgery was performed on all patients (100%) in group 1, and there were ten patients (37%) in group 2 who underwent surgery after angiography. A statistically significant difference was found between the groups (p=0.000).

The number of patients who underwent revision surgery was seven (21.2%) in Group 1 and ten patients (37%) in Group 2. There was no statistically significant difference between the groups (p=0.143).

CT angiography was used as the imaging method in 17 (53.1%) patients in Group 1, Doppler was used in 14 (43.8%), and MRI in 1 (3.1%), while CT angiography was used in 21 (84%) patients, Doppler was used in 3 (12%) and MRI in 1 (4%) patients in Group 2. A statistically significant difference was found between the groups regarding imaging methods (p=0.034) (Table 2).

According to the graft types which were used in Group 1, a saphenous vein graft was applied to 6 (18.2%) patients, Dacron tube grafts were applied to 18 (54.5%), and PTFE ring grafts were applied to 9 (27.3%) patients. Saphenous vein graft was applied to 2 (20%) patients, Dacron tube grafts were applied to 5 (50%), and PTFE ring grafts were applied to 3 (30%) patients in Group 2, where endovascular intervention was not enough or stents found occluded. There was no statistically significant difference between the two groups (p>0.050) (Table 2).

Table 1. Demographic Data of the Patient Groups, co-morbidities with involvement arteries and post-procedure patency rate

		Group 1 (n=33)		Group 2 (n=27)	
		Mean±S.S.	Med (min-max)	Mean±S.S	Med (min-max)	***p***
Age		62.85±11.51	65 (34-83)	61.63±9.63	63 (47-75)	0.663
Gender	**Female**	5 (15.2 %)		3 (11.1%)		0.474
	Male	28 (84.8%)		24 (88.9%)		
Co-Morbidities	**DM**	16 (50%)		11 (40.7%)		0.327
	HT	22 (68.8%)		9 (33.3%)		0.007
	HL	9 (28.1%)		6 (22.2%)		0.415
	CAD	12 (37.5%)		7 (25.9%)		0.253
	COPD	5 (15.6%)		3 (11.1%)		0.455
	Smoking	20 (62.5%)		19 (70.4%)		0.360
	Alcohol	5 (15.6%)		3 (11.1%)		0.455
Presentation status	**Acute**	17 (51.5%)		15 (55.6%)		0.480
	Chronic	16 (48.5%)		12 (44.4%)		
Location of obstruction		**Group 1 (n=33) %**		**Group 2 (n=27) %**		
Iliac		7 (21.2%)		18 (66.7%)		
Femoral		24 (72.7%)		3 (11.1%)		
Popliteal		0 (0%)		2 (7.4%)		
Femoral+popliteal		0 (0%)		1 (3.7%)		
Iliac+femoral		2 (6.1%)		2 (7.4%)		
Iliac+femoral+popliteal		0 (0%)		1 (3.7%)		
Patency						
	location	**grafting**		**stenting**		
	Iliac	7 (100%)		6 (33%)		
	Femoral	17 (70%)		1 (33%)		
	Popliteal	0		0		
	Femoral+popliteal	0		0		
	Iliac+femoral	2 (100%)		2 (100%)		
	Iliac+femoral+popliteal	0		0		

DM: Diabetes mellitus, HT: Hypertension, HL: Hyperlipidemia, CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease

Table 2. Types of surgical procedures, imaging methods, and graft types performed on patients in Group I and Group II

		Group 1 (n=33) n (%)	Group 2 (n=27) n (%)	*p*
Types of procedure				
Stent	No	30 (90.9%)	0 (0%)	0.000
	Yes	3 (9.1%)	27 (100%)	
Balloon	No	30 (90.9%)	13 (48.1%)	0.000
	Yes	3 (9.1%)	14 (51.9%)	
Patency	No	10 (45.5%)	12 (54.5%)	0.194
	Yes	23 (60.5%)	15 (39.5%)	
Surgery	No	0 (0%)	17 (63%)	0.000
	Yes	33 (100%)	10 (37%)	
Revision	No	26 (78.8%)	17 (63%)	0.143
	Yes	7 (21.2%)	10 (37%)	
Imaging Method	CT-Angiogram	17 (53.1%)	21 (84%)	0.034
	Doppler USG	14 (43.8%)	3 (12%)	
	MRI	1 (3.1%)	1 (4%)	
Types of Grafts for group 1 and for the Revision of group 2				
Saphenous vein graft		6 (18.2%)	2 (20%)	0.935
Dacron tube graft		18 (54.5%)	5 (50%)	
PTFE ring graft		9 (27.3%)	3 (30%)	

All the patients who underwent revision were evaluated; in Group 1, four patients underwent graft embolectomy, one patient had hematoma evacuation, one had abdominal exploration, one had an endovascular intervention, and one had been revised for bleeding control. In Group 2, ten patients were referred to open surgery, and revision surgery was performed with graft bypass (Table 3).

