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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

An Examination of the Flora and pH Changes in the External Auditory Canal in Patients with Seborrheic Dermatitis

Seboreik Dermatitli Hastalarda Dış Kulak Yolu Florası ve pH Değişiminin İncelenmesi

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ABSTRACT

Aim: *To investigate flora and pH changes in the external auditory canal (EAC) in patients with seborrheic dermatitis (SD).*

Material and Method: *Twenty-five patients with SD presenting to the Ataturk University Faculty of Medicine Ear, Nose and Throat and Dermatology Departments and 20 healthy controls were included in this prospective study. All individuals' EAC floras were collected using culture swabs, and EAC pH was measured using a surface pH meter.*

Results: *Coagulase-negative staphylococci and diphtheroid bacilli, representing normal flora elements, grew in 15 patients in the SD group and 10 in the healthy control group. pH values in the SD patient group ranged between 4.17 and 4.89, with a mean value of 4.47±0.2. pH values among the healthy controls ranged between 4.33 and 4.94, with a mean value of 4.63±0.2.*

Conclusion: *SD did not affect EAC flora or EAC pH values*

Key words: *external auditory canal flora; external auditory canal pH; seborrheic dermatitis*

ÖZET

Amaç: *Bu çalışmada seboreik dermatit (SD)'li hastalarda dış kulak yolu (DKY) florası ve pH değişiminin araştırılması amaçlanmıştır.*

Materyal ve Metot: *Prospektif olarak planlanan çalışmaya Atatürk Üniversitesi Tıp Fakültesi Kulak Burun Boğaz ve Dermatoloji Anabilim Dalı polikliniğine başvuran 25 SD'li hasta ve 20 sağlıklı kontrol grubu dâhil edildi. Tüm bireylerin DKY florası kültür çubukları yardımıyla ve DKY pH'ı ise yüzey pH metre ile ölçülerek değerlendirildi.*

Bulgular: *SD'li hasta grubundaki 15 hastada ve sağlıklı kontrol grubundaki 10 hastada koagülaz negatif stafilokoklar ve difteroid basiller yani normal flora elemanları üredi. SD'li hasta grubunun pH değerleri 4,17 ve 4,89 arasında değişmekte olup pH değerleri ortalaması 4,47±0,2 olarak bulundu. Sağlıklı kontrol grubundaki bireylerin pH değerleri 4,33 ve 4,94 arasında değişmekte olup pH değerleri ortalaması 4,63±0,2 olarak bulundu.*

Sonuç: *Bu çalışmaya göre SD hastalığının DKY florası ve pH değeri üzerine etkisinin olmadığı tespit edilmiştir.*

Anahtar kelimeler: *dış kulak yolu florası; dış kulak yolu pH'ı; seboreik dermatit*

Introduction

Seborrheic dermatitis (SD) is a chronic inflammatory skin disease characterized by erythematous plaques or patches in areas containing sebaceous glands. The mild form of the disease is seen in 15–20% of the population, and it is more common in males. It may be associated with genetic and environmental factors[1]. The entity mostly affects parts of the body rich in sebaceous glands, such as hairy skin, the face, and the upper trunk[2]. The lesions are distributed symmetrically and typically worsen in winter. There is no specific treatment, the main aim being the control of symptoms[1].

Although the etiology is uncertain, various factors can give rise to SD, such as androgens, fatigue, stress, depression, microangiopathic vascular diseases, atmospheric changes such as pressure variation, seasonal factors, clothing, diet and food allergies, autoimmunity, climacteric changes, Pityrosporum ovale (Malassezia furfur), medications, and riboflavin, pyridoxine, and biotin deficiencies[2–4].

The cerumen consists of glandular secretions and epithelial wastes in the cartilaginous part of the external auditory canal (EAC). It is hydrophobic and slightly acidic and thus prevents bacterial growth[5,6]. The

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normal flora in the EAC and cerumen is gram (+), frequently consisting of Staphylococcus aureus and Staphylococcus epidermidis. Diphtheroids, streptococci, and enterococci are less common. Pseudomonas aeruginosa and fungal agents are rare in the normal EAC[5].

Seborrheic dermatitis has been reported to be capable of affecting the EAC[7]. However, to the best of our knowledge, the effect of SD on the EAC flora and pH values has not previously been investigated. This study aimed to explore the EAC flora and whether any changes in EAC pH levels occur in patients with SD.

Material and Method

Approval for this prospective study was granted by the Ataturk University Faculty of Medicine Clinical Research Ethical Committee (decision no. 1 dated 02.09.2013). Twenty-five patients (16 female and nine male) presenting to the Ataturk University Faculty of Medicine Ear, Nose and Throat and Dermatology Departments and diagnosed with SD and 20 healthy controls (10 female and 10 male) were included in the study. Written informed consent was obtained from all patients who participated in this study.

Individuals who had not used antibiotics for at least one month and had not experienced upper respiratory tract infection, with no chronic disease such as diabetes mellitus (DM), and without chronic or acute otitis or external otitis were enrolled. Patients with any chronic skin infection other than SD were excluded.

Cultures were taken using culture swabs from the patient, and control groups were sent to the microbiology laboratory. Culture collection was based on the swab remaining in contact with the patient's EAC for at least 10 sec. Specimens sent to the microbiology laboratory were immediately inoculated onto blood broth and eosin methylene-blue (EMB) agar media. Daily growth of specimens inoculated onto growth was checked for a minimum of three and a maximum of five days. External auditory canal pH was measured in all individuals with a surface pH meter with an appropriate probe. After measurement, the probe was placed in a neutral solution supplied with the pH meter to avoid false results.

Statistical Analysis

Data analysis was performed on IBM Statistical Package for Social Sciences program version 20.0 (IBM Corporation, New York, NY, USA). Data were expressed as number, percentage, and mean plus standard deviation. The Kolmogorov-Smirnov test was applied to determine whether the data for variables included in the analysis were normally distributed. The Mann-Whitney U test was used to compare age and pH values between the patient and control groups and the sexes, while the chi-square test was used to determine growth status between the patient and control groups. The growth status between the sexes between the patient and control groups was analyzed using Fisher's exact test. p values <0.05 were regarded as significant for all analyses.

Results

Ages in the patient group ranged from 18 to 57, with a mean age of 37.6±13.6. Ages in the healthy control group ranged from 18 to 54, with a mean age of 30.2±11.4. No statistically significant difference was observed in terms of age between the patient and control groups (p=0.1; Mann-Whitney U value=179).

No growth occurred in 10 (40%) patients in the SD patient group and in 10 (50%) healthy control group members. Coagulase-negative staphylococci and diphtheroid bacilli, representing normal flora elements, grew in 15 patients in the SD group and 10 (50%) healthy control group members (Table 1). The difference between the two groups was not significant (p=0.5; chi-square value=0.45).

Patient group pH values ranged between 4.17 and 4.89, with a mean value of 4.47±0.2. pH values in the healthy control group ranged between 4.33 and 4.94, with a mean pH of 4.63±0.2. The difference between the two groups was statistically significant (p=0.01; Mann-Whitney U value=145) (Table 2).

Table 1. *Isolated microorganisms*

	Patients	Control group	p value	Chi-square value
Microbiological evaluation (isolated microorganisms)	Coagulase (-) staphylococci Dipheroid bacilli	Coagulase (-) staphylococci Dipheroid bacilli	0.5	0.45

Table 2. *pH value of patients and control group*

	Patients	Control group	p value	Mann-Whitney U value
pH	4.17-4.89 mean 4.47 ± 0.2	4.33-4.94 mean 4.63±0.2	0.01	145

Table 3. *pH value of the patient and control group with growth*

	Reproductive ones			
	Patients	Control group	p value	Mann-Whitney U value
pH	4.17-4.89 mean 4.43±0.2	4.33- 4.74 mean 4.51±0.1	0.14	49
Total	25 patients			

pH values in the patient group with normal floral bacteria growth in culture ranged between 4.17 and 4.89, with a mean value of 4.43±0.2. The pH values of the healthy control group with normal flora bacteria ranged between 4.33 and 4.74, with a mean value of 4.51±0.1. The difference between the two groups was not statistically significant (p=0.14; Mann-Whitney U value=49) (Table 3).

Discussion

Since SD is a frequently seen chronic skin disease that may be refractory to treatment and that has a damaging psychosocial impact on patients, it has been the subject of considerable research. The EAC can also be affected in diseases with widespread skin involvement, such as SD, allergic dermatitis, atopic dermatitis, and psoriasis[8]. Seborrheic dermatitis frequently causes pruritus when it affects the EAC, and pruritic ears were present in approximately 90% of the patients in the present study.

Malassezia species are part of the normal skin flora in humans. These species can cause pityriasis versicolor, Malassezia folliculitis, SD, and atopic dermatitis[9,10]. No growth of Malassezia species was observed in the present study.

The EAC is normally slightly acidic, with a pH value of approximately 4–6. Micro-organisms are, therefore not easily able to colonize this region. However, infection may occur under conditions such as sterile equipment not being employed during ear cleaning or pH increasing due to frequent contact with water. Dampness prepares the ground for infection by numerous micro-organisms, particularly fungi[11,12]. External auditory canal pH was acidic in both the patient and control groups in the present study. External auditory canal pH values were also acidic among individuals with normal floral bacteria growing in EAC culture in both groups. On the basis of these findings, it may be concluded that SD has no effect on EAC pH.

The two micro-organisms most frequently isolated from the EAC are the normal flora elements coagulase-negative staphylococci and diphtheroid bacilli (Corynebacteria spp.)[11,13]. Coagulase-negative staphylococci and diphtheroid bacilli (Corynebacteria spp.) also grew in patients with SD and members of the healthy control group in the present study. P. aeruginosa is a micro-organism with high mortality and morbidity that spreads rapidly and causes malignant (invasive) otitis externa, particularly in individuals with immune suppression, such as malignancy, in DM patients, and the elderly[14,15]. P. aeruginosa was not isolated in the present study since cases from this patient group were not included.

Stroman et al.[5] reported gram-positive growth at a rate of 92% in EAC and cerumen cultures from normal healthy individuals, gram-negative growth at 1%, fungus growth at 7%, and gram-positive growth at 93% in EAC isolates, gram-negative micro-organism growth at a rate of 4.5%, and fungal growth at 2.5%. The most commonly detected strain in both groups was coagulase-negative S. auricularis, and detailed examination of both cerumen and EAC isolates revealed that similar micro-organisms exhibited very close growth rates[5]. The present study also detected predominant coagulase-negative staphylococci and diphtheroid growth in SD patients and the healthy control group.

In addition to structural, environmental, and traumatic factors leading to inflammatory changes, infectious

(bacterial, fungal, and viral) and reactive (eczema, SD, neurodermatitis, etc.) factors are also involved in otitis externa[16]. This common disease affects 10% of the population, particularly in summer. The most commonly isolated bacterial agents are P. aeruginosa and S. aureus, while the most frequent fungal agents are Aspergillus and Candida albicans[17,18]. Patients with chronic or acute otitis externa were excluded from the present study.

The principal limitation of the present study is the low number of patients. Further extensive studies investigating the effect of SD on the pH and microbiology of the EAC are now needed.

Conclusion

Seborrheic dermatitis is a chronic skin disease whose etiology is not yet fully understood. External auditory canal involvement is common in patients diagnosed with SD, and these patients present to ENT and dermatology clinics due to pruritus. Seborrheic dermatitis was found to cause no change in EAC microbiology or EAC pH in the present study.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Has the Pneumothorax's Course Changed in COVID-19?

COVİD-19'da Pnömotoraksın Seyri Değişti mi?

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ABSTRACT

Aim: *During severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, pneumothorax is a frequent consequence. This study investigated the treatment methods and clinical course of 27 patients with a pneumothorax who were followed up in the intensive care unit for 3 months.*

Material and Method: *The inclusion criteria included patients with Coronavirus disease 2019 (COVID-19) who were admitted to our hospital's intensive care unit and underwent a tube thoracostomy because of the development of a pneumothorax while receiving invasive mechanical ventilation therapy. Over three months, these patients were evaluated based on age, sex, tube thoracostomy duration, and mechanical ventilation parameters.*

Results: *Among the 27 patients, 17 (62.9%) were men and 10 (37.1%) were women. The mean age of the patients was 68 (39–92) years. Chest tubes were removed in 4 of 27 patients within a week.*

Conclusion: *Tube removal takes longer than that in patients with non-COVID-19-related pneumothorax. Care should be taken to ensure full expansion to prevent pneumothorax recurrence after tube thoracostomy.*

Keywords: *SARS-CoV-2; pneumothorax; thoracostomy*

ÖZET

Amaç: *SARS-CoV-2 enfeksiyonu nedeniyle pnömotoraks tipik bir komplikasyon olarak ortaya çıkar. Bu çalışmada, yoğun bakımda üç ay izlenen 27 pnömotorakslı hastanın tedavi yöntemleri ve klinik seyri araştırıldı.*

Materyal ve Metot: *Dâhil edilme kriterleri, hastanemizin yoğun bakım ünitesine kabul edilen ve invaziv mekanik ventilasyon tedavisi alırken pnömotoraks gelişmesi nedeniyle tüp torakostomi uygulanan COVID-19 hastalarını içeriyordu. Üç aylık bir süre boyunca bu hastalar yaşlarına, cinsiyetlerine, tüp torakostomi sürelerine ve mekanik ventilasyon parametrelerine göre değerlendirildi.*

Bulgular: *27 hastanın 17'si (%62,9) erkek, 10'u (%37,1) kadındı. Hastaların ortalama yaşı 68 (39–92) yıldı. Tüp torakostomi uygulanan 27 hastanın sadece dördü bir hafta içinde göğüs tüpünü çıkarabildi.*

Sonuç: *Tüpün çıkarılması, COVID-19 olmayan pnömotoraks hastalarında olduğundan daha uzun sürer. Tüp torakostomi sonrası pnömotoraksın tekrarını önlemek için tam genişlemenin sağlanmasına özen gösterilmelidir*

Anahtar kelimeler: *COVID-19, pnömotoraks, torakostom*

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a viral agent that can cause a variety of clinical manifestations, from cold-like flu to acute respiratory failure syndrome (ARDS). Coronavirus disease 2019 (COVID-19) has a wide range of systemic effects in humans; however, it is mostly responsible for lower respiratory tract diseases. Various clinical manifestations of secondary bacterial infections include cardiac arrhythmia, cardiomyopathy, acute renal injury, and liver dysfunction[1].

Pneumomediastinum, pneumopericardium, pleural effusion, subcutaneous emphysema, and pneumothorax complications are among the thoracic abnormalities associated with COVID-19 in patients monitored in intensive care units[1,2].

Because SARS-CoV-2 infection is frequent in the lung parenchyma and causes damage up to fibrosis, pneumothorax can occur as a typical complication. The interaction of the fibrotic parenchyma and long-term high-pressure breathing can cause pneumothorax.

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This study investigated the treatment methods and clinical course of 27 patients with pneumothorax who were followed up in the intensive care unit for 3 months.

Materials and Methods

Ethical approval for this study was obtained from a relevant institute (E1–20–612). Patients with SARS-CoV-2 who were admitted to the intensive care unit and monitored while receiving invasive mechanical ventilation therapy between 1 April 2020 and 30 June 2020 were examined clinically and radiologically retrospectively. Various complications were noted. During these three months, 39 patients from the intensive care unit presented to our hospital for complications such as pneumothorax, pneumomediastinum, pneumopericardium, and subcutaneous emphysema. The patients' mechanical ventilator parameters and the levels of lung parenchymal involvement on radiological imaging were recorded. All patients underwent thorax tomography. The inclusion criteria included patients with SARS-CoV-2 who were admitted to our hospital's intensive care unit and underwent a tube thoracostomy because of the development of pneumothorax while receiving invasive mechanical ventilation therapy. Over three months, these patients were evaluated based on age, sex, tube thoracostomy duration, and mechanical ventilation parameters. Patients were divided into two groups according to tube thoracostomy durations (<1 week and ≥ one week). Descriptive analyses were presented using the means for the fraction of inspired oxygen rate (FiO2) and positive end-expiratory pressure (PEEP) values.

Results

During these three months, 27 of the 39 patients who presented to our hospital from the intensive care unit had pneumothorax, 10 had pleural effusion, and 2 had hemothorax. Polymerase Chain Reaction (PCR) testing established that all patients had SARS-CoV-2 infection.

No patient had a negative test but a positive CT (computed tomography) result.

Among the 27 patients who underwent tube thoracostomy because of pneumothorax (Figure 1) and were monitored while receiving invasive mechanical ventilation therapy, 17 (62.9%) were men and 10 (37.1%) were women. The mean age of the patients was 68 (39–92) years.

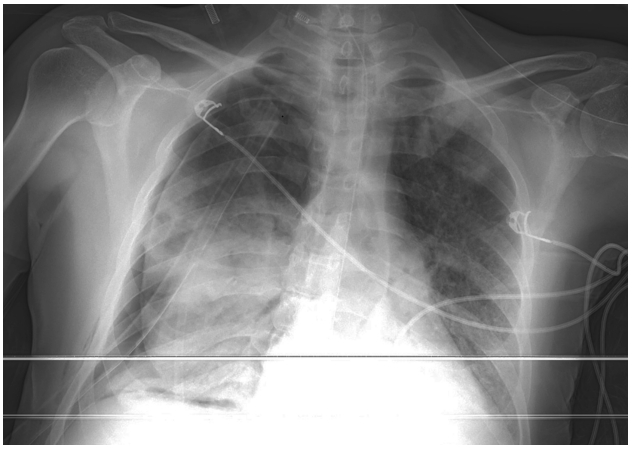

Figure 1. *Patient who underwent tube thoracostomy for pneumothorax*

All patients showed lung involvement and parenchymal infiltrative regions on thorax tomography. At least one lobe of the patients was impacted, and the majority had bilateral parenchymal infiltration.

Of the patients with pneumothorax, 17 (62.9%) had right hemithorax, 8 (29.7%) had left hemithorax, and 2 (7.4%) had bilateral pneumothorax. Of the 27 pneumothorax patients, 25 (92%) had pneumomediastinum, 5 (18.5%) had a mediastinal shift, and 7 (25.9%) had pleural effusion.

Although 4 (14.8%) patients had their chest tube removed within one week, 12 (44.4%) had their tube removed after >1 week. During these three months, the chest tube could not be removed in 11 (40.8%) of the 27 patients, and recurrent pneumothorax developed in 4 (14.8%) within 24 hours of the tube removal. In patients who experienced recurrent pneumothorax, a tube thoracostomy was performed again. The duration after both tube thoracostomies until the tube removal was >1 week in two patients with bilateral tube thoracostomies. The average tube thoracostomy duration of 16 patients with chest tubes removed over three months was 38.4 (2–90) days.

The chest tube could not be removed because, unfortunately, 4 of the 11 patients who had their tube removed died, and the remaining 7 had an expansion defect and severe air leakage. Intermittent negative aspiration was first applied from a chest bottle for unexpanded lungs. Devices with automatic continuous aspiration were used in resistant expansion defects.

In our study, the three-month mortality rate was 14.8%. The cause of death of the patients was determined to be COVID-19.

Table 1. *Results and patient characteristics*

Features		Number of patients/Rate
Sex	Men	17
	Women	10
Hemithorax	Right	17
	Left	8
	Bilateral	2
Concomitant Pathology	Pneumomediastinum	25
	Mediastinal shift	5
	Pleural effusion	7
Chest tube duration	< 1 week	4
	≥ 1 week	12
	Not terminated within 3 months	11
Chest tube duration <1 week	The FIO_2 value required to provide 80% or more oxygen saturation	≤75%
	the required PEEP value	≤8 cm H_2O
Recurrent pneumothorax	4	
3-month mortality	14.8%	

Based on the radiological examination results of the patients and the mechanical ventilation parameters, we compared the duration of chest tube removal in patients who were monitored while receiving invasive mechanical ventilation therapy in the intensive care unit. For patients whose chest tube was removed in <1 week, the highest FiO2 rate necessary to achieve a saturation of ≥80% was ≤75, whereas it was higher in the other group. The PEEP was ≤8 cmH_2O in patients whose tube was removed within one week, whereas it was higher in the other group.

Although both groups had bilateral parenchymal infiltrative involvement, the entire group with tube thoracostomy had bilateral diffuse homogenous infiltration for over a week. The patient characteristics and outcomes are summarized in Table 1.

Discussion

With the progression of the COVID-19 pandemic, clinicians' understanding of uncommon symptoms, radiographic findings, ARDS, clinical stages, and associated complications has improved. Recent studies have focused on disease-related pneumothorax, pleural effusion, and pneumomediastinum. In patients monitored in the intensive care unit, pneumomediastinum, pneumopericardium, pleural effusion, subcutaneous emphysema, and pneumothorax have been identified as complications associated with COVID-19[3–5].

Pneumothorax and pneumomediastinum can appear even without barotrauma associated with mechanical ventilation in patients with SARS-CoV-2 pneumonia[2,6,7]. Pneumothorax is a known complication of intubated patients receiving mechanical ventilation[8,9]. COVID-19-related pneumothorax can be detected during hospitalization,[3] in the delayed period after the SARS-CoV-2 treatment,[4] and at the time of hospitalization[5].

Spontaneous pneumothorax is often detected in younger patients compared with secondary pneumothorax. Sahn et al. reported that primary and secondary spontaneous pneumothoraxes were more common in males than females[10]. They also indicated that the mean age of patients with secondary spontaneous pneumothorax was higher than that of those with primary spontaneous pneumothorax[10]. As in non-COVID-19 secondary pneumothorax cases, the proportion of male patients was higher than that of female patients in cases of pneumothorax due to COVID-19[11,12].

In this study, pneumothorax was 1.7 times more common in male patients than female patients, which is consistent with the literature. Although it is similar to the male-to-female ratio found in non-COVID-19 spontaneous pneumothorax, a statistical comparison requires more patients.

The mean age of patients with pneumothorax who underwent tube thoracostomy as a result of COVID-19 was 68 (39–92) years. COVID-19 increases the demand for intensive care in patients who are older and have more comorbidities. This circumstance is known to apply to all secondary spontaneous pneumothorax patients[7].

In total, 248 hospitalized patients with a diagnosis of COVID-19 were evaluated by Wang et al.[12], and 49 of these patients were diagnosed with ARDS. According to the study, 21 patients with ARDS were started on mechanical ventilation, and 9 received invasive mechanical ventilation therapy. According to Wang et al., pneumothorax was observed in 2.01% of patients overall, 10% in patients with ARDS, 24% in patients receiving mechanical ventilation support, and 56% in patients receiving invasive mechanical ventilation therapy[12]. In several other studies, the rate of COVID-19-related pneumothorax was reported to be 0.66%–0.97% in all patients[13,14].

Pneumothorax is the most common manifestation of alveolar macroscopic damage due to mechanical

ventilation in a hospital[15]. Pneumothorax is a common complication in mechanically ventilated patients, with rates ranging from 4% to 15%[16,17]. Gammon et al. reported a 14% pneumothorax incidence rate in 139 patients receiving mechanical ventilation therapy compared with a 60% pneumothorax incidence in 29 patients with ARDS[18]. Another study reported a 30%–87% incidence rate of pneumothorax, depending on the severity and duration of ARDS as well as the mode of ventilator management[19]. COVID-19 has been associated with barotrauma in up to 40% and pneumothorax in up to 25% of patients receiving invasive mechanical ventilation therapy in the intensive care unit[20].

In pneumothorax, persistent air leakage is defined as air leakage that lasts for >7 days after tube thoracostomy. Only 4 of the 27 patients who underwent a tube thoracostomy were able to have the chest tube removed within one week. Prolonged tube thoracostomy duration was observed in 23 (85.1%) patients.

Surgical treatment is preferred in patients with non-COVID-19 pneumothorax with persistent air leakage[13]. Previous studies have recommended thoracoscopic surgery for COVID-19-associated persistent air leakage patients[13,21]. Surgical treatment is impossible in our patients because of their general state in the follow-up. Depending on whether bullae are identified on a thorax tomography scan, some studies recommend evaluating patients with primary or secondary pneumothorax and persistent air leakage for emergency surgery[22]. In patients with persistent air leakage, chemical pleurodesis with an intrapleural sclerosing agent has a low success rate. Some studies have reported performing chemical pleurodesis through tube thoracostomy in patients with COVID-19-associated pneumothorax[13]. Because our patients had substantial expansion defects, we could not predict the effect in patients with ARDS, and some patients were candidates for lung transplantation. We did not opt for chemical pleurodesis in our study. A suction apparatus was used to create an intermittent vacuum in these patients. However, the patient's overall status was monitored, and surgical intervention was planned.

Although 75% and 61% of primary and secondary spontaneous pneumothorax air leakages, respectively, resolve one week after the tube thoracostomy, approximately 100% and 79% of the cases are settled after 15 days[10]. In our study, 14.8% of patients who had pneumothorax due to COVID-19 and mechanical ventilation had lung expansion within one week of tube thoracostomy, and 44.4% achieved lung expansion after one week and the chest tube was removed. In 40.8% of patients, the tube could not be removed within three months.

Barotrauma due to mechanical ventilation has been reported to be more common in patients with COVID-19[23]. In the early stages of ARDS due to COVID-19, low tidal volume (6–8 ml/kg), increased respiratory rate (35/min), PEEP >5 cmH_2O, and PaO2 55–80 (SaO2 88%–95%) are all classic lung-protective mechanical ventilation parameters. These parameters were used to maintain appropriate, suitable FiO2 levels. Because patients with COVID-19 did not respond to a high PEEP, the pathophysiology of ARDS was considered different, and some studies supported this hypothesis[24,25]. This explains the increased incidence of barotrauma in patients receiving mechanical ventilation therapy. In our study, patients ventilated at a high PEEP (>8 cmH_2O) had longer tube thoracostomy durations and massive air leakages. This condition might have resulted from the significant barotrauma that the patients experienced due to mechanical ventilation.

Parenchymal expansion defects, diffuse consolidation, and infiltration areas were prominent on the chest radiographs of the patients with prolonged tube thoracostomy. There was massive air leakage from the tube thoracostomies. The clinical parameters were poorer in patients with bilateral tube thoracostomy, and the tube thoracostomy duration was more prolonged.

An increased risk of death has been associated with advanced age (>65 years), male sex, hypertension, cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, and cancer. The survival rate of patients with pneumothorax receiving invasive mechanical ventilation therapy has been estimated to be approximately 50%0,[11] In our study, the three-month mortality rate was 14.8%. The fact that parenchymal infiltration areas are bilateral and widespread, as well as the need for high FiO_2 (≥75%) and high PEEP (≥8 cmH_2O) to achieve a saturation of ≥80%, all contribute to the poor prognosis of the patients and the longer duration of tube thoracostomies.