In Group 1, four (12.1%) patients had extremities amputation. In contrast, amputation was performed in Group 2 in one (3.7%) patient. There was no statistically significant difference between the two groups (p=0.246).

Blood transfusion was performed in 15 (46.9%) patients in Group 1 and six (23.1%) in Group 2. No difference was found between the two groups (p>0.050). In Group 1, two patients (6.1%) died, whereas 31 (93.9%) survived. In Group 2, six (22.2%) patients died, and 21 (77.8%) survived. No difference was found between the two groups regarding mortality (p>0.050). Mortality causes varied from untreated CAD in one patient and untreated severe mitral insufficiency and heart failure in the other patient in Group 1, to one CVA, one multiple organ failure, two heart failure, and two pulmonary diseases in Group 2 (Table 3). The hospitalization and follow-up periods were calculated for all the patients. the average hospital stay was 11.33 days in Group 1 and 6.15 days in Group 2. There was no statistically significant difference (p>0.050). Follow-up time in months was calculated too and found to be 30.77 in Group 1 and 20.87 months in Group 2. There was no significant difference, too (p=0.186). The patients' mean preoperative creatinine (Cr) was 1.76 in Group 1, and 1.18 in Group 2. The postoperative creatinine

was 1.89 on average in group 1 and 1.59 on average in group 2. There was no statistically significant difference between the two groups (p=0.064) (p=0.534), respectively. Also, there were no significant differences between groups regarding preop HGB and preop WBC. When the postoperative complications were examined (100-200 meters) claudication was observed in 10 patients in Group 1. In addition, one patient had a foot wound, one patient was immobile, and one patient had a chronic renal failure (CRF) accompanying claudication. There were no complications in the other 20 patients who underwent surgery.

Comparison of vessel patency with radiological and post-procedural findings is given in (Table 4). There was no statistically significant difference between vascular patency and imaging method, presence of revision, and mortality rates (p>0.050). Vascular patency was found in 6 patients with saphenous vein grafts, while it was occluded in two patients. In Dacron grafts, the graft was occluded in five patients compared to 18 patients with normal vascular patency. Four grafts were patent in PTFE ring grafts, while the number of occluded grafts was 8. A statistically significant difference was found between graft patency and graft type (p=0.024) (Table 4).

Table 3. Types of revision surgery and postoperative blood transfusion, mortality and amputation rates

Types of revision surgery	**Group 1 (n=7)**	**Group 2 (n=10)**	
Graft bypass	0	10	
Graft embolectomy	4	0	
Hematoma evacuation	1	0	
Abdominal exploration for bleeding control	1	0	
Endovascular intervention	1	0	
Postoperative blood transfusion, mortality and amputation rates			
	Group 1 (n=33) %	**Group 2 (n=27) %**	***p***
Blood transfusion	15 (46.9%)	6 (23.1%)	
Exitus	3 (13.6%)	5 (13.2%)	0.073
Amputation	4 (12.1%)	1 (3.7%)	

Table 4. Comparison of vessel patency with radiological and post-procedural findings

		Occluded (n=22) %	**Patent (n=38) %**	***p***
Imaging Method	CT angiography	16 (72.7%)	24 (63.2%)	
	USG Doppler	5 (22.7%)	13 (34.2%)	0.620
	MRI	1 (4.5%)	1 (2.6%)	
Graft Type	Saphenous vein graft	2 (25%)	6 (75%)	
	Dacron tube graft	5 (21.73%)	18 (78.26%)	0.024
	PTFE ring graft	8 (66.6%)	4 (33.3%)	
Revision	No	14 (63.6%)	29 (76.3%)	0.224
	Yes	8 (36.4%)	9 (23,7%)	
Mortality	Exitus	3 (13.6%)	5 (13.2%)	0.623
	Survivor	19 (86.4%)	33 (86.8%)	

Discussion

Peripheral vascular disease is associated with significant morbidity and mortality. In terms of vascular patency, the graft patency rate was higher in the surgical group than in the endovascular group. The other advantages and disadvantages of each treatment method for iliofemoral and above-knee femoropopliteal arterial occlusion had been studied and analyzed in this study.