There are some limitations of this study. First, the data retrospectively reflect the data of a limited time in a single center. The study includes only adult patients. Although pneumothorax might occur during SARS-CoV-2 infection, patients may have parenchymal lung illness, which can cause pneumothorax. Furthermore, patients need long-term follow-up data.

Conclusions

In patients with pneumothorax who underwent tube thoracostomy and were monitored in the intensive care unit for COVID-19, conditions such as bilateral, diffuse, and late-healing lung parenchyma involvement, as well as mechanical ventilation support, prolong the duration of tube thoracostomy. Physicians show interest in exploring the impact of disease-induced widespread fibrosis on the onset and progression of pneumothorax.

Tube thoracostomy treats patients with parenchymal involvement and pneumothorax due to COVID-19. In these patients, however, tube removal takes longer than in patients with non-COVID-19 pneumothorax. Care should be taken to ensure complete expansion to prevent pneumothorax recurrence after tube thoracostomy.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Change in Pneumococcal and Influenza Vaccine Awareness in Diabetic Patients in the Last 2 Years

Diyabetik Hastalarda Son İki Yılda Değişen Pnömokok ve İnfluenza Aşı Farkındalığı

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ABSTRACT

Aim: *Our study aimed to evaluate the influenza and pneumococcal vaccination rates in patients diagnosed with Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) and the awareness of these patients about vaccination after the coronavirus disease 2019 (COVID-19) pandemic.*

Material and Method: *Between April and May 2022, a questionnaire was applied to diabetic patients who met the criteria and applied to the internal medicine outpatient clinic at the University of Health Sciences, Kartal Dr. Lütfi Kirdar City Hospital, questioning the status of influenza and pneumococcal vaccination, and their awareness of vaccination before and after the COVID-19 pandemic; and the results were evaluated.*

Results: *A total of 195 cases, 43.6% female and 56.4% male, with a mean age of 58.53±10.73 years, were included in the study. 92.8% of the cases were T2DM; and the mean duration of diabetes mellitus was 15.11±7.64 years. 48.2% of the cases had at least one type of vaccine (83% influenza, 47.9% pneumococcal vaccine) before the pandemic. It was observed that 51.8% of the cases were not vaccinated with influenza and pneumococcal vaccines. Among the reasons why these patients were not vaccinated, the most common reason was that the doctor did not give information about vaccination (44.6%). 70.3% of the cases who had not been vaccinated before decided to have both vaccinations after the COVID-19 pandemic.*

Conclusion: *In our study, it was determined that the pneumococcal and influenza vaccination rates of diabetic patients were low before the COVID-19 pandemic, and the most important factor causing this was the inadequacy of the physician to inform the patients. Patient age, diagnosis of T2DM, duration of diabetes mellitus, and presence of additional disease were positively associated with vaccination. According to the data, the COVID-19 pandemic has positively affected the approach to vaccination of diabetic patients who have not been vaccinated before.*

Keywords: *awareness; diabetes mellitus; influenza vaccine; pneumococcal vaccine*

ÖZET

Amaç: *Çalışmamızda Tip 1 Diabetes Mellitus (T1DM) ve Tip 2 Diabetes Mellitus (T2DM) hastalarında influenza ve pnömokok aşılanma durumu ve COVID-19 pandemisi sonrasında bu hastaların aşı konusundaki farkındalıklarının değerlendirilmesi amaçlanmıştır.*

Materyal ve Metot: *Nisan-Mayıs 2022 tarihleri arasında Sağlık Bilimleri Üniversitesi Kartal Dr. Lütfi Kırdar Şehir Hastanesi Diyabet Polikliniği'ne başvuran diyabetik hastalara yönelik influenza ve pnömokok aşıları hakkındaki bilgi düzeyleri ile COVID-19 pandemisi öncesi ve sonrası aşılanma durumlarını sorgulayan anket uygulanmış olup sonuçları değerlendirilmiştir.*

Bulgular: *Çalışmaya yaş ortalaması 58,53±10,73 yıl olan, %43,6'sı kadın, %56,4'ü erkek toplam 195 olgu alındı. Olguların %7,2'sinin T1DM ve %92,8'inin T2DM olduğu, diyabet sürelerinin ise ortalama 15,11±7,64 yıl olduğu görüldü. Olguların %48,2'sinin pandemi öncesi en az bir tip aşı (%83 influenza veya %47,9 pnömokok aşısı) yaptırdığı, ancak %51,8'sinin ise influenza ve pnömokok aşılarını yaptırmadığı saptandı. Aşı olmama nedenleri arasında ilk sırada doktorun aşıları tavsiye etmemesi (%44,6) olduğu görüldü. Daha önce aşı yaptırmayan olguların %70,3'ünün COVID-19 pandemisi sonrası her iki aşıyı da yaptırmaya karar verdiği çalışmamızda saptandı.*

Sonuç: *COVID-19 pandemisi öncesinde diyabetik hastaların pnömokok ve infuenza aşılarını yeterli oranda yaptırmadıkları ve buna neden olan en önemli faktörün hekim bilgilendirmesinin yetersizliği olduğu saptandı. Hasta yaşı, T2DM tanısı, diyabet süresi ve ek hastalık varlığı aşı yaptırma ile pozitif yönde ilişkili bulundu. Covid 19 daha önce aşı yaptırmayan diyabetik hastaların aşı konusundaki yaklaşımını olumlu yönde etkilemiştir.*

Anahtar kelimeler: *diabetes mellitus; farkındalık; influenza aşısı; pnömokok aşısı*

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Introduction

Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) have become important health problems all over the world in recent years, and their incidence is increasing rapidly due to the change in lifestyle[1]. Diabetes Mellitus (DM) is one of the important causes of morbidity and mortality; prolonged hyperglycemia macrovascular and microvascular complications cause degradation in leukocyte functions in the long term in diabetic patients. Along with immune dysregulation, sensitivity to infections occurs, and pneumococcal pneumonia, which is an acquired phagocytic system defect, is observed more frequently in diabetic patients than in the general population[2–5]. The progression of DM with hypoglycemia-hyperglycemia attacks and failure to reach glycemic targets constitute the basis of the development of pneumonia. Pneumonia is one of the important causes of hospitalization and mortality in diabetic patients[6,7]. Vaccines are biological agents that protect against preventable infections by modulating the immune system. Vaccination in diseases with a high risk of infection, such as DM, is one of the most effective and safe preventive health services that reduce morbidity and mortality against infectious diseases. Turkish Infectious Diseases and Clinical Microbiology Specialization Association[8], American Diabetes Association[9], Diabetes Canada Clinical Practice Guidelines[10], Royal Australian College of General Practitioners[11], Turkish Endocrinology and Metabolism Association[12], Turkish Diabetes Foundation[13], Centers for Disease Control and Prevention[14], which are national and international associations; recommend that diabetic patients be vaccinated against influenza and pneumococcus (Fig. 1)[6,9–14].

The World Health Organization (WHO) defined vaccine hesitancy as a behavior influenced by many factors, including complacency (do not perceive a need for a vaccine, do not value the vaccine) and issues of confidence in the vaccine or provider[15]. Vaccine-hesitant individuals are a group who hold various degrees of indecision about vaccines or vaccination in general. Vaccine-hesitant individuals may accept all vaccines but remain concerned about vaccines; some individuals may not accept all vaccines, and some may refuse some vaccines but accept others[15].

In December 2019, a cluster of patients presented with pneumonia caused by an unknown pathogen in China, and the disease caused by the virus was named coronavirus disease 2019 by the WHO[15]. COVID-19 presents a range of clinical manifestations, from mild flu-like symptoms to life-threatening clinical conditions[16]. The spread of the COVID-19 epidemic to many countries and the increase in the number of deaths due to the disease have affected people deeply. The availability of vaccines is important to minimize new infectious diseases, so it is crucial to vaccinate people.

Our study aims to determine the frequency of pneumococcal and influenza vaccination in the diabetic population in Kartal/Istanbul, to find out why non-vaccinated diabetic patients are not vaccinated, and to evaluate the impact of the Covid-19 pandemic on the views of these patients about vaccination.

Material and Method

Diabetes Mellitus patients admitted to Kartal Dr. Lütfi Kirdar City Hospital Department of Internal Medicine between April and May 2022 were included in this cross-sectional study. One hundred ninety-five patients over 18 years of age who gave informed consent for data collection and had a diagnosis of T1DM and T2DM for at least one year were included in the study. Patients under 18 years of age, patients with secondary diabetes mellitus, pregnant women, active malignancy, and severe neurological and psychiatric disorders were excluded from the study.

Figure 1. *Vaccine recommendations in national and international guidelines*

Guide Name	Recommended Vaccines
American Diabetes Association, Guidelines for Standards of Medical Care in Diabetes	Influenza, Pneumococcus, Hepatitis B
Canada, Guide to Clinical Practice in Diabetes Management and Prevention	Influenza, Pneumococcus, Hepatitis B, Herpes Zoster
Australia, General Practice Guidelines for the Management of Type 2 Diabetes	Influenza, Pneumococcus, Diphtheria-Tetanus-Pertussis (DTP)
TEMD (Turkish Society of Endocrinology and Metabolism), Diagnosis and Treatment Guidelines for Diabetes Mellitus and Its Complications	Influenza, Pneumococcus, Hepatitis B
TÜRKDİAB (Turkish Diabetes Foundation), Diabetes Diagnosis and Treatment Guidelines	Influenza, Pneumococcus, Hepatitis B
EKMUD (Turkish Infectious Diseases and Clinical Microbiology Specialization Association), Adult Immunization Guidelines	Influenza, Pneumococcus, Hepatitis B, Herpes Zoster
ACIP (Advisory Committee on Immunization Practices)	Influenza, Pneumococcus, Hepatitis B, Herpes zoster, Diphtheria-Tetanus-Pertussis (DTP)

A questionnaire was applied using the interview method, which questioned the demographic characteristics of the patients as well as their comorbidities, influenza, and pneumococcal vaccination status in the previous years. If the patients were against vaccination, the reason for this was discussed. In addition, it was questioned whether there was a change in patients' awareness of influenza and pneumococcal vaccines after the COVID-19 pandemic.

For statistical analysis, the Shapiro-Wilk test, Student's t-test, Mann-Whitney U test, Pearson chi-square test, and Fisher's exact test were used together with the NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program. The statistical significance level was accepted as a P value of less than 0.05.

The Ethics Committee of Dr. Lütfi Kirdar Kartal City Hospital approved the study (30 March 2022, no 2022/514/222/4). All the procedures conformed to the ethical standards of the Declaration of Helsinki.

Results

The study was conducted with a total of 195 cases, 43.6% (n=85) female and 56.4% (n=110) male, with a mean age of 59 years. Of the cases participating in the study, 92.8% were diagnosed with T2DM and 7.2% with T1DM, and the mean duration of DM was 15.11±7.64 years.

Regarding educational status, most cases were primary school graduates (49.8%), while the rate of university graduates was the lowest with 7.7%. There was at least one additional disease in 94.4% (n=184) of the cases included in the study. Hyperlipidemia (80.4%), hypertension (77.2%), and coronary artery disease (27.7%) constituted the majority of cases with comorbidities (Table 1).

48.2% (n=94) of the cases had been vaccinated with one or both influenza and pneumococcal vaccines before the COVID-19 pandemic. 83% (n=78) of the cases declared that they had been vaccinated with influenza; 47.9% (n=45) of the cases proclaimed that they had been vaccinated with the pneumococcal vaccine, and all of these cases stated that they would be vaccinated this year as well. When the unvaccinated group was examined, it was determined that 44.6% (n=45) of the cases were not recommended for vaccination by the doctor, 24.8% (n=25) did not believe that the vaccines were beneficial, 14.9% (n=15) were negligent, 11.9% (n=12) of them thought that the vaccines were harmful and 4% of them could not reach the vaccine. 70.3% (n=71) of the unvaccinated cases decided to get vaccinated after the COVID-19 pandemic (Table 2).

Table 1. *Distribution of sociodemographic characteristics*

Age (year)	Mean ± Standard Deviation (SD)	58.5±10.73
	Median (Min-Max)	59 (25–84)
Gender; n (%)	Woman	85 (43.6)
	Man	110 (56.4)
Education status; n (%)	Primary school and below	97 (49.8)
	Middle school	28 (14.4)
	High school	55 (28.2)
	University	15 (7.7)
		n (%)
Comorbidity	No	11 (5.6)
	Yes	184 (94.4)
	COPD	13 (7.1)
	CAD	51 (27.7)
	HT	142 (77.2)
	HL	148 (80.4)
	CKD	26 (14.1)
	CVD	5 (2.7)
	Rheumatological Disease	4 (2.2)
	Malignancy	7 (3.8)
	Hypothyroidism	13 (7.1)
	Other	17 (9.2)
Type of DM n (%)	Type 1	14 (7.2)
	Type 2	181 (92.8)
Duration of DM (year)	Mean ± SD	15.11±7.64
	Median (Min-Max)	15 (1–40)

COPD: Chronic Obstructive Pulmonary Disease; CAD: Coronary Artery Disease; HT: Hypertension; HL: Hyperlipidemia
CKD: Chronic Kidney Disease; CVD: Cerebrovascular Diseases; DM: Diabetes Mellitus

Table 2. *Situations of getting the recommended pneumococcal and influenza vaccine and barriers to vaccination*

		n (%)
Have you ever been vaccinated with one or both of the influenza and pneumococcal vaccines before the COVID-19 pandemic?	No	101 (51.8)
	Yes	94 (48.2)
Influenza (n=94)	No	16 (17.0)
	Yes	78 (83.0)
Pneumococcal (n=94)	No	49 (52.1)
	Yes	45 (47.9)
If your answer is no, why? (n=101)	Has harmful effects	12 (11.9)
	Not informed by any doctor	45 (44.6)
	Don't think it's helpful	25 (24.8)
	Lack of access to vaccine	4 (4.0)
	Negligence	15 (14.9)
Have you been vaccinated with influenza and pneumococcal vaccines post-pandemic? (n=101)	Yes	71 (70.3)
	No	30 (29.7)
If you were vaccinated with influenza and pneumococcal vaccines before the pandemic, will you be vaccinated this year?	Yes	94 (100.0)

Table 3. *Evaluation of sociodemographic characteristics by vaccination status*

		Have you been vaccinated?		p
		No (n=101)	Yes (n=94)	
Age (year)	Mean ± SD	55.23±10.92	62.07±9.36	[a]0.001**
	Median (Min-Max)	56 (26–84)	63 (25–81)	
Gender	Woman	50 (49,5)	35 (37,2)	[b]0.084
	Man	51 (50,5)	59 (62,8)	
Education status	Primary school and below	48 (47,5)	49 (52,1)	[b]0.869
	Middle school	14 (13,9)	14 (14,9)	
	High school	31 (30,7)	24 (25,5)	
	University	8 (7,9)	7 (7,4)	
Comorbidity	No	9 (8,9)	2 (2,1)	[b]0.040*
	Yes	92 (91,1)	92 (97,9)	
Duration of DM (year)	Mean ± SD	13.73±7.61	16.59±7.43	[d]0.008**
	Median (Min-Max)	14 (2–40)	16 (1–40)	
Type of DM	Type 1	11 (10,9)	3 (3,2)	[b]0.037*
	Type 2	90 (89,1)	91 (96,8)	

[a]Student t-Test [b]PearsonChi-Square Test [d]Mann-Whitney U Test
*p<0.05 **p<0.01

The vaccinated cases' age was statistically significantly higher than the unvaccinated cases (p=0.001; p<0,01). The distribution of gender and educational status does not show a statistically significant difference according to vaccination status (p>0,05). The vaccination rate of cases with comorbidity was statistically significantly higher than cases without comorbidity (p=0.040; p<0,05). The duration of diabetes in the vaccinated cases was statistically significantly higher than in the unvaccinated cases (p=0.008; p<0,01). When we look at the distribution of the cases, the vaccination rate of the cases with T2DM was found to be statistically significantly higher than the cases with T1DM (p=0.037; p<0,05) (Table 3).

Discussion

March 11, 2020, when the first case was seen in Türkiye, was also the day that Covid-19 was declared a "global pandemic" by the WHO. In addition, the first death from COVID-19 in Türkiye occurred on March 17, 2020. In the last two years, with the pandemic, various measures have been taken to prevent the spread of the virus in Türkiye, as in many countries in the world. One of them was the vaccination policy. Many studies have been conducted on vaccination in our country before the pandemic, and it has been observed that vaccination awareness is low throughout our country. Whether the COVID-19 pandemic, which affected the whole world and caused many deaths, raised awareness about vaccination among people was a matter of curiosity by scientists in Türkiye and worldwide. In this context, we wanted to contribute to the literature with our study to increase academic studies on vaccination awareness after the pandemic, especially in patients with chronic diseases such as DM. In this regard, we have completed the ethics committee approval phase for the scientific relevance of the research we planned to do during the preparation process of the survey on the subject. In April and May, when the number of applications to our diabetes mellitus outpatient clinic was high, we asked our survey questions to all our patients who met the criteria we determined after obtaining their written and verbal consent. No patient refused to participate in the survey during our study. According to our clinical experience, we evaluated that the data collected from our patients who applied to our clinic between April and May 2022 were safe and consistent in terms of the healthy results of our research.

Many studies show that vaccination rates for preventing adult infectious diseases remain well below the target[17]. Öncü et al.[18], in their study conducted in 2019, demonstrated that 12.53% (n=55) of 439 diabetic patients had pneumococcal vaccine and 24.6% (n=108) of them had influenza vaccine. Arslan et al.[5] showed that awareness of vaccination and vaccination rates are very low in our country compared to other countries. A study conducted in metropolitan cities in our country showed a pneumococcal vaccination rate of 0.1% in diabetic patients[17]. Another study in our country showed that only 0.9% of 2383 cases were vaccinated, and 10% of these patients were aware of the pneumococcal vaccine[20]. Our study, which included

195 patients, found that 23.07% (n=45) had pneumococcal vaccine, and 40% (n=78) had influenza vaccine. Before the pandemic, 51.2% of diabetic cases were not vaccinated; similar to the studies of Öncü et al.[18], 44.6% (n=45) of the unvaccinated group stated that their doctor did not provide information about the vaccine, and 70.3% of this group said that they would have at least one of the pneumococcal and influenza vaccines after the pandemic. Adult patients with comorbidities should be especially aware and willing to be vaccinated. One of the most common reasons for reluctance to vaccinate is the thought that vaccine protection is insufficient[21–23]. In our study, like other studies, of 101 diabetic patients who did not want to be vaccinated, 24.8% stated that they did not believe vaccines were beneficial, and 11.9% said vaccines were harmful. A study on influenza vaccines with healthcare workers observed that the vaccination rate of individuals in the community increased when health workers were informed about vaccination[19]. In addition, a study by Ünal et al., twenty-four showed that vaccination rates quadrupled within six months with a one-day training program given to family physicians. The diaVAX study published in 2013 showed that vaccination rates of 5682 diabetic patients increased from 27% to 63.3% for influenza vaccine and from 9.8% to 47.7% for pneumococcal vaccine after the training given to physicians[25].

The COVID-19 pandemic has been declared a public health emergency by the World Health Organization (WHO). It has greatly affected daily life worldwide, and COVID-19 vaccination is carried out in our country, as in other countries. With the development of COVID-19 vaccines, a more positive approach to other vaccines has been observed, especially in patients with severe comorbidities. In their study with 1425 cases in 2021, Kiskaç et al.[26] showed that the COVID-19 pandemic increased awareness about pneumococcal and influenza vaccines. Similarly, in our study, it was observed that the pneumococcal and influenza vaccination level was low in diabetic patients before the COVID-19 pandemic, and there was an increase in the awareness level and vaccination rate about these vaccines after the pandemic.

In community-acquired pneumonia, DM is closely associated with pleural effusion, empyema, and mortality, and the presence of DM is one of the poor prognoses[27]. A study investigating the effects of pneumonia and influenza on mortality rates in diabetic patients in the USA showed that in adults aged 24–65, regardless of age and socioeconomic status, the rate of DM in patients with pneumonia and flu is significantly higher[28]. The effectiveness of pneumonia and influenza vaccination programs causes a decrease in mortality in DM cases.

Host response to epidemic-causing agents such as Streptococcus Pneumonia and Seasonal Influenza Virus is lower in diabetic patients than non-diabetic patients. Thus, diabetic patients are more susceptible to these infectious agents than non-diabetic patients, and these patients have a more extended recovery period and a higher risk of hospitalization and mortality. Although we want to support our view with a cost-effectiveness study conducted specifically for the diabetic group between pneumonia-influenza infections and pneumonia-influenza vaccines, no cost-effectiveness study is explicitly conducted for pneumonia in diabetic cases.

Our study data showed an increase in awareness of vaccination in patients with severe comorbidities such as DM during the COVID-19 pandemic. There is increased social awareness about vaccination with the developed vaccine programs and continuous publications for COVID-19, regardless of the patients' educational status and the presence of coronavirus infections.

The most common answer given by diabetic patients in our survey's open-ended questions was that the patients were not vaccinated because the doctor did not recommend the current vaccination program. How important are oral antidiabetic and insulin treatments for DM patients? Implementing the vaccination schedule, our prophylactic approaches, and preventive medicine against preventable factors such as pneumonia-flu are just as important and cost-effective. If the importance of vaccination is emphasized to patients despite the intensity of the outpatient clinic, the rate of physician-induced non-vaccination will decrease. As for the reason for not being vaccinated, approximately one out of 4 patients answered that the vaccines were ineffective. This approach can be minimized with media and public service announcements.

Other common causes, such as 'negligence' and 'vaccinations are harmful,' can be resolved through doctor's information, media, and public service announcements if pneumococcal and influenza vaccines are not included in the essential vaccination program of the Ministry of Health. The pneumococcal vaccine was included in

the adult vaccination program in 2016 by the Ministry of Health of the Republic of Türkiye. It was included in the expanded program on the same date for DM.

Our study shows how important it is to motivate patients with comorbidities such as diabetes mellitus to get vaccinated in terms of public health and cost-effectiveness.

In conclusion, as shown by our study, the frequency of pneumococcal and influenza vaccination in diabetic patients is still not at the desired level in the Istanbul Kartal region. The most common and most important reason for the low success rate in the vaccination was the physicians' failure to inform the patients adequately. However, after the COVID-19 pandemic, there was a significant increase in the desire to be vaccinated in diabetic patients who did not want to be vaccinated before. In this study, it was determined that the most important factor in increasing the success of vaccination was that healthcare professionals informed patients more about vaccination.

Limitations of the study

Our study does not include data on physicians' vaccination awareness, which limits our study. Also, since the study was conducted in a tertiary health center in Istanbul, it may not reflect the country's vaccination rate. Patients who stated that they had been vaccinated in the past do not clearly remember how many doses of influenza vaccine were administered. Even though all those who had PCV-13 among those who had pneumococcal vaccine were registered in our unit, patient information about the PPV23 vaccine is unclear.

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Disclosure of potential conflicts of interest

There is no conflict of interest between the authors.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Evaluation of Cardiac Findings Before Laboratory-Based Polysomnography

Laboratuvar Temelli Polisomnografi Öncesi Kardiyak Bulguların Değerlendirilmesi

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ABSTRACT

Aim: *This study aimed to determine the demographic characteristics of the patients who applied to the Cardiology Out-patient Clinic before laboratory-based polysomnography was performed.*

Material and Method: *123 adult patients undergoing laboratory-based polysomnography were included in this retrospective study. The demographic features of the patients were recorded. Routine laboratory tests, electrocardiography (ECG), transthoracic echocardiography (TTE), 24-hour ambulatory blood pressure monitoring, cardiovascular stress test, and laboratory-based polysomnography were performed in the same hospital. The apnea-hypopnea index (AHI) was used to diagnose obstructive sleep apnea syndrome (OSAS).*

Results: *In this study, we found the following patient findings. The mean age of the patients was 49±1.61 years. The majority of the patients were in the middle age group. Forty-two (34.10%) patients were female and 81 (65.90%) were male. Normal AHI value was detected in 5 (4.2%) patients. Mild OSAS was detected in 30 (24.2%) of the patients, moderate OSAS in 24 (18.9%), and severe OSAS in 64 (51.6%). Continuous positive airway pressure (CPAP) device report was given to 67 (54.7%) of the patients. The most accompanying comorbidity of the patients was hypertension (HT). There was a weak positive correlation between AHI and HT; r=0.280, N=95, and the relationship was statistically significant (p=0.006). Nine (7.30%) of the patients were newly diagnosed with hypertension. Invasive coronary angiography was recommended for two (1.6%) patients with positive test results, and coronary computed tomographic angiography was recommended for four (3.2%) patients. Myocardial perfusion scintigraphy was recommended to nine (7.3%) patients who could not perform cardiovascular stress tests.*

Conclusion: *Cardiac evaluation should be performed before laboratory-based polysomnography is performed. Electrocardiography and TTE recording should be performed on the patients, and 24-hour ABPM should be inserted. Additional tests should be performed to investigate ischemia in patients with myocardial ischemia findings.*

Keywords: *obstructive sleep apnea syndrome; polysomnography; cardiology*

ÖZET

Amaç: *Bu çalışmanın amacı, laboratuvar temelli polisomnografi yapılmadan önce Kardiyoloji Polikliniğine başvuran hastaların demografik özelliklerini belirlemekti.*

Materyal ve Metot: *Bu retrospektif çalışmaya laboratuvar temelli polisomnografi yapılan 123 yetişkin hasta dâhil edildi. Hastaların demografik özellikleri kaydedildi. Rutin laboratuvar testleri, elektrokardiyografi (EKG), transtorasik ekokardiyografi (TTE), 24 saatlik ambulatuvar kan basıncı monitörizasyonu (AKBM), kardiyovasküler stres testi ve laboratuvar bazlı polisomnografi aynı hastanede yapıldı. Apne-hipopne endeksi (AHİ), obstrüktif uyku apne sendromunu (OUAS) teşhis etmek için kullanıldı.*

Bulgular: *Bu çalışmada hastalarda aşağıdaki bulguları bulduk. Hastaların yaş ortalaması 49±1,61 yıl idi. Hastaların 42'si (%34,1) kadın, 81'i (%65,9) erkekti. Hastaların beşinde (%4,2) normal AHİ saptandı. Hastaların 30'unda (%24,2) hafif OUAS, 24'ünde (%18,9) orta OUAS ve 64'ünde (%51,6) ağır OUAS saptandı. Hastaların 67'sine (%54,7) sürekli pozitif hava yolu basıncı (CPAP) cihazı raporu verildi. Hastalara en fazla eşlik eden komorbidite hipertansiyon idi. Apne-hipopne endeksi ile HT arasında zayıf pozitif korelasyon vardı; r=0,280, N=95, bu ilişki istatistiksel olarak anlamlıydı (p=0,006). Dokuz (%7,3) hastaya ambulatuvar kan basıncı takibi (AKBM) yapıldı ve yeni hipertansiyon tanısı konuldu. Test sonucu pozitif olan iki (%1,6) hastaya invaziv koroner anjiyografi, dört (%3,2) hastaya koroner bilgisayarlı tomografik anjiyografi önerildi. Kardiyovasküler stres testi yapamayan dokuz (%7,3) hastaya miyokardiyal perfüzyon sintigrafisi önerildi.*

Sonuç: *Laboratuvar tabanlı polisomnografi yapılmadan önce kardiyak değerlendirme yapılmalıdır. Hastalara EKG, TTE ve 24 saatlik AKBM yapılmalıdır. Miyokardiyal iskemi bulguları olan hastalarda iskemiyi araştırmak için ek testler yapılmalıdır.*

Anahtar kelimeler: *obstrüktif uyku apne sendromu; polisomnografi; kardiyoloji*

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Introduction

Obstructive sleep apnea syndrome (OSAS) is a common disorder, and it is a cardiovascular disease risk factor. Snoring, apnea, frequent awakening at night, and constant drowsiness during the day occur in patients with OSAS. Recurrent apneas and hypopneas during sleep are accompanied by hypoxia, increased sympathetic activity, and frequent awakenings. Some studies showed that OSAS is associated with an increased risk of cardiovascular morbidity and mortality. Obstructive sleep apnea syndrome has clearly been shown to be an independent risk factor for the development of hypertension and has also been implicated in the pathogenesis of pulmonary hypertension, congestive heart failure, cardiac arrhythmias, stroke, and atherosclerosis. Continuous positive airway pressure (CPAP) therapy was effective in treating OSAS[1]. Polysomnography is performed to diagnose OSAS. We have done laboratory-based polysomnography in our hospital. Before laboratory-based polysomnography is performed, patients are evaluated in Neurology, Otolaryngology, and Cardiology outpatient clinics. This study aimed to determine the demographic characteristics of patients who applied to the Cardiology Out-patient Clinic before laboratory-based polysomnography was performed.