There is a dramatic increase in the prevalence of PAD with advanced age [10, 11]. In a similar study conducted in 2005, 244 patients were examined; The mean age of the patients who underwent endovascular intervention has been reported as 67 and 66 in those who had bypass surgery. In the same study, men were found to be the majority in endovascular treatment with 62.6% and surgery with 75.2% [11].

In our study, men were higher than the rates in the literature; It was the majority with 24 patients (88.9%) who underwent endovascular surgery and 28 patients (84.8%) who underwent surgery. Habits like smoking, irregular diet, and doing little exercise are common in men's lifestyle in our country, which is possible for this higher rate.

Patients who had presented with acute symptoms and underwent immediate surgery were about 51.5%, while 48.5% had chronic symptoms. Endovascular interventions were performed in 55.6% who had acute admission and 44.4% of patients with chronic symptoms.

Hypertension (HT) is a significant risk factor for peripheral arterial diseases. In a study done in 2014, HT was found relatively high in patients who had undergone endovascular intervention (77%) and surgery in the lower extremities (77.82%), respectively [12]. In another study with the same criteria, HT was reported in the range of 75-92% [13]. In our study, there were nine patients (33.3%) diagnosed with HT in the endovascular intervention group and 22 patients (68.8%) in the surgery group. Compared to the literature, the rate of HT in peripheral arterial patients was lower in our study. Although there was a statistically significant difference between the groups in our study, we did not consider the HT risk factor as a determining factor when choosing surgery or endovascular intervention in clinical application.

Hyperlipidemia (HL) is considered a significant risk factor in cardiovascular diseases. Malas et al. [12] reported the rate of HL as 82% in patients treated with angiography and 74% in patients treated with surgical treatment. According to our study, there were nine patients (28.1%) in the surgical group, and six patients (22.2%) in endovascular interventions who had hyperlipidemia; this rate was lower than in the literature, we did not accept it as a determining factor among the patients who underwent endovascular/surgical treatment since there was no significant difference between the groups.

In our study, the most frequently involved location in peripheral artery patients who underwent surgery was the femoral artery (72.7%), followed by the iliac artery. However, in our patients who underwent endovascular treatment, the most common site of involvement was the iliac artery, with a rate of 66.7%. Catheter-based procedures in patients undergoing endovascular intervention with stenting have been used more widely in recent years than the surgical approach in the lower extremities. An important indicator is the length and grade of the lesion detected during the clinical presentation of patients who are planned to be treated with stent or balloon angioplasty [14].

In a study done in 2009, the 24-month patency rate in stented patients was 75% and 81% in bypass grafting; thus, results in favor of surgery have been reported [15]. However, in another study conducted in 2010, it was reported that there was no significant difference in patency rates between stenting and bypass surgery [16].

Malas et al. [12] reported that 88% of patients who underwent angioplasty were stented. After a 24-month, the patency rate of patients with stent was reported as 67%, while it was 75% in patients with surgical bypass grafting. Our study observed that three (9.1%) of 33 patients who underwent surgery had a history of stenting. In the control imaging of two of these patients, intrastent vascular patency was not preserved. Stents were placed in 27 patients (100%) who underwent endovascular intervention. We found that stent patency was preserved in fifteen (39.5%) of these patients. Our stent patency rate in patients who underwent endovascular intervention was slightly lower than in the

literature. This low patency rate may be due to the irregularity of patients in the use of blood thinner drugs. On the other hand, the patency rate (60.5%) in our patients who underwent surgery was in line with the literature and was superior to endovascular intervention.

In addition, when we evaluated the localization and graft patency in Group 1, we found that seven of seven patients (100%), who had iliac localization, had patent iliac grafts, and 17 of 24 patients (70%) with femoral localization had femoral patent grafts. In Group 2, the grafts were patent in 6 (33%) of 18 patients with iliac stents and 1 (33%) of the three patients with femoral stents. As a result, it was observed that grafting was superior to the stent in all localizations.

In a study of patients who underwent surgery with endovascular intervention, the grafts' patency rates gave better results after two years of follow-up. However, the failure rate of angioplasty was 25% higher. In addition, the revision rate was found to be significantly higher than in surgery [16].