Materials and Methods

Study design and setting

This study was an observational-retrospective study. One hundred and twenty-three adult patients undergoing laboratory-based polysomnography and cardiac evaluation in the Cardiology Out-patient Clinic before laboratory-based polysomnography were included in this study. Patients who applied to our clinic between October 15, 2021, and October 15, 2022, were included in the study. Past medical histories, examination notes, and laboratory results were obtained from our hospital's electronic records. Demographic features of the patient's age, gender, chronic diseases [diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HL), documented coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), stroke history, impaired fasting glucose, thyroid gland disease, anemia, polycythemia, splenomegaly, cancer ratio were recorded. We recorded the laboratory data from electronic medical records using a standardized data collection form. The patients admitted to the sleep unit had the following complaints: Snoring (100%), awakening with the feeling of suffocation (76.4%), witnessed apnea (66.6%), and excessive daytime sleepiness (66.6%). Laboratory findings, i. e., glucose (normal range: 74–106 mg/dL), hemoglobin (normal range: 12.6–17.4 g/dL), white blood cells (WBC) (normal range: 4.23–9.07 $10^3/\mu L$), platelet (normal range: 150–360 $10^3/\mu L$), C reactive protein (CRP) (normal range: 0–5 mg/L), creatinine (normal range: 0.67–1.17 mg/dL), alanine aminotransferase (ALT) (normal range: 0–50 U/L), thyroid stimulating hormone (normal range: 0.35–4.94 mIU/L), thyroxine (normal range: 0.70–1.48 ng/dL), low-density lipoprotein (normal range: 0–130 mg/dL), triglyceride (normal range: 0–150 mg/dL were recorded on admission, and they were evaluated as normal or abnormal according to the normal range of the laboratory assays. Anaemia was defined as a hemoglobin (Hb) level of <12.0 g/dL in women and <13.0 g/dL in men. If the plasma fasting glucose was 100–126 mg/dL, it was defined as impaired plasma fasting glucose.

After taking the anamnesis of the patients, electrocardiography (ECG) was taken, and transthoracic echocardiography (TTE) was performed for all patients. Cardiology outpatient clinic nurses performed ECG. Normal sinus rhythm and atrial fibrillation were recorded on the ECG of the patients. Transthoracic echocardiography was conducted by the cardiologists at the Cardiology Out-patient Clinic. Left ventricular ejection fraction (LVEF) was measured. Left ventricular ejection fraction below 50% was defined as systolic heart failure. A 24-hour ambulatory blood pressure monitoring (ABPM) was inserted in patients who did not have hypertension before but whose blood pressure increased occasionally. The cardiovascular stress test was performed on patients with chest pain, shortness of breath, and effort dyspnea. Invasive coronary angiography or coronary computed tomography angiography was recommended for patients who were found to have signs of ischemia as a result of the test. Myocardial perfusion scintigraphy was recommended for patients who could not perform cardiovascular stress testing.

Polysomnography was performed in our hospital's sleep laboratory. The apnea-hypopnea index (AHI) was used to diagnose OSAS. The AHI value is obtained by dividing the sum of the apnea and hypopnea numbers by the individual's sleep time. Hourly AHI values were used. Apnea-hypopnea index values were categorized as Normal: AHI <5, mild sleep apnea: 5 ≤ AHI <15,

moderate sleep apnea: 15 ≤ AHI <30, and severe sleep apnea: AHI ≥30.

Inclusion criteria

- Be over 18 years old
- Admission to the hospital with complaints of snoring, awakening with the feeling of suffocation, witnessed apnea and excessive daytime sleepiness
- Admitted to the Cardiology Out-patient Clinic for cardiac control

Exclusion criteria

- Be under the age of 18
- Patients whose laboratory results could not be reached

Data Analysis

IBM Statistical Package for Social Sciences (SPSS) software for Windows release 25.0.0 (IBM, Chicago, IL) was used for statistical analysis. The Kolmogorov-Smirnov test was used to determine the normality of the variables. Continuous variables were reported as mean ± standard deviation (SD) or median (IQR). Categorical variables were reported as the number (percentage) of participants. A p-value of less than 0.05 was considered statistically significant. P values were two-tailed. Mann-Whitney U test was used to compare medians of two independent groups. A Pearson correlation analysis was used to measure the strength of the linear relationship between two variables.

Ethical Consideration

The study was ethically conducted by the Declaration of Helsinki. Approval was obtained from the local Clinical Research Ethics Committee for the study (approval number: 2022–51).

Results

One hundred and twenty-three consecutive patients were included in the study. The patients were over 18, undergoing polysomnography in our hospital sleep laboratory, and had cardiac evaluations in the Cardiology Out-patient Clinic before polysomnography. The mean age of the patients was 49±1.61 years. The majority of the patients were in the middle age group. Forty-two (34.1%) patients were female and 81 (65.9%) were male. The accompanying comorbidities of the patients were as follows: HT 45 (36.6%), impaired fasting plasma glucose 31 (25.2%), DM 27 (22.0), HL 23 (18.7%), CAD 12 (9.8%), COPD 9 (7.3%), smoker 14 (11.4%), stroke history 3 (2.4%), metallic valve prosthesis 1 (0.8%), hypothyroidism 3 (2.4%), hyperthyroidism 3 (2.4%), anemia 5 (4.1%), polycythemia 1 (0.8%), splenomegaly 1 (0.8%), pancreatic cancer 1 (0.8%), endometrial cancer 1 (0.8%), breast cancer 1 (0.8%). The basic clinical characteristics of the patients, including age, gender, and comorbidities, are listed in Table 1.

Cardiac findings of the patients were as follows: Atrial fibrillation 1 (0.8 %), right bundle branch block 2 (1.6%), metallic valve prosthesis 1 (0.8 %), patent foramen ovale (pfo) 1 (0.8%), mild mitral regurgitation 10 (8.1%), mild aortic regurgitation 6 (4.9%), mild tricuspid regurgitation 5 (4.1%), bicuspid aorta 2 (1.6 %), aortic aneurysm 5 (4.1%), mild left ventricular hypertrophy 10 (8.1%), left ventricular diastolic dysfunction 10 (8.1%), left ventricular systolic dysfunction 1 (0.8%) and LVEF was median 60% (32–65). Cardiac findings of patients on admission are shown in Table 2.

Table 1. *The basic clinical characteristics of the patients*

Total patients number	123
Gender (female/male) (n) (%)	42/81 (34.1/65.9)
Age (years) mean ± SD	49±1.61
Hypertension (n) (%)	45 (36.6)
Diabetes mellitus (n) (%)	27 (22.0)
Hyperlipidemia (n) (%)	23 (18.7)
Coronary artery disease (n) (%)	12 (9.8)
Impaired fasting glucose (n) (%)	31 (25.2)
Chronic Obstructive Pulmonary Disease (n) (%)	9 (7.3)
Metallic valve prosthesis (n) (%)	1 (0.8)
Stroke history (n) (%)	3 (2.4)
Smoker (n) (%)	14 (11.4)
Hypothyroidism (n) (%)	3 (2.4)
Hyperthyroidism (n) (%)	3 (2.4)
Anemia (n) (%)	5 (4.1)
Polycythemia (n) (%)	1 (0.8)
Splenomegaly (n) (%)	1 (0.8)
Pancreatic cancer (n) (%)	1 (0.8)
Endometrial cancer (n) (%)	1 (0.8)
Breast cancer (n) (%)	1 (0.8)

n: number; SD: standard deviation

Table 2. *Cardiac findings of the patients*

Atrial fibrillation (n) (%)	1 (0.8)
Right bundle branch block (n) (%)	2 (1.6)
Patent foramen ovale (n) (%)	1 (0.8)
Mild mitral regurgitation (n) (%)	10 (8.1)
Mild aortic regurgitation (n) (%)	6 (4.9)
Mild tricuspid regurgitation (n) (%)	5 (4.1)
Aortic aneurysm (n) (%)	5 (4.1)
Mild left ventricular hypertrophy (n) (%)	10 (8.1)
Left ventricular diastolic dysfunction (n) (%)	10 (8.1)
Bicuspid aorta	2 (1.6)
Left ventricular systolic dysfunction (n) (%)	1 (0.8)
Left atrial dilatation (n) (%)	5 (4.1)
LVEF % median (IQR)	60 (32–65)

n: number; IQR: Inter Quantile Range; LVEF: Left ventricular ejection fraction

Table 3. *Laboratory findings of patients*

Glucose (mg/dl) median (IQR)	104.00 (79.00–301.00)	74–106 mg/dl
Creatinine (mg/dl) median (IQR)	0.79 (0.44–1.70)	0.5–0.9 mg/dL
Glomerular filtration rate (mL/min/1.73 m2) median (IQR)	102 (42–128)	-
Alanine aminotransferase (U/L) mean ± SD	29.46±12.58	0–33 U/L
CRP (mg/L) median (IQR)	2.60 (0.14–39.30)	0–5 mg/L
Thyroid stimulating hormone (mIU/L) median (IQR)	1.75±1.19	0.35–4.94 mIU/L
White blood count (10^3/mL) mean ± SD	7.91±2.04	3.98–10.04 10^3/mL
Hemoglobin (g/dL) mean ± SD	14.18±1.58	11.7–16.0 g/ dL
Platelet count (10^3/µL) mean ± SD	255.46±63.26	150–360 10^3/µL
Low density lipoprotein mg/dL mean ± SD	117.60±36.85	0–130 mg/dL
Triglyceride mg/dL mean ± SD	159.06±69.50	0–150 mg/dL

n: number; IQR: Inter Quantile Range; SD: standard deviation; mg/dl: milligram/deciliter; mmol/L: millimole/liter; mIU/mL: micro international unit/milliliter; mg/mL: microgram/ milliliter; mL: microliter; mg/L: milligram/liter; pg/mL: picogram/ milliliter; CRP: C reactive protein; ng/L: nanogram/liter; U/L: unit/liter

Median glucose level was 104.00 (79.00–301.00) mg/dl; median creatinine level was 0.79 (0.44–1.70) mg/dL, mean alanine aminotransferase level was 29.46±2.58 U/L, median CRP level was 2.60 (0.14–39.30) mg/L, mean thyroid stimulating hormone level was 1.75±1.19 IU/ mL, mean hemoglobin level was 14.18±1.58 g/dL, mean WBC level was 7.91±2.04 10^3/mL, mean platelet count was 255.46±63.26, mean low-density lipoprotein level was 117.60±36.85 mg/dL, mean triglyceride level was 159.06±69.50 mg/dL. Laboratory findings of patients are shown in Table 3.

Normal AHI value was detected in 5 (4.2%) patients. Mild OSAS was detected in 30 (24.2%) of the patients, moderate OSAS in 24 (18.9%), and severe OSAS in 64 (51.6%). The median AHI value was 30.98 (10.79–56.13). When the patients with and without HT were compared, the AHI values of the HT group were statistically higher (p=0.001). There was a weak positive correlation between AHI and HT, r=0.280, N=95; the relationship was statistically significant (p=0.006). No statistically significant relationship was found between the presence of other comorbidities and AHI values (obesity p=0.735, smoking p=0.979, HL p=0.086, CAD p=0.503, DM p=0.257, COPD p=0.145, stroke history p=0.758, impaired fasting plasma glucose p=0.922 and male gender p=0.904).

Continuous positive airway pressure (CPAP) device report was given to 67 (54.7%) of the patients.

Discussion

Obstructive sleep apnea syndrome is particularly common in middle-aged and older adults[2]. Similarly, the patients in our study were in the middle age group. Recent studies show that the prevalence of OSAS is 2–4% in average-aged men and 1–2% in women[3]. Compared to the rates in the literature, we found the prevalence of OSAS in women to be higher in our study. Obstructive sleep apnea syndrome prevalence was found to be high in our region, as women's socioeconomic levels are higher, and the rate of admission to our hospital is higher for women. Obstructive sleep apnea syndrome is also associated with cardiovascular comorbidities such as arrhythmias, hypertension, stroke, coronary atherosclerosis, and overall increased cardiovascular mortality as well as metabolic dysfunction. Voulgaris et al. conducted a study to investigate the prevalence of comorbidities in a patient cohort of OSAS and COPD-OSAS overlap syndrome patients[4]. They found the prevalence of HT 58.9%, dyslipidemia 28.2%, DM 17.8%, cardiovascular disease 13.5%, atrial fibrillation 4.3%. Their findings were similar to those of this study. When the literature was searched, studies revealing the relationship between OSAS and HT were found. Nieto et al. conducted the Sleep Heart Health Study[5]. This study is a community-based study. It includes 6132 subjects from ongoing population-based studies (age >40 years) and investigates sleep-disordered breathing. They observed that incident HT increased compared to baseline. Bixler et al. conducted another prospective two-stage study involving >16000 individuals in the first phase and >1700 individuals in the second phase; sleep-disordered breathing was independently associated with HT[6]. Increasing evidence suggests that OSAS may be cause related to

various metabolic abnormalities, including insulin resistance, glucose intolerance, DM and metabolic syndrome regardless of adiposity[7–12]. Otake et al found that in an Asian population, >25% of OSAS patients were diagnosed with DM[13]. In Hispanic and African Americans, the prevalence of DM was 30% in OSAS patients[14]. Meslier et al. reported that the frequency of DM in OSAS patients was 30%, and Levinson et al. reported that the frequency of DM in OSAS patients was 11% in the study of the European population[15–16]. When compared with these studies, it was observed that the rate of DM was lower in our country. Impaired glucose intolerance in these studies was higher than ours. In the Sleep Heart Health Study, individuals with severe sleep apnea were shown to be four times more likely to have AF[17]. However, our study found that the prevalence of AF was similar to the normal population. The probability of finding Af in the resting ECG is low, and it would be appropriate to insert a 24-hour rhythm holter in these patients. Javaheri et al. and Sin et al., found the prevalence of heart failure 11% and 37%, respectively, in patients with OSAS[18–19]. Compared to these studies, the heart failure rate was lower in our patients. In our daily practice, dyspnea is usually associated with heart failure, and OSAS is ignored. When the demographic characteristics of the patients in our study were compared with the studies in the literature, it was observed that they were similar.

Laboratory-based polysomnography or home-based polysomnography can be used to diagnose OSAS[20]. Laboratory-based polysomnography was preferred in our hospital. Continuous positive airway pressure (CPAP) device report is given to patients diagnosed with moderate and severe OSAS. The diagnosis of OSAS in patients should encourage treatment with CPAP[21]. Wuest W et al. evaluate the long-term effect of CPAP therapy on cardiac functional parameters with cardiac Magnetic Resonance Imaging[22]. They found that left ventricular stroke volume and right ventricular ejection fraction were significantly improved with CPAP treatment ($p=0.04$). They found that all other cardiac parameters did not change significantly, while mean systolic and diastolic blood pressure improved significantly ($p<0.01$).

We included the adult patients who undergoing laboratory-based polysomnography in our hospital in this study. The most common chronic disease is HT in the patients. There was a weak positive correlation between AHI and HT, the relationship was statistically significant. 24-hour ABPM was performed in nine patients and a new diagnosis of hypertension was made. Antihypertensive treatment was started in these patients. Cardiovascular stress test was performed on 22 patients. Invasive coronary angiography was recommended for two patients and coronary computed tomographic angiography was recommended for four patients with positive test results. Myocardial perfusion scintigraphy was recommended to nine patients who could not perform cardiovascular stress test.

One patient was diagnosed with pfo. Transesophageal echocardiography was recommended for this patient. More than half of the patients participating in the study had a CPAP device report.

This study has several limitations. Firstly, the study was a single-center and retrospective analysis. Secondly, the number of patients participating in the study was small. Third, our hospital has no coronary angiography unit and nuclear medicine imaging center. For this reason, patients go to other hospitals to have the tests we recommend to investigate myocardial ischemia. Patients have yet to come to show test results. Therefore, the test results could not be written.

Cardiac evaluation should be performed before laboratory-based polysomnography is performed. Fasting blood glucose measurement, ECG, and TTE recording should be performed on the patients, and 24-hour ABPM should be inserted. Additional tests should be performed to investigate ischemia in patients with myocardial ischemia findings. It would be appropriate to check the cardiac functions of patients receiving CPAP treatment at specific intervals.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Relationship of Clinicopathological Features, Ki-67 Proliferation Index, IDH1, EGFR, and p53 Mutations with Prognosis in Glioblastomas

Glioblastomlarda Klinikopatolojik Özelliklerin, Ki-67 Proliferasyon Endeksi, IDH1, EGFR ve p53 Mutasyonlarının Prognoz ile İlişkisinin Değerlendirilmesi

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ABSTRACT

Aim: *Glioblastoma is the most common malignant brain tumor. In the literature, few reports examine the relationship between morphologic findings of glioblastomas and patient prognosis. This study investigates the effects of morphological conclusions, IDH1, EGFR, p53 expressions, and Ki-67 proliferation index on patient prognosis in glioblastoma patients.*

Material and Method: *This study evaluated 166 patients diagnosed with glioblastoma between 2014 and 2017 in the Faculty of Medicine, Department of Pathology. Morphological findings (broad necrosis, focal necrosis, palisaded necrosis, microvascular proliferation, atypia, cellularity, lymphocyte infiltration, mitosis, cell type) were classified according to their presence/absence or intensity. IDH1, EGFR (Epidermal Growth Factor Receptor), p53 expressions, and Ki-67 proliferation indexes were grouped according to staining/non-staining conditions or staining percentages. The relationship between these findings and postoperative survival time was investigated.*

Results: *There was no statistically significant relationship with survival between morphologic findings, IDH1, EGFR, p53 expressions, and Ki-67 index.*

Conclusion: *Morphological and immunohistochemical features are insufficient to predict glioblastoma prognosis. Referring to molecular methods in estimating the prognosis may be more appropriate.*

Keywords: *glioblastoma; IDH1; p53; ki-67; EGFR*

ÖZET

Amaç: *Glioblastoma en sık görülen malign beyin tümörüdür. Literatürde glioblastomların morfolojik bulguları ile hasta prognozu arasındaki ilişkiyi inceleyen az sayıda yayın bulunmaktadır. Bu çalışmanın amacı, glioblastoma hastalarında morfolojik bulgular, IDH1, EGFR, p53 ekspresyonları ve ki-67 proliferasyon endeksinin hasta prognozu üzerindeki etkilerini araştırmaktır.*

Materyal ve Metot: *Bu çalışmada Tıp Fakültesi Patoloji Anabilim Dalı'nda 2014–2017 yılları arasında glioblastoma tanısı konulan toplam 166 hasta değerlendirildi. Morfolojik bulguların (geniş nekroz, fokal nekroz, palizadlanan nekroz, mikrovasküler proliferasyon, atipi, sellülarite, lenfosit infiltrasyonu, mitoz, hücre tipi) varlığı/yokluğu veya yoğunluğuna göre sınıflandırıldı. IDH1, EGFR, p53 ekspresyonları ve ki-67 proliferasyon endeksleri boyanma/boyanmama durumları veya boyanma yüzdelerine göre gruplandırıldı. Bu bulgular ile postoperatif sağkalım süresi arasındaki ilişki araştırıldı.*

Bulgular: *Morfolojik bulgular, IDH1, EGFR, p53 ekspresyonları, ki-67 endeksi ile hasta sağkalımı arasında istatistiksel olarak anlamlı bir ilişki bulunamadı.*

Sonuç: *Morfolojik ve immünohistokimyasal özellikler glioblastomaların prognozunu tahmin etmek için yeterli olmayabilir. Prognozu tahmin etmede moleküler yöntemlere başvurmak daha uygun olabilir.*

Anahtar kelimeler: *glioblastoma; IDH1; p53; ki-67; EGFR*

Introduction

Glioblastoma is a grade IV diffuse astrocytic tumor. It is the most common malignant brain tumor in adults, constituting 15% of all intracranial neoplasms and 45–50% of primary malignant brain tumors[1,2]. While diagnosing Central Nervous System (CNS) tumors was based solely on microscopic morphological features, the World Health Organization (WHO) suggested in 2016 that molecular parameters should be used in central nervous system tumor classification and morphological features[3]. As knowledge of the molecular basis of tumors

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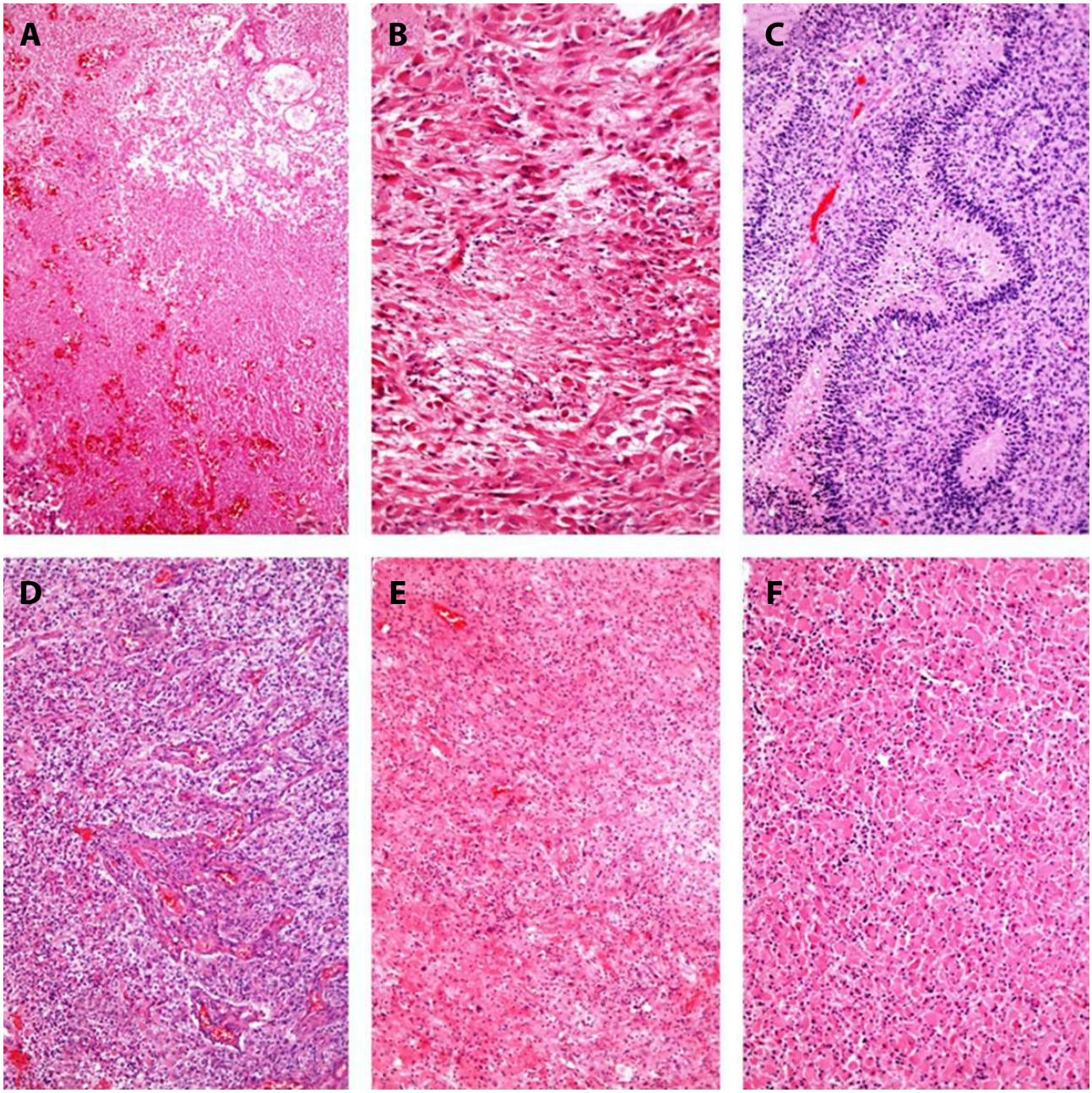

Figure 1. a–f. *Large necrosis (×200)(a). Focal necrosis (×200)(b). Palisaded necrosis (×200)(c). Microvascular proliferation (×200)(d). Lymphocyte infiltration (×400) (e). Gemystocytic cell presence (×200)(f).*

increases, the latest 2021 classification has provided a more precise classification of many CNS tumors[4]. In the previous classification, diffuse gliomas of adults were divided into 15 entities. In the latest classification, it is divided into only three groups: astrocytoma, IDH-mutant; oligodendroglioma, IDH-mutant and 1p/19q codeleted; and glioblastoma, IDH-wild type[4].

Isocitrate dehydrogenase 1 (IDH1) mutation status is one of the most important prognostic factors determining patient survival. Studies have shown that IDH-mutant type glioblastoma has a better prognosis than IDH-wild type glioblastoma and IDH-wild type anaplastic astrocytoma[5].

Glioblastomas progress rapidly despite surgical resection, radiotherapy, and treatment consisting of the chemotherapeutic agent temozolomide (TMZ), and the average survival time is 15 months [6–8]. This study investigates the effects of immunohistochemically detected p53, IDH1, EGFR mutations, Ki-67 expression, and clinicopathological features on patient prognosis.

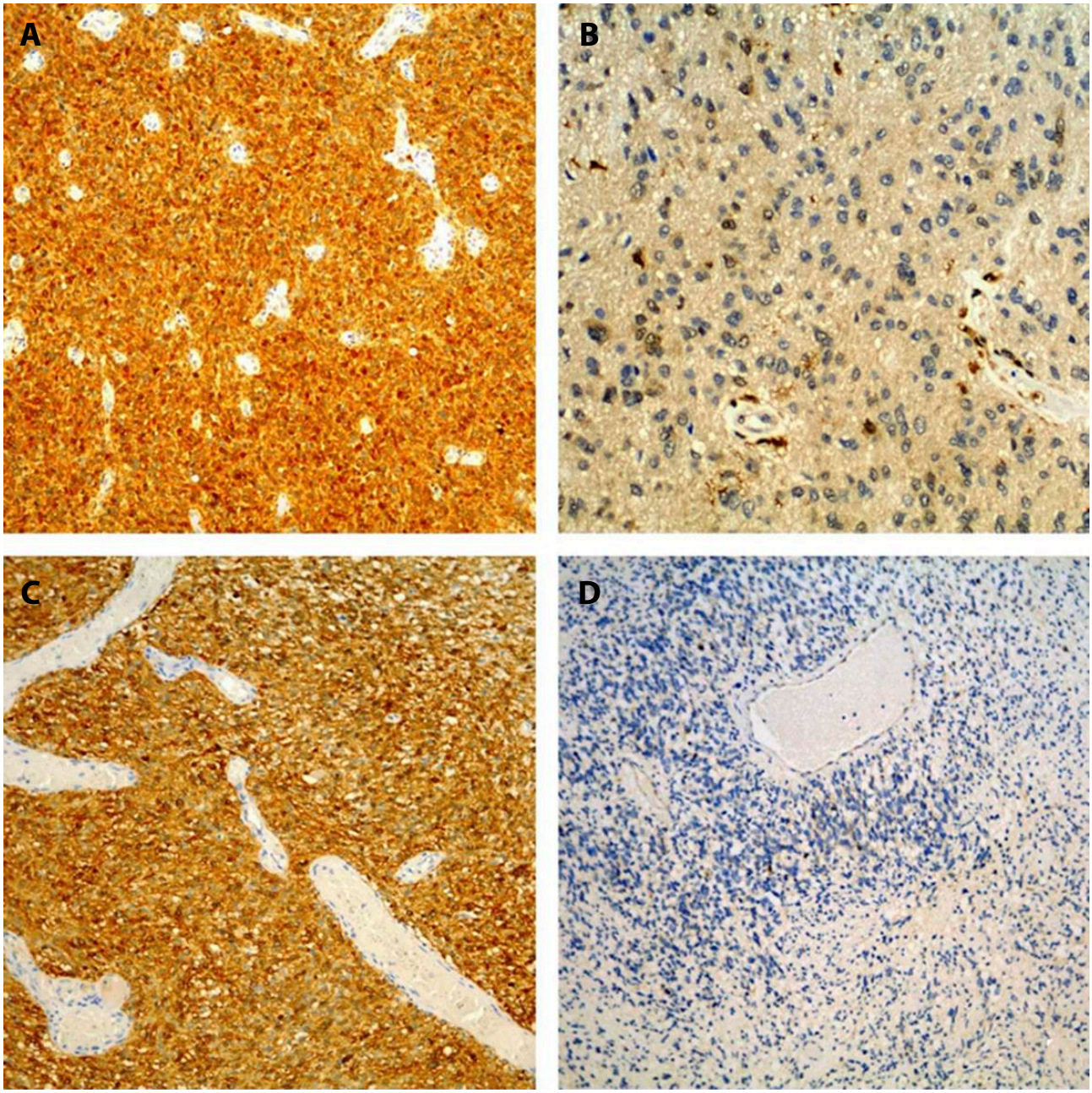

Figure 2. a–d. *Positive staining with IDH1 (×400)(a). Negative staining with IDH1 (×400)(b). Positive staining with EGFR (×400)(c). Negative staining with EGFR (×400)(d).*

Materials and Methods

The records of patients diagnosed with glioblastoma in 2014–2017 in the Pathology Department of the Faculty of Medicine were accessed by examining the electronic hospital database. Age, gender, clinical and radiological information of the patients were taken from the hospital database and recorded.

While patients diagnosed with glioblastoma and whose clinical and radiological data can be accessed regardless of age and gender were included in the study, patients without clinical and radiological data, tissues with poor fixation-follow-up quality, and tissues for which Hematoxylin-Eosin (HE) stained preparations were not available were excluded from the study. Survival data between the days of the operation and September 2019 were used to calculate the prognosis. The survival information of the patients was accessed through the Death Notification System.

Paraffin blocks and preparations were obtained from the archive. Hematoxylin-Eosin stained preparations

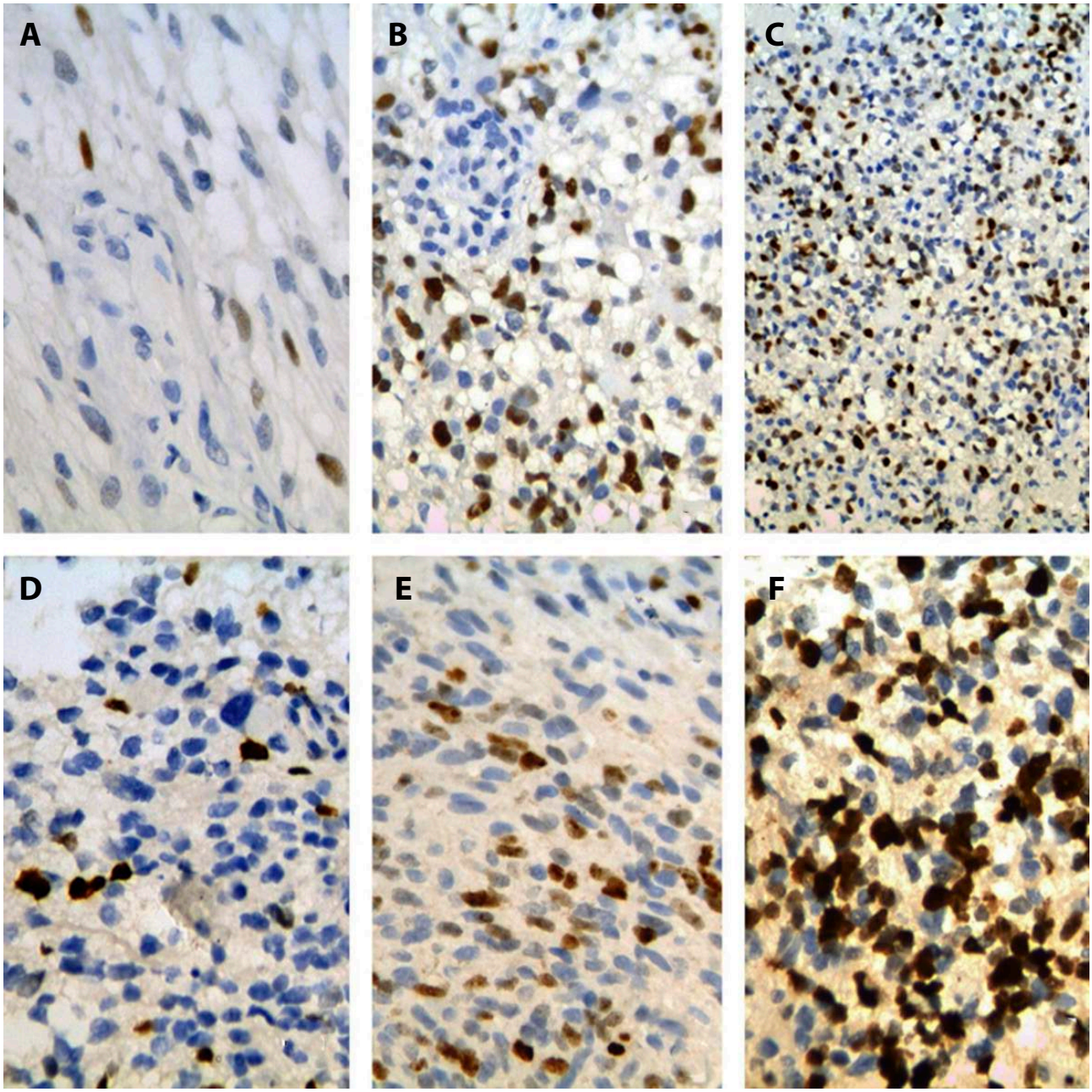

Figure 3. a–f. *Score 1 staining with p53 (×400)(a). Score 2 staining with p53 (×400)(b). Score 3 staining with p53 (×400)(c). Score 1 staining with Ki-67 (×400)(d). Score 2 staining with Ki-67 (×400)(e). Score 3 staining with Ki-67 (×400)(f).*

belonging to the patients were classified according to the presence/absence or density of morphological findings (large necrosis, focal necrosis, palisaded necrosis, microvascular proliferation, atypia, cellularity, lymphocyte infiltration, mitosis, cell type) (Fig. 1). The most suitable preparations for immunohistochemical staining were selected, and for immunohistochemical staining, 4 micrometer thick sections were cut from these blocks. The properties of the immunohistochemical marker used in the study are given in Table 1. For IDH1 evaluation, cases with significant cytoplasmic staining in tumor cells were positive, whereas cases without staining or weak staining in tumor cells were considered negative. For EGFR evaluation, cases with cytoplasmic and membraneous staining of tumor cells were evaluated as positive, while cases without staining were considered negative (Fig. 2). P53 and Ki-67 scores were classified as follows: score 1: <10% positivity of tumor cells, score 2:10–30% positivity of tumor cells, and score 3: >30% positive tumor cells (Fig. 3).

Table 1. *The properties of the immunohistochemical marker*

Immunohistochemical marker	Brand	Clone	Dilution	Positive control
IDH1	Histonova	H09	1/20	Glial tumor
p53	Novocastra	DO-7	1/800	Colon adenocarcinoma
EGFR	Novocastra	EGFR.113	1/10–1/20	Placenta
Ki-67	Novocastra	SP 6	1/100–1/500	Lymph node

Table 2. *The clinical findings of the cases*

Age	
The mean age	59.88
Distribution range	19–85
Gender	
Male	96 (57.8%)
Female	70 (42.2%)
Most common localizations	
Frontal lobe	53 (31.9%)
Temporal lobe	52 (31.3%)
Parietal lobe	31 (18.7%)
Multifocality	
Present	28 (16.9%)
Absent	125 (75.3%)
Radiotherapy	
Received treatment	104 (62.7%)
Not received treatment	49 (29.5%)
Chemotherapy	
Received treatment	86 (51.8%)
Not received treatment	67 (40.4%)
Survival	
Alive	14 (8.4%)
Dead	152 (91.6%)

Table 3. *The histopathological findings of the cases*

Cellular composition	
Presence of gemystocyte	78 (47%)
Presence of giant cells	30 (38.1%)
Presence of oligodendroglial cells	77 (46.4%)
Presence of sarcomatous component	6 (3.6%)
Presence of small cell component	42 (25.3%)
Classification according to Primary and Secondary	
Primary glioblastoma	56 (33.7%)
Secondary glioblastoma	70 (42.2%)
Broad necrosis	
Present	135 (81.3%)
Absent	31 (18.7%)
Focal necrosis	
Present	91 (54.8%)
Absent	75 (45.2%)
Palisading necrosis	
Present	81 (48.8%)
Absent	85 (51.2%)
Microvascular proliferation	
Present	126 (75.9%)
Absent	40 (24.1%
Cellularity increase	
Present	94 (56.6%)
Absent	72 (43.4%)
Marked atypia	
Present	23 (13.9%)
Absent	143 (86.1%)
Mitotic count	
<10	107 (64.5%)
10–25	37 (22.3%)
>25	22 (13.3%)
Lymphocytic infiltration	
Present	67 (40.4%)
Absent	99 (59.6%)

Statistical Analysis

The statistical analyses of the results were performed using IBM Statistical Package for Social Sciences (SPSS) statistical software package. Continuous variables were expressed as median (minimum-maximum) and mean ± standard deviation, while categorical variables were expressed as n (%). Pearson's chi-square test was used to compare categorical variables. The log-Rank test was used to determine the difference between survival times, and average survival times were given by the Kaplan-Meier method. P values less than 0.05 were considered statistically significant.

Results

Our study evaluated one hundred sixty-six cases diagnosed with glioblastoma between 2014 and 2017. The mean age of 166 cases was 59.88, and the median age was 61 years. The ages of the cases ranged from 19 to 85 years. 42.2% (n=70) of the cases were women; 57.8% (n=96) were male. The male/female ratio was found to be 1.37/1. The cases are most frequently localized in the frontal lobe, temporal lobe, and parietal lobe, and their incidence rates are 31.9% (n=53), 31.3% (n=52), and 18.7% (n=31), respectively. 42.2% (n=70) of 126 cases (75.9%) were IDH1 mutant glioblastoma; 33.7% (n=56) was IDH1 wild glioblastoma; IDH1 staining could not be applied to the remaining 40 cases (24.1%) because it was exhausted in our department or nonspecific results were obtained

Table 4. *The staining rates with immunohistochemical markers*

IDH1	Positive	42.2% (n=70)
	Negative	33.7% (n=56)
p53	Score 1	61.4% (n=102)
	Score 2	15.1% (n=25)
	Score 3	18.7% (n=31)
Ki-67	Score 1	10.2% (n=17)
	Score 2	56% (n=93)
	Score 3	30.1% (n=50)
EGFR	Positive	54.2% (n=90)
	Negative	3% (n=5)

from the staining. The mean age of IDH1 mutant cases was 60.26, while the mean age of IDH1 wild cases was 59.44. According to the data until September 2019, 8.4% (n=14) of 166 cases were still alive, and 91.6% (n=152) had died. The clinical findings of the cases are given in Table 2, histopathological findings in Table 3, and the staining rates with immunohistochemical markers are given in Table 4.

The patients' mean overall survival (OS) was 15.52±1.22 months, and the median was 11 months. No significant difference was found between overall survival times according to cellular components. According to the other histopathological characteristics of the cases (large necrosis, focal necrosis, palisaded necrosis, microvascular proliferation, pronounced cellularity, significant atypia, number of mitoses, lymphocytic infiltration), no significant difference was detected between overall survival times. P values were p=0.123, p=0.951, p=0.112, p=0.668, p=0.765, p=0.845, p=0.097, p=0.875, respectively.

There was a statistically significant difference between the overall survival times of the patients according to their focality, radiotherapy status, and chemotherapy status ($p < 0.05$).

There was no statistically significant difference between the overall survival times of the cases according to the expression of IDH1 and EGFR. P values were p=0.896 and p=0.268, respectively. No statistically significant difference was found between the groups regarding overall survival according to p53 expression and the Ki-67 index. P values were p=0.110 and p=0.241, respectively.

Discussion

Glioblastoma is the most common malignant brain tumor in adults, constituting approximately 45–50% of primary malignant brain tumors[1,2]. In the study conducted by Bouvier et al.[9] on 63 glioblastomas, the mean age of the cases was 56±13 years, and in the study by Popova et al.[10], the mean age was 48 years. Our study's average age of 166 glioblastoma cases was 59.88 years.

Isocitrate dehydrogenase mutations were first described in 2008 and reported by Parsons[11]. In this study, the authors said that patients with IDH1 mutation were mostly secondary glioblastoma, the patients were young, and their overall survival rate was higher. IDH1 mutation is observed in less than 10% of primary glioblastoma; and seen in about 70% of secondary glioblastoma. IDH1 antibody results were available in 126 of 166 glioblastoma cases in our study, and we found IDH1 expression in 70 (42.2%) of 126 cases. IDH1 staining could not be applied to the remaining 40 cases (24.1%) because it was exhausted in our department, or nonspecific results were obtained from the staining. The presence of IDH1 and IDH2 mutations in glioblastomas is a good prognostic factor. Hartmann et al.[5], in their study of 382 cases, showed that the prognosis was better in tumors with IDH1 mutations than in tumors without IDH mutations. Our study did not observe a statistically significant difference in mean overall survival between patients with IDH1 mutation and patients without IDH mutation.

In the literature, the results of studies that investigated the effect of the Ki-67 index on the clinical course of GBM patients were variable. In our study, we scored Ki-67 values in three groups, as in the study performed by Popova et al.[10] on 219 glial tumors, and we did not detect a statistically significant difference between Ki-67 values and overall survival. It could be because the sampled tissue did not reflect the entire tumor, the tumors had heterogeneous characteristics, and Ki-67 evaluations differed between the observers.

Epidermal growth factor receptor amplification is seen in approximately 40% of primary glioblastomas. The results in the literature investigating the effect of EGFR on the clinical course of GBM patients were variable. In the study conducted by Bouvier-Labit et al.[9] on 63 glioblastoma cases, no significant relationship was found between EGFR and survival. When we looked at the relationship between EGFR expression status and overall survival, no statistically significant difference was found between them. This was thought to be due to the poor compatibility of EGFR immunohistochemistry results with EGFR amplification.

P53 and IDH mutations occur in the early stage of gliomagenesis. The results in the literature investigating the effect of p53 mutation status on the clinical course of GBM patients were variable. In our study, we divided the p53 score into three groups, as in the study performed by Popova et al.[10]. When we examined the relationship between p53 staining rate and overall survival, we did not find a statistically significant difference.

The diagnosis of glioblastoma is based on tissue pattern rather than cell type. There are few studies examining the effects of morphological findings on prognosis. Two comprehensive malignant glioma studies show that necrosis results in a significantly worse prognosis in anaplastic glioma with both oligodendroglial and astrocytic components; patients with tumor necrosis were found to have considerably shorter mean survival than patients without tumor necrosis[12,13]. Bigner et al.[14] examined the relationship of histopathological features with EGFR amplification status and found no significant relationship between necrosis, palisaded necrosis, multinuclear giant cells, and microvascular proliferation. It was found to be borderline significant with lymphocytic infiltration. In the study of Palma et al.[15], consisting of 42 cases, it was reported that lymphocytic infiltration in the tumor positively affected survival. In our study, we classified the cases according to the presence/absence of morphological features such as large necrosis, focal necrosis, palisaded necrosis, microvascular proliferation, cellularity, atypia, infiltration, and cell type, and we examined their overall survival. We detected that none of the morphological findings had any effect on survival.

Ahmadipour et al.[16] investigated the effect of proliferation markers and multifocality on survival in their study of 565 cases. They found the overall survival to be 13.5 months in single lobe involvement, 11.4 months in multifocal involvement of the same hemisphere, and 9.3 months in contralateral hemisphere involvement. As a result of their studies, they mentioned that multifocality can be used as an independent prognostic factor. Our study found that the mean overall survival time in multifocal cases was 9.857±1.444 months, and the mean overall survival time was 16.896±1.521 months in unifocal cases. We observed a statistically significant difference between the overall survival times of the cases according to their focality.

In the phase 3 study conducted by Perry et al.[17], adding TMZ to short-term radiotherapy was associated with significantly longer survival. In our study, we found the mean overall survival time in patients who received radiotherapy was 18.294±1.565 months, and it was 9.347±1.592 months in patients who did not receive radiotherapy; the average overall survival time in patients who received chemotherapy was 20.374±1.786 months, and it was 9.164±1.246 months in patients who did not receive chemotherapy. We observed a statistically significant difference between the overall survival times of the patients according to their radiotherapy and chemotherapy status.

Conclusion

Our study investigated the effects of clinical and morphological features, IDH1, EGFR, Ki-67, and p53 expression states on patient prognosis in glioblastoma cases. As a result, the data showed that the morphological features, IDH1, p53, EGFR expressions, and Ki-67 proliferation index did not significantly affect glioblastoma survival.

The survival of patients who received radiotherapy and TMZ chemotherapy was statistically significantly longer than those who did not receive treatment. It had been observed that multifocality had a negative effect on patient survival. Morphological and immunohistochemical features are not sufficient to predict the prognosis of glioblastomas. Referring to molecular methods in estimating the prognosis may be more appropriate.

Statement of Ethics

The approval for this study was obtained from Uludag University, Faculty of Medicine, Ethics Committee of Medical Research, dated April 09, 2019, and numbered 2019–7/28.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Evaluation of Frequency and Etiological Factors in Pterygium and Pinguecula Cases in Bolu Region

Bolu Bölgesinde Pterjiyum ve Pingekula Olgularındaki Sıklık ve Etiyolojik Faktörlerin İncelenmesi

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ABSTRACT

Aim: *To evaluate the frequency of pterygium and pinguecula in patients living in Bolu region and to assess the relationship between advanced age, UV exposure, smoking status, education level, systemic disease and presence of pterygium and pinguecula.*

Material and Method: *This study comprises 1014 patients between 18–80 years of age. Demographic data, including age and sex, occupation, education level, systemic disease, smoking status and UV exposure, are questioned. A comprehensive ophthalmological examination was performed, and the presence of pterygium and pinguecula was evaluated. A p-value under 0.05 was taken to be statistically significant.*

Results: *Among 1014 patients included in this study, 553 patients were in the control group (54.5%); 101 patients had pterygium (9.96%) and 360 patients had pinguecula (35.5%). Three hundred eighty-two patients were men and 632 were women. The mean age of patients was 46.33±15.83 years. Bilaterality was more evident in the pinguecula group compared to the pterygium group (p<0.001). Patients with outdoor occupation were higher in the pterygium and pinguecula group compared to the control group (p<0.001). UV exposure time was significantly different between groups (p<0.001). While UV exposure time was the highest in the pterygium group (median: 3.0, IQR: 0–4.5), it was approximately 2 hours in the pinguecula group (median: 2, IQR: 0–4). Education levels were lower in the pterygium and pinguecula group compared to the control group (p<0.001).*

Conclusion: *Advanced age, extended UV exposure, outdoor occupation and low education levels are associated with developing pterygium and pinguecula. This cross-sectional study could contribute to prevalence studies with large series that would be held in our country in the future.*

Keywords: *pterygium; pinguecula; UV exposure; age; education level*

ÖZET

Amaç: *Bolu bölgesindeki hastalarda pterjiyum ve pingekulanın sıklığını değerlendirmek ve ileri yaş, UV maruziyeti, sigara kullanımı, eğitim durumu, sistemik hastalık gibi faktörlerin pterjiyum ve pingekula ile ilişkisini araştırmak.*

Materyal ve Metot: *Kliniğimize başvuran 18–80 yaş arası 1014 hasta çalışmaya dâhil edildi. Hastaların yaş ve cinsiyet gibi demografik bilgileri, meslekleri, eğitim düzeyleri, sistemik hastalıkları, sigara kullanımları ve gün içerisindeki UV maruziyetleri sorgulandı. Tam bir oftalmolojik muayene yapılarak, pterjiyum ve pingekula varlığı değerlendirildi. Sonuçlar p <0,05 düzeyinde anlamlı kabul edildi.*

Bulgular: *Çalışmaya dâhil edilen 1014 hastanın 553'ü kontrol grubunda (%54,5); 101'i pterjiyum grubunda (%9,96) ve 360'ı pingekula grubunda yer aldı (%35,5). Hastaların 382'si erkek; 632'si kadındı. Hastaların yaş ortalaması 46,33±15,83 idi. Pingekulanın pterjiyuma göre daha fazla oranda bilateral seyrettiği izlendi (p<0,001). Pterjiyum ve pingekula grubunda dış mekanda çalışanların oranı kontrol grubuna göre belirgin olarak yüksekti (p<0,001). UV maruziyetine bakıldığında gruplar arası anlamlı farklar tespit edildi (p<0,001). Pterjiyum grubundaki hastaların gün içindeki ortalama UV maruziyeti en üst sıradayken (median: 3,0, IQR: 0–4,5); pingekula grubunda bu süre ortalama iki saat olarak tespit edildi (median: 2, IQR: 0–4). Kontrol grubuyla karşılaştırıldığında eğitim düzeyinin pterjiyum ve pingekula grubunda daha düşük olduğu saptandı (p<0,001).*

Sonuç: *İleri yaş, uzamış UV maruziyeti, dış mekanda çalışma ve düşük eğitim seviyesi pterjiyum ve pingekula gelişimi ile yakından ilişkilidir. Bu kesitsel çalışma ileride ülkemizde yapılabilecek geniş serili prevalans çalışmalarına katkı sağlayabilecek niteliktedir.*

Anahtar kelimeler: *pterjiyum; pingekula; UV maruziyeti; yaş; eğitim seviyesi*

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Introduction

Pterygium, which usually extends to the cornea, is a degenerative, hyperplastic, and fibrovascular tissue originating from the interpalpebral bulbar conjunctiva[1]. Patients with pterygium frequently apply to the ophthalmology clinics with ocular irritation and blurred vision that results from corneal astigmatism[2].

Pinguecula presents as a yellowish, slightly elevated, lipid-like degeneration frequently seen in the conjunctiva on the nasal side of the limbus[3]. Although pinguecula is often asymptomatic, it can lead to chronic irritation by disrupting the tear film layer[4].

It is known that exposure to ultraviolet light (UV) plays an important role in the development of both conditions[5,6]. While there are many studies in the literature about the prevalence of pterygium ranging from 1.2% to 33%, the data on the frequency of pinguecula are relatively limited. Available data indicate a higher prevalence than pterygium; this rate may reach 70% in some regions[7,8].

This study aimed to evaluate the frequency of pterygium and pinguecula in patients who applied to an ophthalmology clinic in the Bolu region. In addition, we aimed to assess the relationship between advanced age, ultraviolet light (UV) exposure, smoking status, education level, systemic disease and the presence of pterygium and pinguecula.

Materials and Methods

This study consists of 1014 patients between 18–80 years of age who applied to our ophthalmology clinic between February 2020 and February 2021. This prospective cross-sectional study is held in Bolu, in the western Blacksea Region of Türkiye.

Demographic data, including age, sex, occupation, education level, systemic diseases, smoking status and daily UV exposure, were questioned. Patients' workplaces are considered outdoors if they spend most of their working hours in the open air; they are defined as indoor if they spend most of their working hours inside buildings. Patients were divided into groups according to their educational status as primary school graduate, high school graduate, university graduate and above.