The most crucial complaint in endovascular interventions and surgery is claudication. Surowiec et al. [10] reported 32% of patients with moderate to mild limping and 32% of patients with severe fatigue and limping. In the same study, the rate of patients with 200 meters claudication was reported as 49% in patients who underwent dacron-PTFE grafting and 32% in patients who underwent saphenous vein grafting. The rate of claudication above 100 meters in patients who underwent stenting was 66%. Karch et al. [17] reported 60% complications in stented angiography. The majority (75%) of complications included fatigue and severe pain when walking. When the patients in our study were examined, five patients in Group 1 experienced claudication at 100 meters, two patients at 150 meters. In Group 2, three patients experienced claudication above 200 meters. In addition, foot wounds in Group 1 and in Group 2 chronic renal failure and immobilization were seen in one patient.

Short-term postoperative complications in lower extremity surgery may include acute renal failure, arterial injury, acute MI, arrhythmia, and congestive heart failure [16, 18]. Also, post-surgical dissection, bleeding, occlusion, infection need to be revised. In revision, various operations such as embolectomy, hematoma evacuation, bleeding control, bypass, and endovascular intervention (atherectomy, drug-coated balloon angioplasty, stenting) may be required [18].

Krankenberg et al. [11], in their studies with stent versus surgery in the femoral-popliteal location, reported revisions for reasons such as dissection, hematoma evacuation, thromboembolic, aneurysm, and arteriovenous fistula. In addition, Karch et al. [17] observed hematoma in five patients in the study group whose 4-year long-term results were examined.

Our study achieved re-revascularization by embolectomy in four of seven patients who underwent surgical revision, abdominal exploration for bleeding control in one and endovascular intervention was performed in another patient. The revision numbers in our study were similar to the literature.

Digital Subtraction Angiography (DSA) was the primary way of evaluating peripheral arterial disease and is considered the gold standard compared to other methods. However, (DSA) may be insufficient to visualize the distal vascular bed in patients with proximal lesions. In contrast, Doppler USG imaging allows both functional and anatomical evaluation. Therefore, this non-invasive imaging method can be considered one of the best preoperative radiological methods, especially in patients with contrast material allergy or renal failure [19].

A study done by Katsamouris et al. [20] detected 12 open tibial arteries with Doppler in 10 extremities that angiography could not demonstrate. Doppler examination of the above-knee popliteal artery gave a significantly correct diagnosis in all four extremities, but arteriography gave false results.

Catalano et al. [21] compared the infrarenal aorta and lower extremity arteries with CT Angiography and DSA in 50 patients with peripheral artery disease. They found the sensitivity 96%, specificity 93%, and accuracy 94%. With the development of multidetector CT technology, CT Angiography has replaced DSA in most cases. In the last ten years, many surgeons have preferred CT Angiography to DSA, which is less invasive and cheaper than DSA. It allows three-dimensional imaging and

contains less radiation than DSA too. While CT Angiography provides anatomical evaluation, Doppler US provides additional functional evaluation. On the other hand, CT Angiography does not reflect the hemodynamic results of the lesion and may not be sufficient alone for diagnosis because only the vascular lumen is shown. Therefore, the correlation of CT Angiography with Doppler USG is considered the most accurate approach [22].

In our study, 17 patients were operated on with endovascular intervention, and 21 patients with surgery underwent control imaging with CT angiography. Thrombosis was seen in 16 patients, and vascular patency was normal in 24 patients. However, one of the two patients checked with MRI had occlusion. Creatinine level may affect the choice of imaging methods. In patients with high creatinine levels, Doppler USG is preferred. However, since it is impossible to evaluate clearly with Doppler in some localizations, MRI is preferred in the second plan, and CT angiography is preferred as the last resort. Here, 17 patients were visualized with Doppler, and thrombosed veins were seen in 5 patients. Since different imaging methods were not applied to the same patients, we correctly accepted the Doppler imaging results. A comparison of imaging methods was not made, and it was used as a tool to evaluate graft patency. If the patient's creatinine level was appropriate (creatinine <1.5), it was seen that the most preferred imaging method was CT Angiography. The rate of using CT angiography is higher in patients undergoing endovascular intervention than in surgical patients. Contrast-induced nephropathy is not a concern, as the creatinine levels of patients likely to be treated with endovascular intervention are low.

Autogenous saphenous vein graft is preferred for bypass grafting for its' long-term patency rates in lower extremity ischemia since the patient has autogenous tissue [23]. In addition, dacron tube graft and PTFE ring grafts have become widespread in recent years.