A complete ophthalmological examination was performed, and pterygium and pinguecula were evaluated. The bilaterality of the lesions was examined.

Patients under the age of 18 and over the age of 80 and those with a history of pterygium/pinguecula excision were excluded from the study.

Ethical Board Approval

Before the study, ethical board approval was obtained from the Scientific Research Ethics Committee (Decision no: 2020/20). The study was carried out in accordance with the Declaration of Helsinki, and informed consent was obtained from each patient.

Statistical Analysis

Data distribution presents descriptive data as numbers (percentage) or median (interquartile range, IQR: 25th to 75th percentile) or mean ± standard deviation. One-way ANOVA and post-hoc Games-Howell tests were used to compare normally distributed variables. Group comparisons were made with the Kruskal Wallis test and post-hoc Dunn's test for nonnormal data. Single logistic regression models were used to estimate the effect of each variable on the diagnosis of pterygium and pinguecula. In addition, multiple logistic regression analysis was performed to identify independent risk factors for these diseases. In the multiple regression analysis, variables with $p<0.20$ in the simple regression model were included in the model as independent variables. Odds ratios (OR) were calculated with 95% confidence intervals. Hosmer Lemeshow tests confirmed the goodness of fit of the models. Statistical analyses were performed using Statistical Package for Social Sciences 25.0 for Windows (IBM Inc., Chicago, Illinois, USA) and R 4.0.2 statistical software. The results were considered significant at the $p<0.05$ level.

Results

While 553 of 1014 patients included in the study did not have pinguecula or pterygium (54.5%), 101 patients (9.96%) were in the pterygium group and 360 patients (35.5%) were in the pinguecula group. Overall, 382 of the patients were male and 632 of them were women. The mean age of the patients was 46.33±15.83 years. The clinical and demographic characteristics of the patients included in the pterygium and pinguecula group and those without pterygium or pingueculae are shown in Table 1 comparatively.

It was observed that there was a significant difference between the groups in terms of age ($p<0.001$).

Table 1. *Comparison of clinical characteristics of the groups*

	Group without pterygium/ pinguecula (n=553)	Pterygium (n=101)	Pinguecula (n=360)	p
Age	41.37±16.58[a,b]	54.99±12.68[a]	51.52±12.39[b]	**<0.001**
Sex				**0.004**
Male	183 (33.1)	42 (41.6)	157 (43.6)	
Female	370 (66.9)	59 (58.4)	203(56.4)	
Laterality				**<0.001**
Right	NA	34 (33.7)	105 (29.2)	
Left	NA	31 (30.7)	48 (13.3)	
Bilateral	NA	36 (35.6)	207 (57.5)	
Working place				**<0.001**
Indoor	515 (93.1)	72 (71.3)	295 (81.9)	
Outdoor	38 (6.9)	29 (28.7)	65 (18.1)	
UV exposure, hour/day	0 (0–0) [a]	3 (0–4.5) [a]	2 (0–4)[a]	**<0.001**
Smoking				0.600
No	369 (66.7)	68 (67.3)	225 (62.5)	
Yes	116 (21.0)	19 (18.8)	79 (21.9)	
Ex-smoker	68 (12.3)	14 (13.9)	56 (15.6)	
Education level				**<0.001**
Elementary school	200 (36.2)	82 (81.2)	217 (60.3)	
Middle-school	58 (10.5)	6 (5.9)	55 (15.3)	
High-school	162 (29.3)	7 (6.9)	60 (16.7)	
University	133 (24.1)	6 (5.9)	28 (7.8)	
Hypertension	74 (13.4)	35 (34.7)	88 (24.4)	**<0.001**
Asthma	17 (3.1)	2 (2.0)	16 (4.4)	0.376
Diabetes Mellitus	47 (8.5)	20 (19.8)	66 (18.3)	**<0.001**
Coronary Artery Disease	14 (2.5)	9 (8.9)	23 (6.4)	**0.002**
Hypothryoidism	34 (6.1)	4 (4.0)	14 (3.9)	0.272
Hyperthryoidism	3 (0.5)	1 (1.0)	3 (0.8)	0.812
Rheumatoid Arthritis	6 (1.1)	2 (2.0)	2 (0.6)	0.415
Hyperlipidemia	5 (0.9)	2 (2.0)	14 (3.9)	**0.008**

Pearson's chi-square or Fisher exact test was used for categorical variables. If mean values are given, one-way ANOVA; if median values are given, the Kruskal-Wallis test was used. The a, b values show significant differences between the two groups evaluated with the Games-Howell or Dunn's test; CAD: Coronary Artery Disease; Bold p values indicate statistical significance at p<0.05.

It was shown that the mean age was higher in the pterygium and pinguecula groups. A significant relation was found between gender distribution and groups (p=0.004). It was observed that pinguecula was seen more frequently bilateral when compared to pterygium (p<0.001). The rate of patients who work outdoors in the pterygium and pinguecula groups was significantly higher than the control group (p<0.001). Considering the UV exposure, a significant difference was detected between the groups (p<0.001). While the patients in the pterygium group had the highest mean daily UV exposure (median: 3 hours), the duration of UV exposure was 2 hours in patients with pinguecula.

It was determined that the education level was lower in the pterygium and pinguecula groups (p<0.001). Systemic diseases such as hypertension, diabetes, coronary artery disease and hyperlipidemia were observed more frequently in the pterygium and pinguecula groups.

Occupational distributions among the groups are shown in Table 2. Notably, the rate of farmers in the pterygium group is 18.6%.

Pterygium

A significant correlation was found in simple regression analysis between advanced age, working outdoors, prolonged UV exposure, low education level, hypertension, diabetes, coronary artery disease and the risk of developing pterygium (Table 3). When variables with p<0.20 in the simple regression model were added and other factors were controlled with the multiple regression model, gender and working outdoors lost their significance. (Table 2). It was determined that 1 hour

Table 2. *Distribution of occupation according to groups*

Occupations	Group without pterygium/pinguecula (n=553)	Pterygium (n=101)	Pinguecula (n=360)
Chef	11 (2.8%)	1 (1.2%)	6 (2%)
Farmer	12 (3.1%)	16 (18.6%)	33 (10.9%)
Retired worker	9 (2.3%)	5 (5.8%)	20 (6.6%)
Retired office worker	23 (5.9%)	5 (5.8%)	23 (7.6%)
Tradesman	14 (3.6%)	0 (0%)	11 (3.6%)
Housewife	186 (48.1%)	42 (48.8%)	140 (46.1%)
Factory worker	43 (11.1%)	5 (5.8%)	33 (10.9%)
Construction worker	5 (1.3%)	3 (3.5%)	6 (2%)
Unemployed	13 (3.4%)	0 (0%)	1 (0.3%)
Office worker	24 (6.2%)	1 (1.2%)	9 (3%)
Teacher	24 (6.2%)	1 (1.2%)	7 (2.3%)
Driver	10 (2.6%)	7 (8.1%)	12 (3.9%)
Medical technician	13 (3.4%)	0 (0%)	3 (1%)
Other	166 (30.0%)	15 (14.8%)	56 (15.5%)

Table 3. *Simple and multiple logistic regression analysis of risk factors for pterygium (n=101)*

	Simple logistic regression			Multiple logistic regression		
Variables	OR	95% CI	p	OR	95% CI	p
Age	1.053	1.038–1.068	**<0.001**	1.001	0.978–1.025	0.914
Sex						
Female	Reference					
Male	1.439	0.933–2.220	0.100	1.725	0.940–3.168	0.079
Working Place						
Indoor	Reference					
Outdoor	5.459	3.173–9.391	**<0.001**	0.745	0.335–1.656	0.470
UV exposure, hour/day	1.986	1.745–2.259	**<0.001**	1.824	1.556–2.138	**<0.001**
Smoking						
No	Reference					
Yes	0.889	0.513–1.540	0.674			
Ex-smoker	1.117	0.595–2.099	0.731			
Education level						
Elementary school	Reference			Reference		
Middle-school	0.252	0.105–0.608	**0.002**	0.254	0.093–0.696	**0.008**
High-school	0.105	0.047–0.234	**<0.001**	0.155	0.057–0.417	**0.000**
University	0.110	0.047–0.259	**<0.001**	0.233	0.082–0.665	**0.006**
Hypertension, yes	3.433	2.130–5.533	**<0.001**	1.618	0.830–3.153	0.158
Asthma, yes	0.637	0.145–2.800	0.550			
Diabetes Mellitus, yes	2.658	1.498–4.717	**0.001**	1.039	0.491–2.199	0.920
Coronary artery disease, yes	3.766	1.584–8.955	**0.003**	0.788	0.246–2.524	0.688
Hypothyroidism, yes	0.629	0.218–1.814	0.391			
Hyperthyroidism, yes	1.833	0.189–17.802	0.601			
Rheumatoid arhritis, yes	1.842	0.366–9.256	0.458			

OR: Odds ratio; CI: Confidence Interval; Dependent variable: Pterygium; Goodness of fit of the models were confirmed with Hosmer-Lemeshow tests (p>0.05); Bold p-values indicate statistical significance at p<0.05.

Table 4. *Simple and multiiple logistic regression analysis of risk factors for pinguecula (n=360)*

	Simple logistic regression			Multiple logistic regression		
Variables	OR	%95 CI	p	OR	%95 CI	p
Age	1.044	1.035–1.054	**<0.001**	1.017	1.003–1.030	**0.014**
Sex						
Female	reference			reference		
Male	1.564	1.190–2.055	**0.001**	0.761	0.535–1.084	0.131
Working Place						
Indoor	reference					
Outdoor	2.986	1.952–4.568	**<0.001**	0.747	0.431–1.297	0.300
UV exposure, hour/day	1.617	1.480–1.766	**<0.001**	1.474	1.327–1.638	**<0.001**
Smoking						
No	reference					
Yes	1.117	0.803–1.554	0.512			
Ex-smoker	1.351	0.914–1.996	0.132			
Education level						
Elementary school	reference			reference		
Middle-school	0.874	0.577–1.325	0.526	1.085	0.671–1.753	0.739
High-school	0.341	0.240–0.486	**<0.001**	0.589	0.375–0.926	**0.022**
University	0.194	0.124–0.304	**<0.001**	0.396	0.231–0.679	**0.001**
Hypertension, yes	2.094	1.486–2.951	**<0.001**	0.938	0.609–1.442	0.769
Asthma, yes	1.466	0.731–2.941	0.281			
Diabetes mellitus, yes	2.417	1.619–3.608	**<0.001**	1.309	0.828–2.07	0.250
Coronary artery disease, yes	2.628	1.334–5.177	**0.005**	1.157	0.536–2.495	0.710
Hypothyroidism, yes	0.618	0.327–1.168	0.238			
Hyperthyroidism, yes	1.541	0.309–7.675	0.598			
Rheumatoid Arthritis, yes	0.509	0.102–2.537	0.410			
Hyperlipidemia, yes	4.435	1.583–12.421	**0.005**	2.537	0.82–7.853	0.106

OR: Odds ratio; CI: Confidence Interval; Dependent variable: Pinguecula; Goodness of fit of the models were confirmed with Hosmer-Lemeshow tests (p>0.05); Bold p-values indicate statistical significance at p<0.05.

more daily exposure to UV light increased the probability of developing pterygium by 82.4% (OR=1.824, 95% CI: 1.556–2.138, p <0.001). In addition, it was observed that the risk of developing pterygium was lower in patients with higher education levels. In multiple logistic regression analyses, systemic comorbidities such as age, hypertension, diabetes and coronary artery disease lost their significance.

Pinguecula

In simple regression analysis, a significant correlation was found between advanced age, male gender, working outdoors, prolonged UV exposure, low education level, hypertension, diabetes, coronary artery disease and hyperlipidemia, and the risk of developing pinguecula (Table 4). According to the multiple logistic regression model, age (OR=1.017, 95% CI: 1.003–1.030, p=0.014), UV exposure (OR=1.474, 95% CI: 1.327–1.638, p <0.001) and education level (high school vs. primary school: OR=0.589, 95% CI: 0.375–0.926, p=0.022; university and above vs. primary school) were found as independent risk factors for pinguecula.

Discussion

There are many studies interested in the prevalence of pterygium in different geographies. The rate is 9.5% in South India[5], 1.3% in Tehran[9], 6.1% in China[10] and 5.9% in Spain[6]. According to a cross-sectional study by Tuncer et al. 11. from Türkiye, the frequency of pterygium was approximately 12.1%. In a cross-sectional study conducted by Gümüş et al.[12], the incidence of pterygium was reported to be 7.8%. Although our study was not a prevalence study, the frequency of pterygium among patients admitted to our clinic was found to be similar to many studies in the current literature (9.9%).

Considering the studies on the prevalence of pinguecula in the literature, the rates are in a wide range, reported as 11.3% in South India[5], 22.5% in Tehran[9], 75.5% in China[13], and 47.9% in Spain[6]. Tuncer et al.[11] reported the incidence of pinguecula among the patients admitted to the outpatient clinic to be 87.7%. In our study, the frequency of pinguecula was 35.5% among the patients who applied to our clinic. In the study of Viso et al.[6], the rate of bilateral involvement was reported as 33.3% and 88.9% for pterygium and pinguecula, respectively. In a

study from Türkiye, bilaterality was found to be 34.6% for pterygium[12]. Similar to these studies, we found the rate of bilaterality in the pterygium group to be 35.6%. However, we found the incidence of bilaterality to be 57.5% in the pinguecula group. Our findings support that pinguecula is more frequently seen bilaterally than pterygium. It has been suggested that the incidence of pterygium is higher in the region, including 37° south and north of the equator, also known as the pterygium belt[14]. Increased UV exposure causes limbal ischemia by damaging Langerhans cells in the limbus. As a result, the conjunctival epithelium undergoes hyperplasia and progresses towards the cornea.[15] High amounts of sunlight exposure and particles that may cause microtrauma, such as sand and dust in the working places, may also contribute to developing pterygium[14]. Publications suggest that the incidence of pterygium and pinguecula is higher, especially in rural areas[5,13,16,17]. A study conducted in our country found that pterygium is most common in agricultural workers and farmers; the pterygium size has also been larger in this occupational group[12]. Our study detected the pterygium group farmers' rate as 18.6%. It was also observed that patients with pterygium and pinguecula were exposed to higher amounts of sunlight (an average of 3 and 2 hours for pterygium and pinguecula, respectively). Bolu province is located at 40° north latitude, relatively far from the equatorial belt. Nevertheless, it can be said that besides the agricultural activities in the province, the marketing made outdoors throughout the year increases UV exposure and increases this frequency. Many studies suggest a strong relationship between pterygium and pinguecula and advanced age[5,6,11,18]. In our study, the mean age in the pterygium and pinguecula groups was significantly higher than in patients without pterygium or pinguecula ($p<0.001$). It was observed that age may be an independent risk factor, especially for developing pinguecula. Since exposure to environmental risk factors will increase as age increases, other data can interpret this result. While many studies suggest that pterygium and pinguecula are more common in males, there are also studies claiming the opposite[6,7,9,13,19,20]. Although it is possible to explain the higher prevalence of pterygium and pinguecula in men by spending more time under the sun, this may not be true for all geographic regions. In this study that we conducted in Bolu province, the rate of pterygium and pinguecula were lower in the female sex group. When the other variables were evaluated, it was found that gender was not a significant risk factor for either group. Many studies have investigated the possible relationship between developing pterygium and pinguecula and smoking, but no significant relationship was found in multivariate analysis[9,13]. Our study did not find a correlation between pterygium, pinguecula, and smoking. Several publications suggest that a low education level is related to developing pterygium and pinguecula[6], while others claim that education level is not important[5,9]. Our study found that the education level and the development of pinguecula and pterygium were inversely proportional in both groups. This data was also correlated with other results of our study (increased UV exposure, outdoor work, occupational distribution). As explained in the literature, it can be assumed that patients with higher education levels prefer jobs that can be carried out indoors[6] The presence of systemic disease in patients with pterygium and pinguecula was also investigated in several studies[5,13]. Although systemic conditions such as diabetes, hypertension, and coronary artery disease were more common in the pterygium and pinguecula groups, in our research, this data lost its significance in multivariate analysis.

The study's limitations are that since it was conducted with patients who applied to our clinic, it does not reflect the real prevalence of pterygium and pinguecula in the normal population.

Conclusion

Advanced age, prolonged UV exposure, outdoor work, and low education are closely associated with developing pterygium and pinguecula. Although Bolu province is located far from the equator, these risk factors have significantly affected the development of pterygium and pinguecula. This cross-sectional study is the first in the literature to examine the frequency and risk factors of pterygium and pinguecula in Bolu.

This study will contribute to the prevalence studies with a large series that can be done in our country in the future.

Statement of Ethics

Before the study, ethical board approval was obtained from the Scientific Research Ethics Committee (Decision no: 2020/20). The study was carried out in accordance with the Declaration of Helsinki, and informed consent was obtained from each patient.

Conflict of Interest Statement

All the authors declare no conflict of interest.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Frequency and Affecting Factors of COVID-19 Vaccine Hesitancy in Patients with Diabetes

Diyabetli Hastalarda COVID-19 Aşı Tereddütünün Sıklığı ve Etkileyen Faktörler

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ABSTRACT

Aim: *While vaccines are the most important strategy in combating the COVID-19 epidemic, their effectiveness can be greatly affected by vaccine hesitancy. Diabetes mellitus is known as an immune-compromised condition, and COVID-19 is associated with an increased risk of morbidity and mortality in patients with diabetes mellitus. For this reason, the COVID-19 vaccine is strongly recommended for diabetic patients. This study investigated the social, demographic, and clinical factors affecting vaccination frequency and vaccine hesitancy in diabetics.*

Material and Method: *A cross-sectional study used a questionnaire to determine the demographic, social, and individual characteristics of the participants who were vaccinated or unvaccinated. Patients with diabetes mellitus and having the physical and mental capacity to answer the pre-prepared questions by face-to-face survey method according to the order of admission were included. There were two groups of participants: those who received the COVID-19 vaccination (Group 1=180) and those who did not (Group 2=26).*

Results: *The frequency of vaccination was 87.4%. A comparison of the vaccinated and unvaccinated groups did not show any significant differences in terms of gender, age, type, and duration of diabetes, smoking, insulin use, marital and educational status, living place, history of COVID-19, family members with COVID-19, and relatives who died due to COVID-19. Influenza vaccination rates were also significantly lower in the unvaccinated group than in the vaccinated group (7.7% vs. 26.1%, p=0.047).*

Conclusions: *Although the COVID-19 vaccination rates of our diabetic patients were higher than the general provincial rate, they were the same as in Türkiye, regardless of demographic, social, and individual characteristics. As evidenced by the COVID-19 experience, additional information and support regarding vaccination-preventable diseases could raise the rate of other recommended vaccinations among diabetic patients.*

Keywords: *COVID-19 vaccine; diabetes mellitus; vaccine hesitancy*

ÖZET

Amaç: *Aşılama süreci, COVID-19 salgınıyla mücadelede en etkin stratejilerden biridir. Ancak, etkinliği aşı reddinden büyük ölçüde etkilenebilmektedir. Diyabetes mellitus, genel olarak immünsupresif bir durum olarak kabul edilmektedir ve bu hasta grubunda COVID-19'a bağlı morbidite ve mortalitede artış söz konusu olmaktadır. Bu nedenle diyabet hastalarına COVID-19 aşısı şiddetle tavsiye edilmektedir. Bu çalışmada diyabetiklerde aşılama sıklığı ve aşı reddini etkileyen sosyal, demografik ve klinik faktörlerin araştırılması amaçlanmıştır.*

Materyal ve Metot: *Bu kesitsel çalışmada aşı olan ve olmayan katılımcıların demografik, sosyal ve bireysel özelliklerini belirlemek için anket kullanıldı. Diyabeti olan ve soruları cevaplayabilecek fiziksel ve zihinsel kapasiteye sahip hastalar başvuru sırasına göre çalışmaya dâhil edildi. Önceden hazırlanmış sorular yüz yüze anket yöntemiyle katılımcılara soruldu. Katılımcılar COVID-19 aşısı olan (Grup 1=180) ve olmayan (Grup 2=26) olmak üzere iki gruba ayrıldı.*

Bulgular: *Aşılanma sıklığı %87,4 idi. Aşılı ve aşısız gruplar arasında yaş, diyabet yaşı, cinsiyet, diyabet tipi, insülin kullanımı, sigara kullanımı, medeni durum, yaşanılan yer, eğitim durumu, COVID-19 geçirme öyküsü, yakınlarından birinin COVID-19 geçirmesi ve yakınlarından birini COVID-19 nedeniyle kaybetmesi açısından fark yoktu. Herhangi bir zamanda influenza aşısı olma oranı aşılanmamış grupta aşılanmış gruba göre anlamlı olarak daha düşüktü (%7,7'ye karşı %26,1, p=0,047).*

Sonuç: *Diyabetik hastalarımızın COVID-19 aşılanma oranları il genelinden yüksek ve Türkiye geneli ile aynıydı ve bu durum demografik, sosyal ve bazı bireysel özelliklerden bağımsızdı. Diyabetik popülasyonda aşı ile önlenebilir hastalıkların ciddi sonuçları hakkında çeşitli ortamlarda daha fazla destek ve bilgi sağlanması, COVID-19'da olduğu gibi, önerilen diğer aşıların da uygulanma oranlarını artırabilir.*

Anahtar kelimeler: *COVID-19 aşısı; diyabetes mellitus; aşı reddi*

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Introduction

The pandemic spread of the novel coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which causes Coronavirus Disease 2019 (COVID-19), has seriously threatened public health worldwide. Great efforts have been made globally to develop and distribute effective vaccines during the pandemic. Manufactured COVID-19 vaccines can effectively prevent serious COVID-19. By October 2022, 68.2% of the world's population had received at least one dose of COVID-19 vaccine[1]. Currently, vaccines are the most important strategy in combating this spread. Although their effectiveness depends on the frequency of vaccination in the population, it can be greatly affected by vaccine hesitancy[2,3].

Mass vaccination programs target herd immunity. This aims to vaccinate a large portion of the population to slow disease transmission. It also protects vulnerable individuals who cannot be vaccinated[4]. There is an increased incidence of vaccine hesitancy, defined as delayed acceptance of a vaccine despite its availability. Vaccine hesitancy has, therefore, led to it being listed by the World Health Organization (WHO) as one of the top 10 global health threats[5,6]. Vaccine hesitancy is a complex problem that disrupts herd immunity and arises depending on the time, place, and type of vaccine[7].

Immune system weakness is present in patients with diabetes mellitus (DM) as the result of dysfunction of neutrophils and macrophages (innate immune response) and dysfunction of T cells (acquired immune response)[8]. It is well known that DM in COVID-19 patients is associated with a significant increase in mortality and severity of COVID-19 compared to non-diabetic patients[9]. For this reason, the priority of the COVID-19 vaccine is given to the population with DM, and the COVID-19 vaccine is strongly recommended for this group[10].

In this study, we aimed to investigate the social, demographic, and clinical factors affecting vaccination frequency and vaccination hesitancy in our patients with DM.

Material and Methods

Study Design

Patients who were followed up in our endocrinology polyclinic diagnosed with DM and had the physical and mental capacity to answer the questionnaire were included in the study according to the admission order. Hereby, a questionnaire including pre-prepared questions was applied to 206 patients. The patient's age, gender, duration, types of DM, and insulin use were recorded simultaneously.

Consent was obtained from the patients who agreed to participate in the study, and their knowledge levels were measured using the face-to-face survey method. Ethics committee approval for the study was obtained from Harran University Clinical Research Ethics Committee on 23.05.2022 with a decision numbered HRU/22.10.05 in accordance with the ethical principles for human investigations, as outlined by the Helsinki Declaration.

Patients who answered the questionnaire were divided into two groups according to COVID-19 vaccination: vaccinated patients as Group 1 (n=180) and unvaccinated as Group 2 (n=26).

Statistical Analysis

Categorical data were presented as % (n), and other data as mean ± standard deviation (SD). Categorical data between groups were compared with the Chi-square test, and those that were constantly variable were compared with the Student T, or Mann Whitney U tests according to the data distribution. Statistical analyses were performed with IBM Statistical Package for Social Sciences (SPSS) program version 20.0 (IBM Inc., Chicago, Illinois, USA). $p<0.05$ was considered significant.

Results

The frequency of vaccination was 87.4% (n=180). According to the number of COVID-19 vaccines, the frequency was 6.3% (n=13) for one vaccine, 53.0% (n=109) for two vaccines, 22.3% (n=46) for three vaccines, 4.9% (n=10) for four vaccines and 0.9% (n=2) for five vaccines. There was no difference between the vaccinated and unvaccinated groups regarding age, diabetes duration, gender, type of diabetes, insulin use, smoking, marital status, living place, and educational status. Moreover, these two groups of patients had a history of having COVID-19 with almost similar frequency. Additionally, there was no significant difference between vaccinated and unvaccinated patients in having a relative with COVID-19 and losing one of their relatives as a result of the disease. All of the data are shown in Table 1. As compared to the vaccinated group (26%.1), the unvaccinated group (7.7%) had a significantly lower frequency of influenza vaccination

Table 1. *Comparison of social, demographic, and clinical data between vaccinated and unvaccinated patients*

Parameter	Group 1 (vaccinated) (n=180)	Group 2 (unvaccinated) (n=26)	p
Age (year)	54.9±12.8	50.0±16.0	0.177
Duration of diabetes (year)	8.3±7.3	9.2±8.4	0.704
Gender (n(%))			
Female	106 (58.9)	19 (73.1)	0.201
Male	74 (41.1)	7 (26.9)	
Type of diabetes (n(%))			
Type 1	83 (46.1)	14 (53.8)	0.426
Type 2	97 (53.9)	12 (46.2)	
Use of insulin? (n(%))			
Yes	37 (48.4)	7 (50)	0.531
No	143 (51.6)	7 (50)	
Smoking (n(%))			
Yes	37(20.6)	5 (19.2)	0.999
No	143 (79.4)	21 (80.8)	
Marital status (n(%))			
Married	172 (95.6)	24 (92.3)	0.631
Single	7(3.9)	2 (7.7)	
Widow	1 (.6)	0 (0)	
Living place			
Provincial center	116 (64.4)	15 (57.7)	0.440
District	36 (20.0)	8 (30.8)	
Village	28 (15.6)	3 (11.5)	
Educational status			
Illiterate	83 (55.9)	11 (42.3)	
Primary school	53 (25.8)	13 (50.0)	0.114
Middle-high school	26 (12.9)	1 (3.8)	
University	18 (5.4)	1 (3.8)	
Catching COVID-19			
Yes	118 (65.6)	14 (53.8)	0.501
No	62 (34.4)	12 (46.2)	
Have a relative with COVID-19			
Yes	95 (52.8)	14 (53.8)	0.999
No	85 (47.2)	12 (46.2)	
Died of a relative due to COVID-19			
Yes	10 (5.6)	0 (0)	0.618
No	170 (94.4)	26(100)	
Influenza vaccination in any time			
Yes	47 (26.1)	2 (7.7)	0.047
No	133 (73.9)	24 (92.3)	
Influenza vaccination in last year			
Yes	18 (10.0)	0 (0)	0.137
No	162 (90.0)	26 (100)	
Pneumococcal vaccine			
Yes	21 (11.7)	1 (3.8)	0.322
No	159 (88.3)	25 (96.2)	

(p=0.047). Although none of the patients in the unvaccinated group and 10.0% (n=18) patients in the vaccinated group had influenza vaccine in the last year, this difference was not statistically significant. Similarly, there was no significant difference in the frequency of pneumococcal vaccine between the two groups.

Discussion

In the current study, we examined the frequency of vaccination of outpatients with DM and found it to be 87.4%. During the study period, the Turkish Ministry of Health reported that 93.0% of the population over 18 years had received their first vaccination dose, and 85.3% had received their second vaccination[11]. On the other hand, the frequency of at least two vaccination doses was reported as 62.4% in our province, Sanliurfa (The Türkiye Ministry of Health announced the frequency of vaccination for only at least two doses in each province)[11]. When the study was conducted, our province ranked as one of the five provinces with the lowest vaccination rates. The frequency of at least two vaccination doses in our study group was 81.1%. Our results showed that DM patients were unaffected by high region-specific vaccine hesitancy. They were vaccinated at a rate similar to vaccination frequency across the country.

After the WHO declared a COVID-19 pandemic on March 11, 2020, it has been understood that the virus can affect all age groups and genders, spread rapidly, and cause serious complications and deaths. It was announced that those over 65 years of age and those with comorbidities such as hypertension, DM, chronic obstructive pulmonary disease (COPD), malignancy, and immunodeficiency are in the high-risk group[12,13]. Turkey's vaccination process started on January 13, 2021, and priority was given to chronic patients, including DM[14].

Routine vaccination against pneumococcal disease, influenza, and hepatitis B is highly recommended for diabetics[15,16]. In the studies about vaccination rates in Turkish patients with DM, influenza and pneumococcal vaccination rates were found between 14.6 to 27.0 % and 3.6 to 9.8 %, respectively[17–19]. In our study, influenza vaccination rates were 23.8% overall and 26.1% among vaccinated patients. The influenza vaccination rate was significantly higher in the vaccinated group than in the unvaccinated group. On the other hand, pneumococcal vaccination rates were 10.7 % overall and 11.7 % among vaccinated patients in this study. Therefore, we found almost the same rates as previous studies in Türkiye.

A Spanish study showed that influenza vaccination frequency in patients with type 2 diabetes is quite low. The main barrier to vaccination is a need for more knowledge about the necessity, risks, and advantages

of influenza vaccines[20]. Moreover, according to another study, the main reasons for refusing influenza and pneumococcal vaccines in patients with diabetes were cost, not being fully convinced of their benefits, fear of complications, and, rarely, fear of needles[21]. There is universal opposition to the COVID-19 vaccine, which arises due to fear of its side effects, suspicion about its safety, short duration of immunity, doubts about its necessity and efficacy, and lack of knowledge[22]. Our study showed diabetic patients did not affect regional vaccine hesitancy and had COVID-19 vaccination levels similar to the general population. It has been shown that diabetes in COVID-19 patients is associated with a two-fold increase in mortality and severity[9]. Since effective pharmacological treatment is still not possible, patients with DM must be vaccinated to avoid COVID-19 infection and its serious consequences in case of illness[22]. This may be one of the reasons why COVID-19 vaccination is high in diabetic patients. Another explanation for the high frequency of vaccinations is that the press and social media frequently emphasize the importance of the COVID-19 vaccine and the ease of access to the vaccine, free of charge and without a prescription. In addition, the severe and sometimes fatal course of the disease and the sudden global pandemic, especially in those with chronic diseases, do not allow for questioning the necessity and risks of the vaccine.

We also examined the socio-demographic characteristics of the patients. Vaccinated patients were older than unvaccinated ones, but this difference was insignificant. Studies have shown that vaccination frequency increases with age. This is likely related to the increase in hospital visits with increasing age[19,23–25]. Although it was not significant, female rates were high in the unvaccinated group in our study. Several studies with conflicting results report an association between gender and vaccination rate in the general population and risk groups. The TEMD vaccination study indicates that the majority of vaccinated patients are males[19]. Another study showed that male gender was associated with higher vaccination rates[20]. Vaccinated and unvaccinated patients did not differ significantly based on other socio-demographic characteristics such as marital status, education level, or living location, such as whether they lived in rural or urban areas. We also asked about the type and duration of diabetes and the use of insulin. There was no difference between the conditions. This suggested that fear of needles did not affect vaccination rates. Furthermore, having COVID-19 or their relatives at any time and dying of a relative for COVID-19 were similar between the two groups.

In conclusion, the patients with DM had higher COVID-19 vaccination rates than our overall province rate. Still, they were the same as the general population of Türkiye, regardless of demographical, social, and some individual properties. Therefore, providing greater support and information in various settings about the serious consequences of vaccine-preventable diseases such as influenza, pneumococcus, and hepatitis B in patients with DM could increase the rates of these vaccines, as is provided in COVID-19.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Correlation and Clinical Impact of Ambulatory, Home, and Office Measurements in Hypertensive Patients

Hipertansiyon Hastalarında Ambulatuar, Ev ve Ofis Ölçümlerinin Korelasyonu ve Klinik Etkisi

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ABSTRACT

Aim: *Hypertension is a major cardiovascular risk factor and the most common chronic disease. Being far from the targeted level in diagnosis and treatment has led to frequent questioning of measurement methods. Although out-of-office measurements are gaining prominence, the reliability and clinical effects of office and out-of-office measurement methods are open to question in terms of both usability and correlation. Our aim in this study was to evaluate the correlation of ambulatory, home, and office measurements and their clinical implications in hypertensive patients.*

Material and Method: *We evaluated out-of-office blood pressure measurements of patients who were followed up with a diagnosis of hypertension. Patients who were scheduled to have ambulatory blood pressure measurements were asked to have home measurements five days before this measurement. Oscillometric and auscultatory methods used for office measurements. Home measurements were performed with the patient's own calibrated devices. Ambulatory measurements were performed after five days of home follow-up.*

Results: *The study included 463 patients with a mean age of 49±15 years, 52% of whom were women. The number of patients diagnosed with non-regulated hypertension, based on home (256) and office (220) measurements, was much higher than the number of patients based on daytime (63) and average (87) ambulatory measurements. Among the patients considered regulated according to daytime ambulatory measurements, 157 (71.4%) were evaluated as hypertension according to office blood pressure, and 193 (75.4%) were assessed as non-regulated hypertension according to home blood pressure. When the correlation between blood pressure measurements was analyzed, it was observed that there was a high correlation between home and office blood pressure (r: 0.922, p<0.001) and a low correlation between ambulatory measurements and home and office blood pressure (r: 0.438, r: 0.459, p<0.001).*

Conclusion: *In a significant proportion of patients who were decided to be unregulated while being followed up with a diagnosis of hypertension due to office measurements, it was decided that the follow-up was regulated as a result of ambulatory measurements. The high correlation between home and office measurements and the low correlation between ambulatory measurements, which differs from these methods, are noteworthy.*

Keywords: *hypertension; out-of-office blood pressure; ambulatory blood pressure*

ÖZET

Amaç: *Hipertansiyon majör kardiyovasküler risk faktörü olmasının yanısıra en sık görülen kronik hastalıktır. Tanı ve tedavisinde hedeflenen düzeyden uzak olunması ölçüm yöntemlerinin sıkça sorgulanmasına neden olmuştur. Ofis dışı ölçümler ön plana çıkıyor olmakla birlikte ofis ve ofis dışı ölçüm yöntemlerinin güvenilirliği ve klinik etkileri hem kullanılabilirlik hem de korelasyon açısından sorgulanmaya açıktır. Bizim bu çalışmadaki amacımız hipertansiyon hastalarında ambulatuvar, ev ve ofis ölçümlerinin korelasyonunun ve bunun klinik etkilerinin değerlendirilmesidir.*

Materyal ve Metot: *Hipertansiyon tanısıyla takip edilen hastaların ofis dışı tansiyon ölçümleri değerlendirildi. Ambulatuvar tansiyon ölçümleri planlanan hastaların bu ölçümden beş gün öncesinde ev ölçümlerinin yapılması istendi. Ofis ölçümleri osilometrik ve oskülatuvar yöntemle ölçüldü. Ev ölçümleri hastaların kendi kalibre edilmiş cihazlarıyla yapıldı. Ambulatuvar ölçümler beş günlük ev takiplerinin sonrasında uygulandı.*

Bulgular: *Çalışmaya 49±15 yaş ortalamasına sahip ve %52'si kadın hastalardan oluşan 463 hasta dâhil edilmiştir. Ev (256) ve ofis (220) ölçümlerine göre regüle olmayan hipertansiyon tanısı alan hasta sayısı, gündüz (63) ve ortalama (87) ambulatuvar ölçümlerine göre regüle olmadığı tanısına varılan hastalardan çok daha fazlaydı. Gündüz ambulatuvar ölçümlerine göre regüle kabul edilen hastalardan 157 (%71,4)'si ofis tansiyonlarına göre, 193 (%75,4)'ü ev tansiyonlarına göre regüle olmayan hipertansiyon olarak değerlendirilmiştir. Tansiyon ölçümlerinin kendi aralarındaki korelasyona bakıldığında ev ve ofis tansiyonları arasında yüksek bir korelasyon (r: 0,922, p<0,001), ambulatuvar ölçümleri ile ev ve ilk ofis tansiyonları arasında ise düşük derecede bir korelasyon (r: 0,438, r: 0,459, p<0,001) olduğu gözlendi.*

Sonuç: *Ofis ölçümleri sonucu hipertansiyon tanısıyla takipli iken regüle olmadığına karar verilen hastaların çok önemli bir kısmında ambulatuvar ölçümler sonucu takibin regüle olduğuna karar verilmiştir. Ev ve ofis ölçümleri arasındaki yüksek korelasyon ile bu ölçüm yöntemlerinden ayrışan ambulatuvar ölçüm arasındaki düşük korelasyon dikkat çekicidir.*

Anahtar kelimeler: *hipertansiyon; ofis dışı kan basıncı; ambulatuvar kan basıncı*

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Introduction

Hypertension is the most common chronic disease and continues to be increasingly important as a major cardiovascular risk factor[1]. With the lowering of hypertension thresholds in current guidelines, the increase in its prevalence has become evident[2]. The clinical importance of hypertension is reinforced by the fact that it is a preventable risk factor, and blood pressure control dramatically reduces cardiovascular mortality and morbidity[3]. Every five mmHg decrease in systolic blood pressure is associated with a 13% reduction in the risk of cardiovascular events. In contrast, every two mmHg decrease in diastolic blood pressure is associated with a 12% reduction in the risk of cardiovascular events[4] –data obtained from office measurements. Despite the increasing number of treatment options and improved monitoring methods, treatment success is far from reaching the desired level. In this sense, it is clear that it is time for some innovations, especially in the new diagnosis and follow-up of hypertension, and it is obvious that current strategies need to be revised to achieve the targeted success at most points[5]. Ambulatory blood pressure measurement has significantly improved the disadvantages of office measurements in hypertensive patients and the general population[6].

For follow-up and treatment purposes, clinicians prefer office, home, or ambulatory blood pressure measurements by filtering the advantages and disadvantages of blood pressure measurement methods through their personal experience. Home measurements are often the out-of-office method of choice, and large analyses have demonstrated a clear association with cardiovascular events[7]. The HONEST study showed the prognostic value of morning systolic blood pressure measurements in predicting cardiovascular events. Its prognostic power is higher than office systolic measurements, especially in patients followed under treatment[8]. In the IDHOCO study, home measurements were shown to have a higher prognostic value in patients receiving treatment[7].

Out-of-office blood pressure monitoring is recommended to diagnose hypertension and the high-risk patient group[2,9,10]. Ambulatory blood pressure measurements have started to be given a leading role, with some limitations such as reachment difficulties and cost. The American College of Cardiology/American Heart Association (ACC/AHA) hypertension guidelines, the European Society of Cardiology (ESC) arterial hypertension guidelines, and the Turkish hypertension consensus report increasingly recommend ambulatory blood pressure measurement[2,11,12].

Ambulatory blood pressure measurements have been questioned in cardiovascular risk and mortality studies, and their value in this regard has been demonstrated[13–15]. Another important advantage of this measurement method, which brings an important power to clinicians in diagnosing and following hypertension, is the evaluation of blood pressure phenotypes of cardiovascular importance, such as masked hypertension and white coat hypertension. For example, masked hypertension can be diagnosed in 29.3% of patients diagnosed with prehypertension, and this diagnosis can be easily missed without out-of-office blood pressure measurement[16]. Similarly, superiority to office measurements has been demonstrated in hypertension phenotypes[17–19] known to have increased target organ damage, such as non dipper, nocturnal hypertension, and morning BP surge. Another advantage is the identification of hypotensive states, which may be an important obstacle to patient-physician cooperation in the treatment and follow-up of hypertension[20]. In this way, it can significantly reduce the rate of inadequate or unnecessary medication[11]. Despite all these important advantages and clear guideline recommendations, especially in patients with newly diagnosed hypertension, utilization rates are far from the desired levels[21,22]. At this point, home measurements still seem to be the first choice of clinicians for out-of-office blood pressure measurement due to their inclusion of the patient in the process, ease of application, and accessibility. In addition to the many advantages of ambulatory measurements, factors such as cost, accessibility, and intolerance of some patients can be counted among the disadvantages[2]. Home measurements have been shown to provide better blood pressure control than office measurements[23] and be superior to those in cardiovascular risk assessment[8,24]. Although cardiovascular outcomes and blood pressure control have been evaluated in many studies, the reliability of home measurements or their correlation with other measurement methods has received different attention. Office dependency on blood pressure measurement has been eliminated with technological developments, and the consistency and correlation of out-of-office measurements are especially important in the follow-up and treatment phase.

At this point, it is a clear fact that the success rate in the follow-up of a major risk factor such as hypertension, with treatment rates that are still not at the desired level despite the availability of treatment alternatives, should be increased. In this study, we aimed to investigate the correlation of ambulatory blood pressure

measurements, which have become clinically mandatory at many points, with office and home measurements and the clinical implications of this correlation.

Material and Methods

This study was conducted in two different centers between 01.06.2022–31.11.2022. Ambulatory blood pressure measurement was planned in patients with a diagnosis of hypertension who applied to the outpatient clinic in six months, and 463 patients were included in the study.

Patients over 18 years of age who were not planning to change their treatment and who had been receiving treatment for at least one month were included in the study. Patients were informed about the study, and written informed consent was obtained. Patients who refused to participate in the study and had a previous cardiovascular event were excluded. Office measurements were made by oscillometric and auscultatory methods using regularly calibrated devices with an arm cuff. Ambulatory blood pressure measurements were performed with regularly calibrated ambulatory blood pressure monitors. Patients with a complete 24-hour measurement and at least 70% of the measurements could be evaluated were included in the study. The patient monitored home measurements twice daily, both in the morning and evening, five days before the ambulatory blood pressure evaluation. For home measurements, the patients were required to perform the measurements themselves. Patients who were unable to meet this requirement were excluded from the study.

Limit values and stages for office, home, and ambulatory measurements were assessed as described in the European Society of Cardiology guidelines for managing arterial hypertension.

In office measurements, the patient's blood pressure was measured after resting in a sitting position for 5 minutes. Two measurements were taken, and the averages of these measurements were recorded. An automated device was used in one measurement, while the traditional auscultatory method was used in the other. For office measurements, the average of the two measurements was recorded.

The recommendation for home measurements is that the patient rest in a calm environment for 5 minutes, then sit in a sitting position with the back and arm resting on something. Each time, two measurements were taken in each arm and averaged. No additional recommendations were made beyond those made in routine practice in this way, aiming to reflect real-life data more clearly. All patients were requested to perform this measurement five consecutive days before the ambulatory measurement appointment. For home measurements, the device used by the patient must be on the list of validated devices. Patients who did not have were excluded from the study.

Ambulatory measurements were performed with validated and regularly calibrated Suntech® oscillometric devices. On the day of ambulatory measurements, the patient was advised not to isolate herself and to continue her daily routine similarly. She was told to keep her arm still during the measurement moments. For ambulatory measurements, comparisons were made over day, night and averages.

All patients included in the study were administered a mini-survey of two questions to assess patient comfort with ambulatory measurements (Table 1). The results have been saved.

Table 1. *Mini-questionnaire assessing ambulatory measurement comfort for patients*

Did the ambulatory blood pressure measurement cause any discomfort in your daily life and during sleep at night?
Yes No
If yes, which item best describes this feeling of discomfort?
○ Very mild
○ Mild
○ Moderate
○ Severe
○ Very severe

The local ethics committee's decision approved our study numbered 23/02-06.

Statistical Analysis

Analyses were performed using IBM Statistical Package for Social Sciences (SPSS) program version 20.0 for Windows® statistical program (IBM Inc. Chicago, IL, USA). Number, percentage, mean, standard deviation, median, minimum, and maximum were used to present descriptive data. The Kolmogorov-Smirnov Test evaluated the conformity of the data to normal distribution. The Pearson chi-square and Fisher's Exact tests were used to compare categorical data. The T-test was used to compare two independent numerical data, and the Mann-Whitney U test was used when the data were not equally distributed. The Pearson correlation

Table 2. *Averages of blood pressure measurements*

Parameter	N	Minimum	Maximum	Mean	SD
Mean systolic (mmHg)	463	91	164	118.59	14.67
Mean diastolic (mmHg)	463	53	101	72.12	10.17
Night systolic (mmHg)	463	79	157	118.94	15.22
Night diastolic (mmHg)	463	48	105	71.92	10.73
Daytime systolic (mmHg)	463	86	155	118.43	15.69
Daytime diastolic (mmHg)	463	49	100	72.32	11.24
Home systolic (mmHg)	463	100	160	134.89	17.05
Home diastolic (mmHg)	463	30	100	80.88	9.80
Office first systolic (mmHg)	463	100	160	133.69	15.34
Office first diastolic (mmHg)	463	60	95	81.78	9.08
Office second systolic (mmHg)	463	100	155	131.13	15.76
Office second diastolic (mmHg)	463	60	100	80.94	9.63

SD: Standard Deviation.

Table 3. *Demographic data of hypertension patients*

Parameter	N (n=463)	Percentage (%)
Gender		
Male	221	47.7
Female	242	52.3
Risk Factors		
DM	66	14.3
Hyperlipidemia	87	18.8
Coronary artery disease	57	12.3
COPD	5	1.1
Anxiety	93	20.1
Medications		
ACE/ARB	88	19
Ca channel blocker	267	57.7
Combined	239	51.6
Beta blocker	197	42.5
MRA	6	1.3
Hypertension (mmHg)		
Mean	87	18.8
Night	126	27.2
Daytime	63	13.6
Home	256	55.3
Office first	220	47.5
Office second	171	36.9

DM; Diabetes Mellitus, COPD; Chronic Obstructive Pulmonary Disease, MRA; Mineralocorticoid Reseptor Antagonist;

test was used to determine the correlation between measurements.

Results were considered significant at $p<0.05$.

Findings

The mean age of the 463 patients included in the study was 49.26±15.83 years, and the age distribution range was 19–84. Of the patients, 242 (52.3%) were female and 221 (47.7%) were male. When the mean blood pressure measurements of the patients were analyzed, it was found that home (134.9/80.9 mmHg) and office (133.7/81.8 mmHg) measurements were higher than the other measurements (Table 2). Anxiety 93 (20.1%) and hyperlipidemia 87 (18.8%) were more prevalent than the other diseases (Table 3). The most commonly used antihypertensive drug group was Ca channel blockers [267 (57.7%)] (Table 3).

Table 4. *Correlation between blood pressure measurements*

Parameter		Mean Systolic (mmHg)	Home Systolic (mmHg)	Office First Systolic (mmHg)	Office Second Systolic (mmHg)
Mean Systolic (mmHg)	r value	1.000	0.438	0.459	0.514
	p value	-	<0.001	<0.001	<0.001
Home Systolic (mmHg)	r value	0.438	1.000	0.922	0.759
	p value	<0.001	-	<0.001	<0.001
Office First Systolic (mmHg)	r value	0.459	0.922	1.000	0.811
	p value	<0.001	<0.001	-	<0.001
Office Second Systolic (mmHg)	r value	0.514	0.759	0.811	1.000
	p value	<0.001	<0.001	<0.001	-

The number of patients judged to be unregulated based on home (256) and office (220) BPs was much higher than on daytime (63) and average (87) ambulatory measurements. Among the patients who were considered regulated according to daytime ambulatory measurements, 157 (71.4%) were evaluated as non-regulated according to office blood pressure and 193 (75.4%) according to home measurements (Table 3).

When the correlation between blood pressure measurements was analyzed, it was observed that there was a high correlation between home and first-office blood pressures (r: 0.922, $p<0.001$) and a low correlation between ambulatory measurements and home and office blood pressures (r: 0.438, r: 0.459, $p<0.001$) (Table 4).

The number of patients evaluated as regulated in all blood pressure measurements was 140 (30.2%). When these patients were compared with patients with unregulated hypertension, it was observed that controlled blood pressure was significantly higher in the female gender (p: 0.003), DM (p: 0.021), and hyperlipidemia ($p<0.001$) groups. It was found that blood pressure regulation was lower in the ACE/ARB group ($p<0.001$), while there was no significant difference between the two groups in other antihypertensives (Table 5).

Table 5. *Comparison of patients with regulated and unregulated blood pressure*

Parameter	Regulated (n=140)	Unregulated (n=323)	p value
Age (Mean ± SD)	48.09±8.71	49.76±18.05	0.296
Gender			
Male	52	169	**0.003**
Female	88	154	
Resume			
DM	12	54	**0.021**
Hyperlipidemia	10	77	**<0.001**
Coronary artery disease	12	45	0.107
COPD	1	4	0.523
Anxiety	23	70	0.196
Medications			
ACE/ARB	13	75	**<0.001**
Ca channel blocker	84	183	0.504
Any combination	78	161	0.246
Beta blocker	65	132	0.266
MRA	0	6	0.114

SD: Standard Deviation; DM: Diabetes Mellitus; COPD: Chronic Obstructive Pulmonary Disease; ACE/ARB: Angiotensin Converting Enzyme/Angiotensin Reseptor Blocker; MRA: Mineralocorticoid Reseptor Antagonist.

Discussion

Our main finding in this study was that 71.4% of the patients who were followed up with a diagnosis of hypertension and/or were judged to be unregulated in office conditions were evaluated as regulated by ambulatory blood pressure measurements. Our second important result is that although there is a high correlation between office and home measurements, the correlation between these and ambulatory measurements is low.

Out-of-office blood pressure measurement is recommended at diagnosis and in high-risk patients. The European Society of Cardiology/European Society of Hypertension (ESC/ESH) arterial hypertension guidelines and the American College of Cardiology/American Heart Association (ACC/AHA) hypertension guidelines strongly recommend out-of-office measurement at the diagnostic stage[2,6,15]. It is widely used as out-of-office, home and ambulatory measurements. While some advantages and disadvantages of both measurement methods come to the fore, it is undoubtedly a fact that clinicians use home measurements extensively in practice during follow-up and diagnosis. In this sense, although it provides a serious advantage that the patient is included in the follow-up, the reliability of a method used with this frequency has been questioned by researchers, especially with the recent prominence of ambulatory blood pressure assessment. At this point, office measurements are another corner of the question mark.

Ambulatory blood pressure measurements have become more prominent with the current guidelines. While the ESC/ESH guidelines for arterial hypertension emphasize some special conditions for ambulatory blood pressure measurement, the Turkish HT consensus report's recommendation to be used whenever possible reinforces the importance attributed to this measurement method[6,27].

Ambulatory and home measurements were compared with office measurements, particularly regarding cardiovascular disease risk assessment and outcomes. However, the correlation between these measures and their impact on diagnostic and therapeutic approaches has not received the same attention. Banegas JR[13] et al. concluded that ambulatory measurements are a stronger predictor of all-cause mortality than office measurements. In this study, the mean office systolic blood pressure was 147 mmHg for surviving patients, while the mean daytime systolic blood pressure was 131 mmHg in 24-hour measurements. Despite having different patient groups and study purposes, if these averages are evaluated for diagnostic purposes, similar to the results in our study, clinical measurements in the same patient group would indicate a diagnosis of stage I hypertension.

In contrast, the same would not be true for ambulatory daytime measurements. In another study evaluating type 2 diabetic patients, ambulatory blood pressure measurements were compared with office measurements in terms of cardiovascular risk classification, and a clear difference was obtained in the direction of ambulatory measurements[15]. Along with this unsurprising result, it is noteworthy that the mean clinical systolic measurement in the entire patient population was 148 mmHg at baseline. In contrast, the daytime mean was 131 mmHg in 24-hour measurements.

In the study by Uallachain GM[25] et al., interesting results were obtained comparing ambulatory and office measurements. Treatment was changed in 38% of patients, new treatment was initiated in 32%, and in 14% of patients who were considered hypertensive based on office measurements, the diagnosis was excluded by ambulatory measurement, and medication was discontinued. In our study, 157 (71.4%) patients diagnosed with stage I hypertension in office measurements were excluded by ambulatory measurements. In light of all

these data, there are other problems with office measurements, which are frequently questioned regarding cardiovascular risk assessment and endpoints, and where ambulatory measurements are found to be more valuable than office measurements in most studies. When the impact of office and ambulatory measurements on diagnosis and treatment is evaluated, it is obvious that ambulatory measurements will play a game-changing role at most points.

The correlation between ambulatory and home measurements was evaluated in some studies, and it was concluded that a moderate correlation was found[26]. However, researchers need to have clearer results on this issue. A meta-analysis evaluating these two measures concluded that which measure has better predictive power for CV risk[27] needs to be clarified. In another study, cardiovascular events and mortality endpoints of home and ambulatory measurements were evaluated, and the results obtained were insufficient to make a clear judgment[28]. In parallel, one study emphasized the stronger association of ambulatory measurements with left ventricular mass index and wall thickness, [14] while another study showed that home measurements were more strongly associated[29]. While all these studies focused on endpoints, the home and ambulatory measurement correlation assessment has yet to be emphasized much. The results of our study emphasize the inconsistency of office measurements, the correlation between home and office was also demonstrated. In this respect, the reliability of home measurements is again questionable. Indeed, in addition to evidence[30] that home measurements are not always reported accurately by patients, [31] it has also been shown that they may cause patient preconditioning that causes anxiety in the measurements.