In our study, 12 patients with PTFE grafts had thrombosis (66.6%), while two patients from eight patients with saphenous vein grafts (25%) and 23 patients with dacron grafts had thrombosis at a rate of 21.26%; thus, the type of the graft with the highest patency rate was the dacron graft. However, saphenous vein grafting requires long incisions in the lower extremity. In a study conducted for the femoral-popliteal region in which autogenous saphenous vein graft was used, the 5-year primary patency rate was reported as 70-80% [24]. Our results show parallelism with the literature.

The literature shows that the average length of hospital stay is five days after endovascular intervention and 11 days after surgery. After 30 days, the need for revision is 6% [18]. In our study, the mean hospital stay time for patients who underwent surgery was 11.33 days and the mean follow-up period was 30 months. The mean hospital stay time was 6.15 days, and the mean follow-up time was 20.9 months in patients who underwent endovascular intervention. Our study's length of hospital stay in both endovascular intervention and surgery was consistent with the literature.

The main limitation of this study: We anticipate that comparing our short to mid-term results with the long-term results will yield more accurate results as it becomes possible to renew it with more patients. Since angioplasty is frequently preferred in the iliac region, data on patients undergoing surgery are limited. In future studies, we need to focus on the results of surgical treatment for lesions in the iliac region with a more extensive population.

As a result, in terms of vascular patency, the graft patency rate was 60.5% in patients who underwent surgical treatment, while the stent patency rate was 39.5% in patients who underwent endovascular treatment. Therefore, the success rate of surgical treatment is higher. On the other hand, re-revascularization rates of surgical treatment are lower than endovascular treatment. The graft type with the highest patency rate was seen in the dacron grafts. The saphenous vein is the second most common, while the PTFE ring graft is the third. Patients undergoing surgical treatment received more blood transfusions than patients receiving endovascular treatment. In addition, the hospitalization and follow-up period of the patients in Group 1 is more extended than the Group 2 patients. Mortality rates were higher in Group 2 patients than the patients in Group 1. Considering patient comfort and early mobilization, we suggest that angiography

should be considered the primary choice in the lower extremity, especially in the iliac region, which is challenging to operate in surgery.

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

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Patients' Inform Consent: Inform consent had been written and signed by the patients themselves or their relatives.

Ethics committee approval: This study was approved by Pamukkale University Clinical Research Ethics Committee (date: 28.04.2020 and number: 60116787-020/28611).

Authors' contributions

M.B. designed the study, data collection, literature search, manuscript writing, and final approval of the version to be published. M.A. and M.C.C. drafted the manuscript, designed with the co-writer, and verified the analytical methods and final approval of the version to be published.

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Pamukkale Tıp Dergisi

ERRATUM / DÜZELTME

The article titled with “An early stage Takayasu arteritis case diagnosed with FDG PET-CT and review of the literature” published in the **Pamukkale Medical Journal 2020;13(2):425-429**;

Correction 1: Its authors are included in the article as “Nagehan Didem Sarı, Gülşen Yörük, Gülhan Eren”. On the “Contents” page of our journal, “Nagihan Yalçın” was inadvertently added to the author names of the article and the correct names of the authors are as follows:

CORRECTION 1

“Nagehan Didem Sarı, Gülşen Yörük, Gülhan Eren”

Correction 2: In the imprint information, the ORCID number of the second author Gülşen Yörük is written incompletely as ‘orcid.org/000-0002-0357-5884’ and the correct one is as follows:

CORRECTION 2

orcid.org/0000-0002-0357-5884

Pamukkale Tıp Dergisi 2020;13(2):425-429 sayısında yayınlanmış olan “Tanısı FDG PET-BT ile konan erken evre Takayasu arteriti olgusu ve literatürün gözden geçirilmesi” başlıklı makalenin;

Düzeltme 1: Yazarları “Nagehan Didem Sarı, Gülşen Yörük, Gülhan Eren” olarak makalede yer almaktadır. Dergimizin “İçindekiler” sayfasında makalenin yazar isimlerine sehven “Nagihan Yalçın” eklenmiş olup yazar isimlerinin doğrusu aşağıdaki gibidir.

DÜZELTME 1

“Nagehan Didem Sarı, Gülşen Yörük, Gülhan Eren”

Düzeltme 2: Künye bilgilerinde ikinci yazar Gülşen Yörük’ün orcid numarası ‘orcid.org/000-0002-0357-5884’ şeklinde eksik yazılmış olup doğrusu aşağıdaki gibidir.

DÜZELTME 2

orcid.org/0000-0002-0357-5884