Making a clear assessment of the reliability of home measurements and their correlation with ambulatory measurements is becoming scientific confusion. In this respect, we need to question the reliability and correlation of home and office measurements.

It is known that including the patient in the follow-up in home measurements increases medication adherence[32] and reduces the rate of clinic visits in follow-up[33]. This improves patient compliance and tolerance to home measurements and makes a clear contribution to assessing different blood pressure phenotypes[34]. It has also been shown to increase patient compliance with treatment medication[35].

Home measurements have been compared with office measurements in some studies. A meta-analysis found that blood pressure control was better in home measurements than office measurements[23]. The Finn-Home study shows that home measurements are significantly superior to office measurements regarding cardiovascular risk correlation[24]. Other studies compare the two measurement methods regarding cardiovascular risk and events and show the superiority of home measurements[8,24,36]. From another perspective, when the correlation of home, office, and ambulatory measurements was evaluated in these studies, the mean systolic blood pressure values were found to be 135.2 mmHg for both home and office measurements, especially during the follow-up period in the study by Shimada K[8] et al. However, in some studies, the results are different. In another study comparing home and ambulatory measurements with outcomes of cardiovascular events or mortality, the systolic mean of office measurements was 131.6 mmHg compared to 123.6 mmHg for home measurements[28]. In the study by Ragot S[37] et al., home and ambulatory measurements were correlated at the beginning of treatment, whereas office measurements were not. In our study, the correlation between home and office measurements was significant, whereas ambulatory measurements did not share this correlation. At this point, home measurements, which seem more dependent on personal characteristics, may give different results in societies at different sociocultural levels. The significant correlation observed between office and home measurements in our study may be parallel to these data in the existing literature, and the dissociation of the ambulatory measurement at this point emphasizes the question mark regarding home measurements.

In addition to the advantages of ambulatory measurements, such as reflecting the blood pressure values that the patient is exposed to in their own living space or in daily life conditions that seem more appropriate, a significant negative aspect that can be counted is seen in terms of patient comfort. As a result of the mini-survey in which the study patients were asked to evaluate whether they felt discomfort related to the 24-hour ambulatory measurement, 87% found it moderately or more uncomfortable. Higher compliance with home measurements and patient involvement in their treatment are considered among the advantages of ambulatory measurements reported in some studies. In contrast, patient comfort and less accessibility are considered to be the most prominent disadvantages

of ambulatory measurements. One study said that this aspect of ambulatory measurement limits its practical applicability[36]. Likewise, ambulatory measurements are not well tolerated by patients, especially at night[38]. In this respect, the results of our study support this view. An additional result is the question mark regarding the effect of inconvenience on the ambulatory measurements.

Some studies have evaluated ambulatory measurements as cost-effective[21,39], and our results may support this. Indeed, our results showed that ambulatory measurement, a more competent measurement method, significantly reduced the rate of new initiation or up-titration of medication in our patient population. Considering the positive effects of proper blood pressure control in cardiovascular and many other systems, cost-effectiveness becomes even more significant[40].

Considering all these data, the power of ambulatory blood pressure measurements to guide treatment in hypertensive patients is seen. The data in our study support the existing literature in the same patient group. This may play an important role in initiating pharmacologic treatment or titration of medication in clinical follow-up. The place of home measurements alone in decision-making seems open to question.

Conclusion

In our study, ambulatory measurements significantly excluded the follow-up results decided by office measurements. Considering the correlation between home measurements, where clinicians feel safer regarding patient follow-up and treatment, and office measurements, a serious question arises.

One of the important results of our study is that home measurements, which have been substituted at most points in clinical practice where office measurements are being used less and less as decision-makers, appear to be significantly discordant with ambulatory measurements. This result may reduce the importance attributed to home measurements in many studies, which is important because it is currently one of the most preferred measurement methods in clinical practice. In this sense, our study raises scientific attention about the reliability of home measurements, but large-scale studies are needed to provide a stronger recommendation.

Limitations

Considering the prevalence and incidence of hypertension, the number of patients in our study needs to be increased to make a stronger recommendation. Another limitation is the adjustment of cuff sizes to make blood pressure measurements more personalized. In our study, patients' own devices and standard cuff sizes were used in home measurements, while two sizes of cuffs, large and small, were used in ambulatory measurements. Our third limitation is that follow-up data, which could strengthen the consistency of our results, should have been collected.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Fusidic Acid Sensitivity in Methicillin-Resistant *Staphylococcus aureus* Strains Isolated From Hospital- and Community-Acquired Skin and Soft Tissue Infections

Hastane ve Toplum Kaynaklı Deri ve Yumuşak Doku Enfeksiyonlarından İzole Edilen Metisilin Dirençli Staphylococus Aureus Suşlarında Fusidik Asit Duyarlılığı

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ABSTRACT

Aim: *We aimed to detect the frequency of methicillin-resistant Staphylococcus aureus (MRSA) strains that were isolated from wound infections in our hospital, and then we want to evaluate the in vitro fusidic acid (FA) susceptibility rates of them to determine the place of FA in empirical treatment.*

Material and Method: *A total of 110 S. aureus strains, which were isolated from wound culture samples from various services and outpatients, were included in the study. The bacteria were identified and antibiogram using our microbiology laboratory's classical methods and VITEK 2 (Biomerieux, France) system. Methicillin sensitivity was evaluated according to Clinical and Laboratory Standards Institute (CLSI) criteria, and Fusidic acid sensitivity was assessed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. The data were recorded in the IBM Statistical Package for Social Sciences (SPSS) program version 17, statistical analyses were performed, and P <0.05 was considered significant.*

Results: *51 (46.4%) of S. aureus were isolated from service patients, and 59 (53.6%) were separated from outpatient clinics. The total methicillin resistance of 110 S. aureus strains was 20.9%. Methicillin resistance in outpatient clinics and services was 17.4% and 23.72%, respectively; their difference was not statistically significant (P >0.05). Sensitivity rates of FA in the outpatient clinics and services were found to be 90.2% and 94.9%, respectively, and the difference was not statistically significant (P >0.05). Methicillin-resistant Staphylococcus aureus was primarily isolated from pediatrics, orthopedics, general surgery, and otorhinolaryngology. In these units, FA sensitivities were 93.7%, 96.1%, 100%, and 100%, respectively.*

Conclusion: *FA should be considered an effective and safe empirical treatment option in treating soft tissue and wound infections caused by community and hospital-acquired MRSA.*

Keywords: *fusidic acid; wound infections; methicillin-resistant Staphylococcus aureus*

ÖZET

Amaç: *Hastanemizde yara enfeksiyonlarından izole edilen metisiline dirençli Staphylococcus aureus (MRSA) suşlarının sıklığı ve bunların fusidik asite (FA) in vitro duyarlılığı değerlendirerek FA'in ampirik tedavideki yerini göstermeyi amaçladık.*

Materyal ve Metot: *Çeşitli servis ve polikliniklerden alınan yara kültür örneklerinden izole edilen toplam 110 S. aureus suşu çalışmaya dâhil edildi. Bakterilerin tanımlanması ve antibiyotiklere olan duyarlılığı mikrobiyoloji laboratuvarımızda klasik yöntemler ve VITEK 2 (Biomerieux, Fransa) sistemi ile gerçekleştirildi. Metisilin duyarlılığı, Klinik ve Laboratuvar Standartları Enstitüsü (CLSI) kriterlerine göre ve Fusidik asit duyarlılığı ise European Committee on Antimicrobial Susceptibility Testing (EUCAST) kriterlerine göre değerlendirildi. Veriler IBM Sosyal Bilimlerde İstatistik Paket Programı (SPSS) sürüm 17 programına kaydedilerek istatistiksel analizleri yapıldı (P <0,05 anlamlı kabul edildi).*

Bulgular: *S. aureus şuşlarının 51'i (%46,4) servis hastalarından 59'u (%53,6) polikliniklerinden izole edildi. One hundred and ten S. aureus suşunun total metisilin direnci %20,9 olarak bulundu. Polikliniklerde ve servislerde metisilin direnci sırasıyla %17,4 ve %23,7 idi ve aralarındaki fark istatistiksel olarak anlamlı değildi (P >0,05). Poliklinik hastalarında ve servis hastalarında FA sensitivitesi sırasıyla %90,2 ve %94,9 olarak bulundu ve aradaki fark istatistiksel olarak anlamlı değildi (P >0,05). Methicillin-resistant Staphylococcus aureus çoğunlukla pediyatri, ortopedi, genel cerrahi ve Kulak Burun Boğaz (KBB) ünitelerinden izole edildi. Bu birimlerde FA sensitiviteleri sırasıyla %93,7, %96,1, %100, %100 idi.*

Sonuç: *FA, toplum ve hastane kaynaklı MRSA'ların neden olduğu yumuşak doku ve yara enfeksiyonlarının tedavisinde etkili ve güvenli bir ampirik tedavi seçeneği olarak akılda tutulmalıdır.*

Anahtar kelimeler: *fusidik asit; yara enfeksiyonları; metisiline dirençli Staphylococcus aureus*

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Introduction

Staphylococci are microorganisms which are located in the normal flora of the skin. Digestive and respiratory tracts can affect a wide range of skin and mucosa diseases, from upper respiratory tract infections to severe systemic infections.[1]

Today, methicillin resistance is one of the most important problems in treating staphylococcal infections. Methicillin-resistant staphylococcus strains are considered resistant to penicillins, combinations of β-lactam / β-lactamase inhibitors, cephalosporins, monobactams, and carbapenems. These strains also appear resistant to macrolides, clindamycin, chloramphenicol, tetracyclines, aminoglycosides, and quinolones.[2] There are a limited number of antibiotics that can be used in the treatment of these infections. Glycopeptides are antimicrobials that can be preferred for the treatment of these infections. But, if precautions are not taken, the widespread use of the glycopeptide group (vancomycin and teicoplanin) antibiotics will cause the development of resistant strains of staphylococci, leading to major problems in the treatment of these infections.[3]

Besides the risk of resistance development to glycopeptides, they can be used only parenterally and are also nephrotoxic. All these adverse situations lead researchers to find alternative effective treatments with minimal side effects.

In recent years, fusidic acid has been started to be the preferred antibiotic as a successful alternative treatment for skin and soft tissue infections. This antibiotic is known to be effective on MRSA and *methicillin-resistant Staphylococcus epidermidis* (MRSE). In addition, this antibiotic can be used parenterally, orally, and topically. When taken orally, it is completely absorbed, and it has good penetration to various body tissues, such as joint fluid, bone, and subcutaneous fat tissue.[3,4,5]

It is a drug recommended for the treatment of both systemic and cutaneous staphylococcal infections because of its low toxicity and low allergic reactions. However, it does not cross-react with other antibiotics due to its specificity in the mechanism of action.[6]

FA may be an alternative safe and effective choice of antibiotics in the empirical treatment of wound infections.

We aimed to determine the methicillin resistance of Staphylococcus aureus strains isolated from the polyclinic and service patients. We also wanted to determine the FA sensitivity of these MRSA strains in our hospital. Thus, we wanted to take attention to the place and importance of FA in treating skin and soft tissue infections.

Material and Method

After the ethics committee's approval from Kocaeli University Faculty of Medicine with decision No. 22/16 dated 18.11.2014 was taken, wound culture samples were collected from various services and outpatient clinics. After the wounds were cleaned with 70% alcohol, swabs were taken from the wounds with sterile cotton swabs, and the abscess contents were aspirated with sterile injectors. The samples were immediately transferred to microbiology laboratories and were incubated for 18-24 hours at 37°C in 5% sheep blood agar, Eosin Methylene Blue agar (EMB), and chocolate agar media. After gram staining, the light microscopic examination was done under x100 objective by dripping immersion oil on all samples. If the sample was taken from a sterile body area with few flat epithelial cells, it was considered significant even if leukocytes were detected on gram staining. As a result, 110 S. aureus strains isolated from infected skin lesions such as folliculitis, impetigo, erysipelas, surgical wound infections, and abscesses were included in the study.S. aureus strain was identified both by VITEK 2 Compact System fully automated identification system and by conventional methods such as catalase test, coagulase test. The susceptibility of the strains to antibiotics was determined with the VITEK 2 Compact System system, and the limit values were evaluated under the guidance of CLSI.[7] Since there is no CLSI-approved standard limit value for fusidic acid, those with a minimum inhibitory concentration (MIC) value of ≤1 μg/ml according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were evaluated as sensitive.[8]

If repetitive bacterial growth occurred in the same patient, they were excluded from the study. S. aureus ATCC 29213 and MRSA ATCC 43300 strains were used for quality control. The data were recorded in the SPSS 17 program, and statistical analyses were performed. $P<0.05$ was considered significant.

Results

110 S.aureus strains were obtained from the wound cultures; 51 (46.4%) were isolated from service patients,

Table 1. *Distribution of wound culture samples according to the medical units they were sent*

Units	n	%
Orthopedics	26	23.6
Dialysis	1	0.9
Burn	12	10.9
Infection Diseases	15	13.6
Nephrology	1	0.9
Intensive Care Unite	2	1.8
Plastic surgery	4	3.6
Cardiovascular surgery	5	4.54
Gastroenterology	2	1.8
Brain surgery	3	2.7
Pediatric diseases	16	14.5
Dermatology	9	8.2
Obtetrics and gynecology	3	2.7
Urology	1	0.9
General surgery	7	6.4
Otolaryngology	3	2.7
Total	110	

Table 2. *Methicillin-resistant rates of outpatient clinics and services*

	METHICILLIN		
Units	Sensitive n (%)	Resistant n (%)	Total
Outpatient clinics	42 (82.36)	9 (17.64)	51
Services	45 (76.28)	14 (23.72)	59
Total	87 (79.1)	23 (20.9)	110

Table 3. *Fusidic acid sensitivity rates of outpatient clinics and services*

	FA			
Units	Sensitive n (%)	Resistant n (%)	Less sensitive n (%)	Total
Outpatient clinics	46 (90.2)	4 (7.8)	1 (2)	51
Services	56 (94.9)	1 (1.7)	2 (3.4)	59
Total	102 (92.7)	5 (4.5)	3 (2.7)	110

Fusidic acid: FA.

and 59 (53.6%) were isolated from outpatient clinics. The samples mostly came from orthopedics, pediatrics, infectious diseases, and burn units (Table 1).

The total methicillin resistance of these 110 S. aureus strains was 20.9%. Methicillin resistance in policlinics and services was 17.4% and 23.72%, respectively, and the difference was not statistically significant (p>0.05). (Table 2).

When we examined FA sensitivity, 102 of 110 strains (92.7%) were sensitive, 3 of them (2.7%) were less sensitive, and 5 of them (4.5%) were resistant. In the outpatient clinics and services, the sensitivity rate to FA was 90.2 % and 94.9 %, respectively, and the difference was not statistically significant (Table 3). (P>0.05).

Table 4. *Distribution of MRSA according to units*

Units	n (%)
Orthopedics	4 (17.4)
Dialysis	1 (4.3)
Burn unit	2 (8.7)
Cardiovascular surgery	1 (4.3)
Pediatrics	6 (26.1)
Dermatology	2 (8.7)
Urology	1 (4.3)
General surgery	3 (13)
Otolaryngology	3 (13)
Total	23

Table 5. *Fusidic acid sensitivity rates according to units*

Units	Sensitive n (%)	Resistant n (%)	Less sensitive n (%)	Total
Orthopedics	25 (96.1)	1 (3.9)	0 (0)	26
Dialysis	0 (0)	0 (0)	1 (100)	1
Burn unit	11 (91.6)	0 (0)	1 (8.4)	12
Infection Diseases	14 (93.3)	1 (6.7)	0 (0)	15
Nephrology	1 (100)	0 (0)	0 (0)	1
Coronary intensive care	2 (10)	0 (0)	0 (0)	2
Plastic surgery	4 (100)	0 (0)	0 (0)	4
Cardiovascular surgery	5 (100)	0 (0)	0 (0)	5
Gastroenterology	2 (100)	0 (0)	0 (0)	2
Brain surgery	2 (66.6)	0 (0)	1 (33.4)	3
Pediatric diseases	15 (93.7)	1 (6.3)	0 (0)	16
Dermatology	8 (88.8)	1 (11.2)	0 (0)	9
Ostetrics and gynecology	3 (100)	0 (0)	0 (0)	3
Urology	0 (0)	1 (100)	0 (0)	1
General surgery	7 (100)	0 (0)	0 (0)	7
Otolaryngology	3 (100)	0 (0)	0 (0)	3
Total	102	5	3	110

MRSA was primarily isolated from pediatrics, orthopedics, general surgery, and Otorhinolaryngology (Table 4).

In these units, FA sensitivities were found to be 93.7%, 96.1%, 100%, and 100%, respectively (Table 5).

Discussion

Recent studies have reported increased hospital admission rates due to skin-soft tissue infections. Bacterial infections of the skin are the most common infections in the community. In dermatology outpatient clinics, about 20% of patients were diagnosed with bacterial skin infections.[1,5] In bacterial skin infections, the most

frequently isolated pathogenic microorganism was reported as S. aureus .[9,10]

Resistance against penicillin and penicillinase-resistant antibiotics is a known big problem in treating S. aureus. Strains resistant to all beta-lactams, termed MRSA, were originally defined as hospital-acquired but later began to be isolated from community-acquired infections.[11] Chronic dermatoses, surgical operations, vascular injections, intensive care units, and systemic diseases are risk factors for MRSA infections. Still, recently, it is worrying that MRSA infections are beginning to be seen even in people who do not have these risk factors in society. The increasing resistance rates against commonly used antibiotics and isolation of methicillin-resistant strains in community-acquired infections have been major difficulties in managing S. aureus infections.[12]

Belbase et al. stated that knowing the regional prevalence of methicillin resistance and regional antibiotic susceptibility rates is important in combating MRSA.[13] When we look at the studies reporting the prevalence of MRSA in our country, the MRSA rates isolated from various clinical specimens ranged from 38.2% to 63%.[2,6,14] In our country and the world, the MRSA rates isolated from only wound specimens have been reported between 15% and 35%.[14,15] In our study, *S. aureus* strains isolated from wound specimens were only evaluated in both clinics and outpatient clinics, and the methicillin resistance was found to be 20.9%.

Hospital-acquired MRSA (HA-MRSA) strains were first reported in the United States in 1960.[10] In addition, several studies are investigating the prevalence of HA-MRSA strains. For example, in a study including only wound culture isolates of five centers in the US and Canada, the HA-MRSA ratio was found to be 30 %.[15] From our country, Doğan et al. explored HA-MRSA rates of isolates, including various clinical specimens, in 2001 and 2012, and the methicillin resistance rates were found to be 49.1% and 37.3%, respectively.[16] In our study, the HA-MRSA rate was 23.72%. Compared with the other studies, this ratio is lower than national and international data. Factors such as antibiotic usage guidelines and infection control measures affect the rate of MRSA. In our hospital, the use of restricted antibiotics and the regular work of the infection control committee can explain why our MRSA rates are low.

Community-acquired MRSA (CA-MRSA) strains were first reported in the mid-1990s.[17] CA-MRSA prevalence varies according to countries and regions. From various areas of the World, the CA-MRSA rate in skin and soft tissue infections has been reported to range from 1% to 69%, and from our country, it ranges from 1% to 12.5%.[5,9,18,19] The CA-MRSA rate in our study was found to be 17.64%. This result is similar to other studies in our country and is lower than worldwide studies.

After vancomycin-resistant strains were reported, different antibiotic treatments began to be tried for MRSA infections. Some drug combinations have been proposed as alternatives to vancomycin use in MRSA, such as FA-rifampin or FA-trimethoprim-sulfamethoxazole (TMP-SMZ).[20,21]

When we search the literature, studies report FA sensitivity in MRSA and MSSA isolated from various clinical specimens. In these previous studies, FA susceptibility rates were reported to be ranging from 92.3% to 97% for MRSA and ranging from 94% to 100% for MSSA .[2,14,16,22,23]

In our study, FA sensitivity was 92.7% among all S.aureus strains and 94.25% and 87% for MSSA and MRSA strains, respectively. In addition, susceptibility rates of the FA in outpatient and service patients were found to be 90.2% and 94.9%, respectively. These results have shown that FA may be a good treatment option for wound infections caused by MRSA and MSSA. These ratios make FA a valuable treatment alternative, particularly in outpatient clinics in MRSA where treatment options are limited.

In the previous studies, S.aureus strains isolated from wound infections were reported to be mostly isolated in dermatology, orthopedic, general surgery, and internal diseases clinics.[15,24] When we evaluated the distribution of the samples in our study, the samples were mostly isolated from orthopedics, pediatrics, infectious diseases, and burn units.

When we look at the distribution of the units where MRSA strains are most isolated, it was seen that the studies reported similar results. For example, in a study conducted in Russia, MRSA patients were mainly isolated from burn, intensive care, and orthopedics clinics.[17] In our study, MRSA was mainly isolated from pediatric diseases and orthopedics, followed by general surgery and otolaryngology units

In our study, FA susceptibilities of MRSA isolates in pediatric diseases, orthopedics, general surgery, and otolaryngology

units were found to be 93.75%, 96.15%, 100%, and 100%, respectively.

This data will be informative in starting empirical treatment, especially in wound infections, which will occur in these units. Data related to the types of infectious agents and the development of antibiotic resistance rates must be shared with clinicians. This approach will contribute to reducing resistance rates to antibiotics.

Several studies are comparing the susceptibility of staphylococcal infections to FA and other antibiotics. Altun et al. compared FA with methicillin, ofloxacin, erythromycin, sulbactam/ampicillin (SAM), and TMP-SMZ, and they found that FA was the most effective antibiotic for MRSA strains among these antibiotics.[25]

Demir et al. compared resistance rates of the most commonly used antibiotics, SAM and amoxicillin-clavulanic acid. FA resistance rates were 36.4%, 45.5%, and 18.2%, respectively.[10] This study also shows that F.A. is more effective in empirical treatment than other antibiotics.

In another study, glycopeptides, linezolid, and FA were mentioned to be the most effective antibiotics for *S. aureus* strains. No resistance against these antibiotics was reported.[17] In another study, FA and TMP-SMZ susceptibility rates were compared, and FA was reported to be as effective as TMP-SMZ.[26] Therefore, FA appears to be an alternative agent in MRSA infections, particularly in cases where long-term sequential therapy is required.

According to Atmaca S et al. Fusidic acid resistance in MRSA strains was reported as 18.9%, 22.3%, and 13.2% in 2001, 2011 and 2017, respectively. [27] A similar study conducted later at the same hospital, and it was observed that these rates were 5% in 2018 and 2019 and 4% in 2020.[28] Şanlı K. et al. was also found that the MRSA and MSSA strains isolated from various clinical samples were 8.2%-1.9% resistant to fusidic acid, respectively.[29] Together with these, in another study, the fusidic acid sensitivity of MRSA strains was 76.2%.[30]

In the hospital, MRSA infections are usually treated with intravascular (i.v) antibiotics such as vancomycin and teicoplanin. Treating these patients with antibiotics that can be used orally, such as FA, provides significant benefits for healthcare professionals and patients. It has the advantages of reducing the length of hospital stay, preventing other patients from being infected, not needing tools such as catheters, and reducing the energy loss of health workers. All these make FA the most effective and reliable treatment for skin infections.

Knowing hospital flora and resistance rates of bacteria are the most important factors in choosing empirical treatment of skin and soft tissue infections. Regular follow-up of antibiotic resistance rates may be useful in selecting the right treatment.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Boron Compounds with Antibiofilm and Synergistic Effects on *Escherichia coli* Infection

Escherichia coli Enfeksiyonu Üzerine Antibiyofilm ve Sinerjistik Etkili Bor Bileşikleri

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ABSTRACT

Aim: *Escherichia coli, a bacterium that forms a biofilm, is mainly responsible for intestinal and extraintestinal infections, such as urinary tract infections, peritonitis, meningitis, and septicemia. Different antibiotics (Aminoglycoside, Fluoroquinolones, etc.) treatments are routinely used to overcome E.coli infections. The presence of biofilm increases E.coli resistance against antibiotics. The study aims to evaluate the boron component's effect on the biofilm formation of E.coli in vitro via a liver (HepG-2) infection model.*

Material and Method: *The antibacterial activity of boron compounds on Escherichia coli was evaluated with Minimum inhibitory concentration. Antibacterial activity with the combination of boron compounds was assessed by fractional inhibitor concentration. The non-cytotoxic dose of boron compounds was determined in the infection model created with Escherichia coli in the cell line. Then, the effect on cell viability and pathological examinations were examined histopathologically.*

Results: *Synergistic effects (≤0.5) were watched at 32 µg/mL Etidote + 32 µg/mL Sodium perborate metahydrate, at Etidote 32 µg/mL + 32 µg/mL Zinc borate, and at Zinc borate 32 µg/mL + 32 µg/mL Sodium perborate metahydrate. It was determined that 128 µg/ml Etidote + 512 µg/ml Sodium perborate metahydrate, Etidote 512 µg/mL + Zinc borate 1024 µg/mL, and Zinc borate 512 µg/mL + Sodium perborate metahydrate 128 µg/mL had antibiofilm activity. In the cell line, it was determined that although Zinc borate + Etidote reduced the infection, it did not completely reduce it in its combination. Histopathological analyses also paralleled these results.*

Conclusion: *Boron components can be used against biofilm-formed E.coli.*

Keywords: *biofilm; boron compounds; Escherichia coli; HepG-2; minimum inhibitory concentration; fractional inhibitory concentration*

ÖZET

Amaç: *Biyofilm oluşturan bir bakteri olan Escherichia coli, esas olarak idrar yolu, peritonit, menenjit ve septisemi gibi bağırsak ve bağırsak dışı enfeksiyonlardan sorumludur. Escherichia coli enfeksiyonlarının üstesinden gelmek için farklı antibiyotikler (Aminoglikozid, Florokinolonlar vb.) tedavileri rutin olarak kullanılmaktadır. Biyofilm varlığı Escherichia coli 'nin antibiyotiklere karşı direncini artırır. Bu çalışmanın amacı, in vitro karaciğer (HepG-2) enfeksiyon modelinde bor bileşeninin Escherichia coli biyofilm oluşumu üzerindeki etkisini değerlendirmeye çalışmaktır.*

Materyal ve Metot: *Escherichia coli üzerine bor bileşiklerinin antibakteriyel etkinliği Minimum inhibitör konsantrasyonu ile değerlendirildi. Bor bileşiklerinin kominasyonu ile gösterdiği antibakteriyel etkinlik Franksiyonel inhibitör konsantrasyonu ile değerlendirildi. Hücre hattında Escherichia coli ile oluşturulan enfeksiyon modelinde bor bileşiklerinin sitotoksik olmayan dozu belirlendi. Ardından hücre canlılığına etkisi ve patolojik incelemeler histopatolojik olarak incelenmiştir.*

Bulgular: *Sinerjistik etkiler (≤0,5), 32 µg/mL Etidote + 32 µg/mL Sodyum perborat metahidratta, Etidote 32 µg/mL + 32 µg/mL Çinko boratta ve Çinko borat 32 µg/mL + Sodyum perborat metahidrat 32 µg/mL'de izlenmiştir. 128 µg/ml Etidote + 512 µg/ml Sodyum perborat metahidrat, Etidote 512 µg/mL + Çinko borat 1024 µg/mL ve Çinko borat 512 µg/mL + Sodyum perborat metahidrat 128 µg/mL'nin antibiyofilm aktivitesine sahip olduğu belirlendi. Hücre hattında Çinko borat + Etidote'un enfeksiyonu azaltmasına rağmen kombinasyonunun tamamen azaltmadığı belirlendi. Histopatolojik analizler de bu sonuçlarla paralellik gösterdi.*

Sonuç: *Bor bileşenleri biyofilm oluşturan Escherichia coli'ye karşı kullanılma potansiyeline sahiptir.*

Anahtar kelimeler: *biyofilm; bor bileşikleri; Escherichia coli; fraksiyonel inhibitör konsantrasyon; HepG-2; minimum inhibitör konsantrasyon*

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Introduction

Escherichia coli is imperative in diseases such as septicemia gastrointestinal and urinary tract diseases. Antibiotics such as ampicillin, amoxicillin-clavulanic acid, and ceftriaxone treat *Escherichia coli*. *E.coli* is a facultative, anaerobic, rod-shaped, Gram-negative bacterium[1]. Variables such as different extracellular expansions that contribute to the colonization of the surface of *E.coli* and their finely controlled expression and action lead to the arrangement of mature biofilms[2]. A biofilm could be a community of microorganisms living together, more often than not, joined to strong surfaces in damp environments[3]. The microorganisms in a biofilm discharge different defensive substances called extracellular polymeric substances (EPS) that increment their chances of survival[3]. Biofilm-forming pathogens are troublesome to treat with ordinary anti-microbials, and the microorganisms are less sensitive to antibiotics[4,5]. *E.coli* and *Klebsiella pneumoniae* have been recognized as the prevailing bacterial bunches related to the generation of extended-spectrum beta-lactamases (ESBLs). The danger of biofilms and multidrug-resistant pathogens underscores the need to create anti-microbial with modern components of action[5].

Boron is a compound with numerous subordinates of boric corrosive, the mineral sodium borate, and ultrahard precious stones of boron carbide and boron nitride[6]. As of late, the application of boron compounds has become more well-known due to their chemical properties[7]. Different theories have appeared on the antimicrobial, antifungal, antiviral, and anticancer movement of boron compounds[8–15]. Boron compounds such as boric corrosive, borax, sodium perborate metahydrate (SPM), zinc borate (ZB), and Etidote are broadly utilized in different areas, particularly in well-being care and as antimicrobial drugs. Boric corrosive and borax successfully decreased colony numbers of *Brucella* spp., *E.coli*, and *Staphylococcus* spp. in one study[15]. Research on sodium perborate metahydrate (SPM), zinc borate (ZB), and Etidote is constrained, and we examined the combination of boron components on HepG-2 for the first time.

Material and Methods

Chemicals and Reagents

Tryptic soy broth (Product No: 22092), Dulbecco's altered Eagle's medium (DMEM) (Product No: D5546), phosphate-buffered saline (PBS) (Product No: P3813), fetal calf serum (FCS) (Product No: A1908), Etidote (Cas No: 1303-96-4), sodium perborate monohydrate (Cas No: 231-556-4), Zinc borate (Cas No: 10361-94-1), anti-microbial antimitotic arrangement (100×) (Product No: A5955), Mueller-Hinton broth (Product No: 70192), L-glutamine (CAS No: 56-85-9), trypsin-EDTA (Product No: T4049), paraformaldehyde (Cas No: 30525-89-4), and ethanol (CAS No: 64-17-5) were gotten from Sigma Aldrich (St. Louis, Moment, USA).

Bacterial Strain

Escherichia coli 25922 strain was used in this consideration, and bacterial suspensions with turbidity of 0.5 McFarland were prepared.

Bacterial Production

Escherichia coli 25922 *strain was* inoculated onto both blood agar and 5% sheep blood agar plates. These plates were then incubated at 37°C for 24 hours. Following incubation, bacterial colonies were visualized using a microscope. Subsequently, actively growing colonies were aseptically transferred into 5 ml of Tryptic Soy Broth (TSB) each, and the resulting cultures were further incubated at 37°C for another 24 hours. These enriched cultures served as the stock for subsequent analyses.

Minimum Inhibitory Concentration (MIC)

The MIC values of sodium perborate metahydrate (SPM), zinc borate (ZB), and Etidote against the *E.coli* 25922 strain were detected by the microdilution method[12,16]. Concentrations of the substances were prepared in the range of 1024–0.97 μg/mL in the presence of Mueller Hinton Broth (MHB), and 180 μL was transferred to 96-well plates. At that point, 20 μL suspension of *E.coli* 25922 strain (10^6 CFU/mL) was included in each well and incubated at 37°C for 24 hours. After 24 hours, water-soluble 2.3.5-Triphenyltetrazolium chloride (TTC) saline (5 mg/mL), a natural pointer, was included in each well, and the plates were incubated for 2–3 hours. For control purposes, the medium was arranged as a negative control, while the wells containing bacteria served as a positive control. In the case of the negative control wells, *E.coli* 25922 was not introduced. This method was repeated in triplicate for the other ZBs and Etidote[12,16].

Biofilm Analysis

An add-up to 180 µL of the compounds arranged with TSB medium, the MIC of which was decided, was transferred into a U-based 96-well plate. The glucose-enriched TSB medium was used as a negative control, and the *E.coli* 25922 strain was used as a positive control. At that point, 20 µL (10^6 CFU/mL) of *E.coli* 25922 strain was inoculated into each well without the negative well. Bacteria were incubated at 37°C for 48 hours. Biofilm examination was performed in three replicates[12,16].

Fractional Inhibitory Concentration (FIC)

The most successful MIC concentrations of SPM, ZB, and Etidote compounds were arranged in combination. The comes about were decided to agree to the formula[16]. This refers to the total value obtained by adding the Fractional Inhibitory Concentration (FIC) values of two or more substances when used together. Fractional inhibitory concentration is a measure of the concentration of each substance required to inhibit bacterial growth when used in combination. Fractional inhibitory concentration A and FIC B: These are the FIC values for the individual substances A and B. Σ FIC ≤0.5 (Summation of FIC values less than or equal to 0.5): When the total FIC value (Σ FIC) is 0.5 or less, it suggests synergism. In other words, the combined effect of substances A and B is greater than the sum of their individual effects. Synergy means that the combination is more effective at inhibiting bacterial growth than each substance alone. >0.5 and <1:If the total FIC value falls within this range, it indicates an additive effect. In an additive scenario, the combined effect is similar to what would be expected by simply adding the effects of the individual substances. It's effective, but there is no synergy. ≥1 and ≤4:When the total FIC value is greater than or equal to 1 but less than or equal to 4, it suggests ineffectiveness. In this case, combining substances is not significantly more effective than using each substance individually. The combination may not be a practical choice for treating bacterial infections. >4:If the total FIC value exceeds 4, it implies antagonism. Antagonism means that the combined effect of substances A and B is less effective at inhibiting bacterial growth than the effect of each substance used individually. In such cases, using the substances together may be counterproductive [12,16].

Cell Cultures

The HepG-2 cell line (HB -8065 ATCC) was obtained from Bilecik Seyh Edebali University, Faculty of Medicine, Department of Pharmacology (Bilecik, Türkiye). Cells were uncovered in a new medium (Dulbecco's altered Eagle's medium, DMEM), a combination of 10% fetal bovine serum (FBS) and 1% anti-microbial (Sigma Aldrich, St. Louis, Moment, Joined together States). Cells were seeded in 24-well plates (Corning, Joined together States) containing 5% CO_2 and cultivated at 37°C[17]. Upon reaching the intersection point of 85%, the presentation was organized utilizing a yellow 100-µl pipette tip. At that point, the bacterial suspension was included in the cell line at McFarland 0.5. After 30 minutes of the treatment with the HepG2 cell line, SPM 62.5 µg/mL + Etidote 125 µg/mL, SPM 62.5 µg/mL + ZB 31.25 µg/mL, and ZB 31.25 µg/mL + Etidote 125 µg/ mL were administered[17,18].

3-(4.5-Dimethylthiazol-2-yl)-2.5-Diphenyltetrazolium Bromide (MTT) Assay

After 24 hours of the treatment with Etidot, SPM, and zinc borate, 10 µL of *3-(4.5-Dimethylthiazol-2-yl)-2.5-Diphenyltetrazolium bromide (MTT)* arrangement (Sigma Aldrich, St. Louis, Moment, USA) was added to each plate. After incubating the plates for 4 hours, 100 µL of dimethyl sulfoxide (DMSO) arrangement (Millipore Sigma) was inoculated to all wells to break up the formazan precious stones. The optical thickness of the arrangements was determined at 570 nm employing a Multiskan™ GO Microplate Spectrophotometer Reader (Thermo Fisher, Porto Salvo, Portugal)[19].

Immunofluorescence

Cells developed within the HepG2 cell line were brooded in a paraformaldehyde arrangement for 30 minutes. Hence, the cells were brooded in 3% hydrogen peroxide for 5 minutes. The cells were blended with a 1% Triton-X arrangement, washed with PBS, and hatched for 15 minutes. At that point, protein pieces were dropped onto the cells and put away within the dull for 5 minutes. The essential counteracting agent (8-OHdG Cat. No.: sc-66036, Santa Cruz Biotechnology, Texas, USA, weakening proportion: 1/100) was included and incubated. An immunofluorescent auxiliary counteracting agent was used as a secondary marker (FITC, cat. no.: ab6785, Abcam, Boston, USA) and brooded for 45 minutes within the

dim. Areas were at that point sprinkled with DAPI mounting medium (Cat. No.: D1306, Thermo Fisher, Porto Salvo, Portugal, weakening proportion: 1/200) and brooded for 5 minutes within the dull. The areas were fixed with a coverslip. The recolored areas were inspected beneath a fluorescence magnifying instrument (Zeiss AXIO, Germany)[16].

Statistical Analysis

Results were calculated as cruel ± standard blunder. Factual comparisons between bunches were performed utilizing the one-way strategy ANOVA and Tukey's LSD strategy. All calculations for factual examination were performed with the IBM Statistical Package for Social Sciences (SPSS) program version 20. P <0.05 was acknowledged as a critical contrast for all tests.

Results

Microbiological Analysis

Boron compounds' MIC values were measured between 1024–0.97 ug/mL. It was found to have an impact on these ranges. The comes about of fragmentary inhibitory concentrations appear in Table 1 and Fig. 1. Synergistic impacts (≤0.5) were observed at 32 µg/mL Etidote + 32 µg/mL SPM, at 32 µg/mL Etidote + 32 µg/mL ZB, and 32 µg/mL ZB + 32 µg/mL SPM. Optical thickness (wavelength 570 nm) is summarized in Fig. 2 to evaluate biofilm arrangement. Amid the measure, sterile TSB was inspected and assessed as a negative control. The concentration of 128 µg/ml Etidote + 512 µg/ml SPM had the most noteworthy impact on biofilm arrangement (Fig. 2). In Figure 2B, the most noteworthy impact on biofilm arrangement was found at the concentration of 512 µg/mL Etidote + 1024 µg/mL ZB. In Fig. 2, the most remarkable impact on biofilm arrangement was found at 512 µg/mL ZB + 128 µg/mL SPM.

Table 1. *Summarise all doses used in FIC and MIC analysis*

Boron Compound		
Etidote 1024µg/ml+Spm 1024 µg/ml	ZB 1024µg/ml+Spm 1024 µg/ml	Etidote 1024µg/ml+ZB 1024 µg/ml
Etidote 512µg/ml+Spm 1024 µg/ml	ZB 512µg/ml+Spm 1024 µg/ml	Etidote 512µg/ml+ZB 1024 µg/ml
Etidote 256µg/ml+Spm 1024 µg/ml	ZB 256µg/ml+Spm 1024 µg/ml	Etidote 256µg/ml+ZB 1024 µg/ml
Etidote 128µg/ml+Spm 1024 µg/ml	ZB 128µg/ml+Spm 1024 µg/ml	Etidote 128µg/ml+ZB 1024 µg/ml
Etidote 64µg/ml+Spm 1024 µg/ml	ZB 64µg/ml+Spm 1024 µg/ml	Etidote 64µg/ml+ZB 1024 µg/ml
Etidote 32µg/ml+Spm 1024 µg/ml	ZB 32µg/ml+Spm 1024 µg/ml	Etidote 32µg/ml+ZB 1024 µg/ml
Etidote 1024µg/ml+Spm 512 µg/ml	ZB 1024µg/ml+Spm 512 µg/ml	Etidote 1024µg/ml+ZB 512 µg/ml
Etidote 512µg/ml+Spm 512 µg/ml	ZB 512µg/ml+Spm 512 µg/ml	Etidote 512µg/ml+ZB 512 µg/ml
Etidote 256µg/ml+Spm 512 µg/ml	ZB 256µg/ml+Spm 512 µg/ml	Etidote 256µg/ml+ZB 512 µg/ml
Etidote 128µg/ml+Spm 512 µg/ml	ZB 128µg/ml+Spm 512 µg/ml	Etidote 128µg/ml+ZB 512 µg/ml
Etidote 64µg/ml+Spm 512 µg/ml	ZB 64µg/ml+Spm 512 µg/ml	Etidote 64µg/ml+ZB 512 µg/ml
Etidote 32µg/ml+Spm 512 µg/ml	ZB 32µg/ml+Spm 512 µg/ml	Etidote 32µg/ml+ZB 512 µg/ml
Etidote 1024µg/ml+Spm 256 µg/ml	ZB 1024µg/ml+Spm 256 µg/ml	Etidote 1024µg/ml+ZB 256 µg/ml
Etidote 512µg/ml+Spm 256 µg/ml	ZB 512µg/ml+Spm 256 µg/ml	Etidote 512µg/ml+ZB 256 µg/ml
Etidote 256µg/ml+Spm 256 µg/ml	ZB 256µg/ml+Spm 256 µg/ml	Etidote 256µg/ml+ZB 256 µg/ml
Etidote 128µg/ml+Spm 256 µg/ml	ZB 128µg/ml+Spm 256 µg/ml	Etidote 128µg/ml+ZB 256 µg/ml
Etidote 64µg/ml+Spm 256 µg/ml	ZB 64µg/ml+Spm 256 µg/ml	Etidote 64µg/ml+ZB 256 µg/ml
Etidote 32µg/ml+Spm 256 µg/ml	ZB 32µg/ml+Spm 256 µg/ml	Etidote 32µg/ml+ZB 256 µg/ml
Etidote 1024µg/ml+Spm 128 µg/ml	ZB 1024µg/ml+Spm 128 µg/ml	Etidote 1024µg/ml+ZB 128 µg/ml
Etidote 512µg/ml+Spm 128 µg/ml	ZB 512µg/ml+Spm 128 µg/ml	Etidote 512µg/ml+ZB 128 µg/ml
Etidote 256µg/ml+Spm 128 µg/ml	ZB 256µg/ml+Spm 128 µg/ml	Etidote 256µg/ml+ZB 128 µg/ml
Etidote 128µg/ml+Spm 128 µg/ml	ZB 128µg/ml+Spm 128 µg/ml	Etidote 128µg/ml+ZB 128 µg/ml
Etidote 64µg/ml+Spm 128 µg/ml	ZB 64µg/ml+Spm 128 µg/ml	Etidote 64µg/ml+ZB 128 µg/ml
Etidote 32µg/ml+Spm 128 µg/ml	ZB 32µg/ml+Spm 128 µg/ml	Etidote 32µg/ml+ZB 128 µg/ml
Etidote 1024µg/ml+Spm 64 µg/ml	ZB 1024µg/ml+Spm 64 µg/ml	Etidote 1024µg/ml+ZB 64 µg/ml
Etidote 512µg/ml+Spm 64µg/ml	ZB 512µg/ml+Spm 64µg/ml	Etidote 512µg/ml+ZB 64µg/ml
Etidote 256µg/ml+Spm 64 µg/ml	ZB 256µg/ml+Spm 64 µg/ml	Etidote 256µg/ml+ZB 64 µg/ml
Etidote 128µg/ml+Spm 64 µg/ml	ZB 128µg/ml+Spm 64 µg/ml	Etidote 128µg/ml+ZB 64 µg/ml
Etidote 64µg/ml+Spm 64 µg/ml	ZB 64µg/ml+Spm 64 µg/ml	Etidote 64µg/ml+ZB 64 µg/ml
Etidote 32µg/ml+Spm 64 µg/ml	ZB 32µg/ml+Spm 64 µg/ml	Etidote 32µg/ml+ZB 64 µg/ml
Etidote 1024µg/ml+Spm 32 µg/ml	ZB 1024µg/ml+Spm 32 µg/ml	Etidote 1024µg/ml+ZB 32 µg/ml
Etidote 512µg/ml+Spm 32 µg/ml	ZB 512µg/ml+Spm 32 µg/ml	Etidote 512µg/ml+ZB 32 µg/ml
Etidote 256µg/ml+Spm 32 µg/ml	ZB 256µg/ml+Spm 32 µg/ml	Etidote 256µg/ml+ZB 32 µg/ml
Etidote 128µg/ml+Spm 32 µg/ml	ZB 128µg/ml+Spm 32 µg/ml	Etidote 128µg/ml+ZB 32 µg/ml
Etidote 64µg/ml+Spm 32 µg/ml	ZB 64µg/ml+Spm 32 µg/ml	Etidote 64µg/ml+ZB 32 µg/ml
Etidote 32µg/ml+Spm 32 µg/ml	ZB 32µg/ml+Spm 32 µg/ml	Etidote 32µg/ml+ZB 32 µg/ml

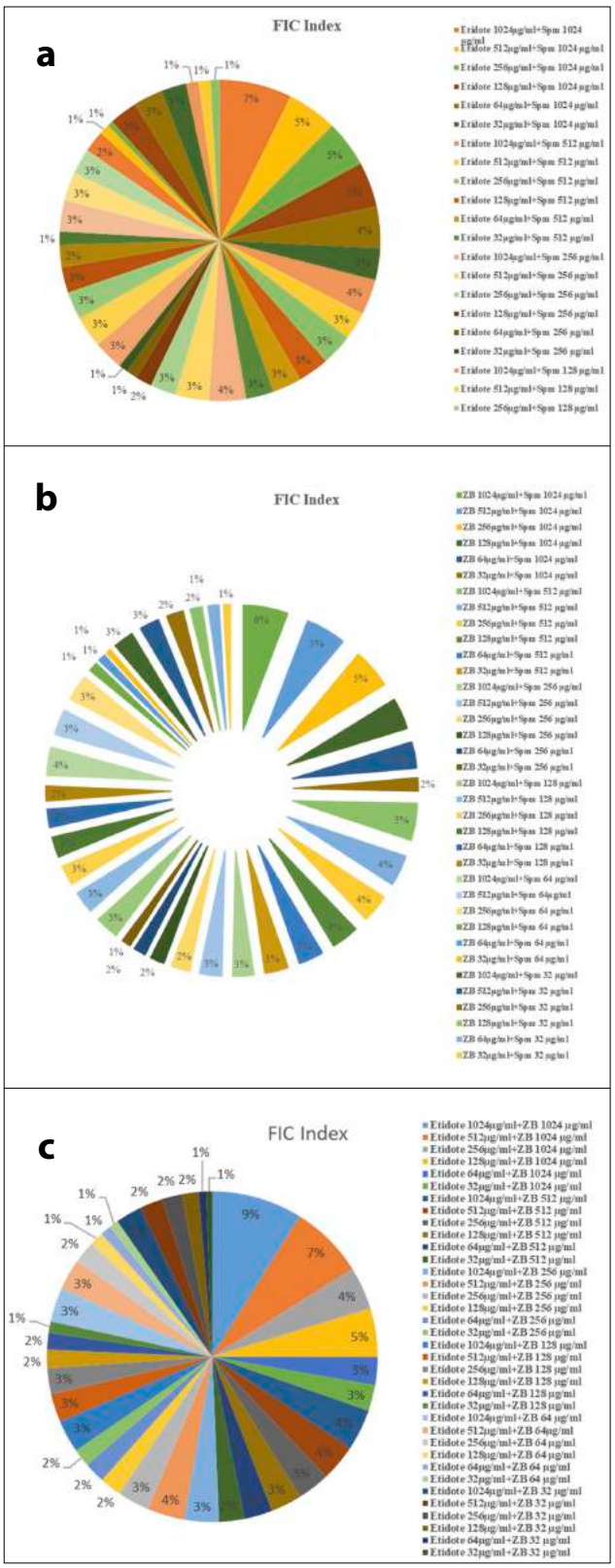

Figure 1. a–c. *FIC index results. Etidote + SPM combination fixation index (a), Etidote + ZB combination fixation index (b), ZB + SPM combination fixation index (c). Value ranges of boron combinations corresponding to Σ FIC index ≤0.5: synergism, >0.5 and <1: additive and ≥1 and 4 ≤: ineffective (indifference).*

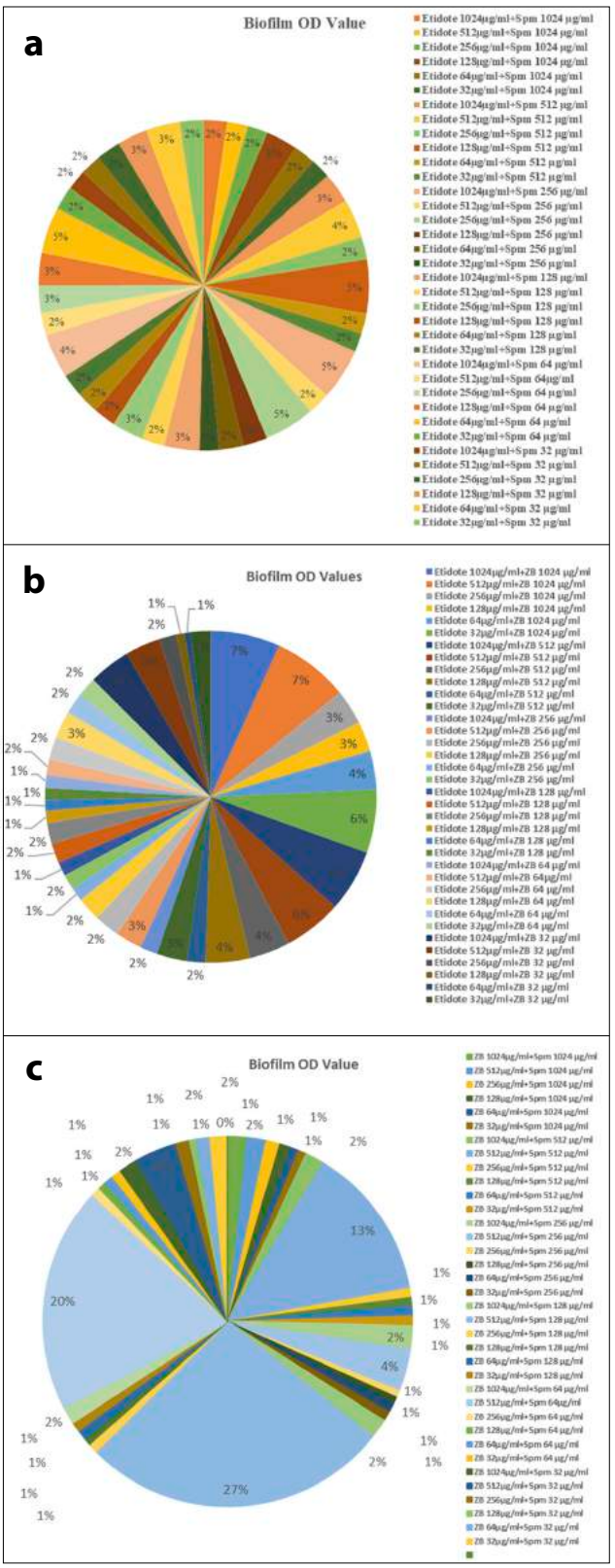

Figure 2. a–c. *Biofilm OD results. Etidote+SPM (a), Etidote+ZB (b), ZB+SPM biofilm OD values (c). The minimum and maximum OD values of Etidote+SPM, Etidote+ZB, and ZB+SPM are at 570 OD.*

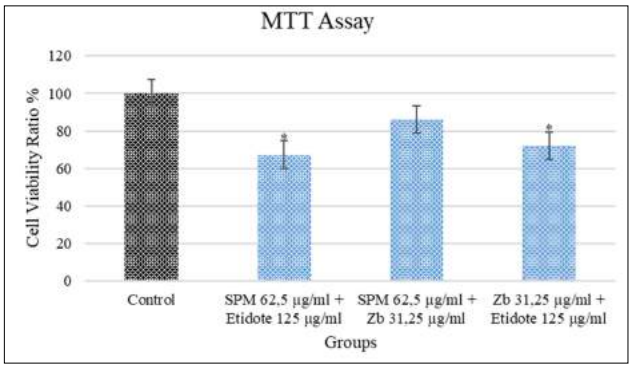

Figure 3. *MTT assay results for the HepG2 cell lines, control group (received only medium), Escherichia coli bacteria were cocultured for 24 h with SPM 62.5 µg/mL + Etidote 125 µg/mL, SPM 62.5 µg/mL + ZB 31.25 µg/mL and ZB 31.25 µg/mL + Etidote 125 µg/mL. (*P<0.05 compared to the control group).*

Cell Viability

The impact of ZB, SPM, and Etidote on the viability of HepG-2 cells is depicted in Fig. 3. Notably, the viability of HepG-2 cells was not significantly compromised when exposed to the combination of ZB + Etidote. Furthermore, it was observed that the combinations of SPM + Etidote and SPM + ZB exhibited inadequate efficacy in protecting against *E.coli* contamination, and these observations held statistical significance ($P < 0.05$) (Fig. 3).

Immunofluorescence Analysis

The co-administration of ZB and Etidote demonstrated a notable reduction in bacterial motility, a protective effect on HepG2 cells, and a reduction in DNA damage. Detailed findings regarding these outcomes are provided in Table 2 and graphically illustrated in Fig. 4.

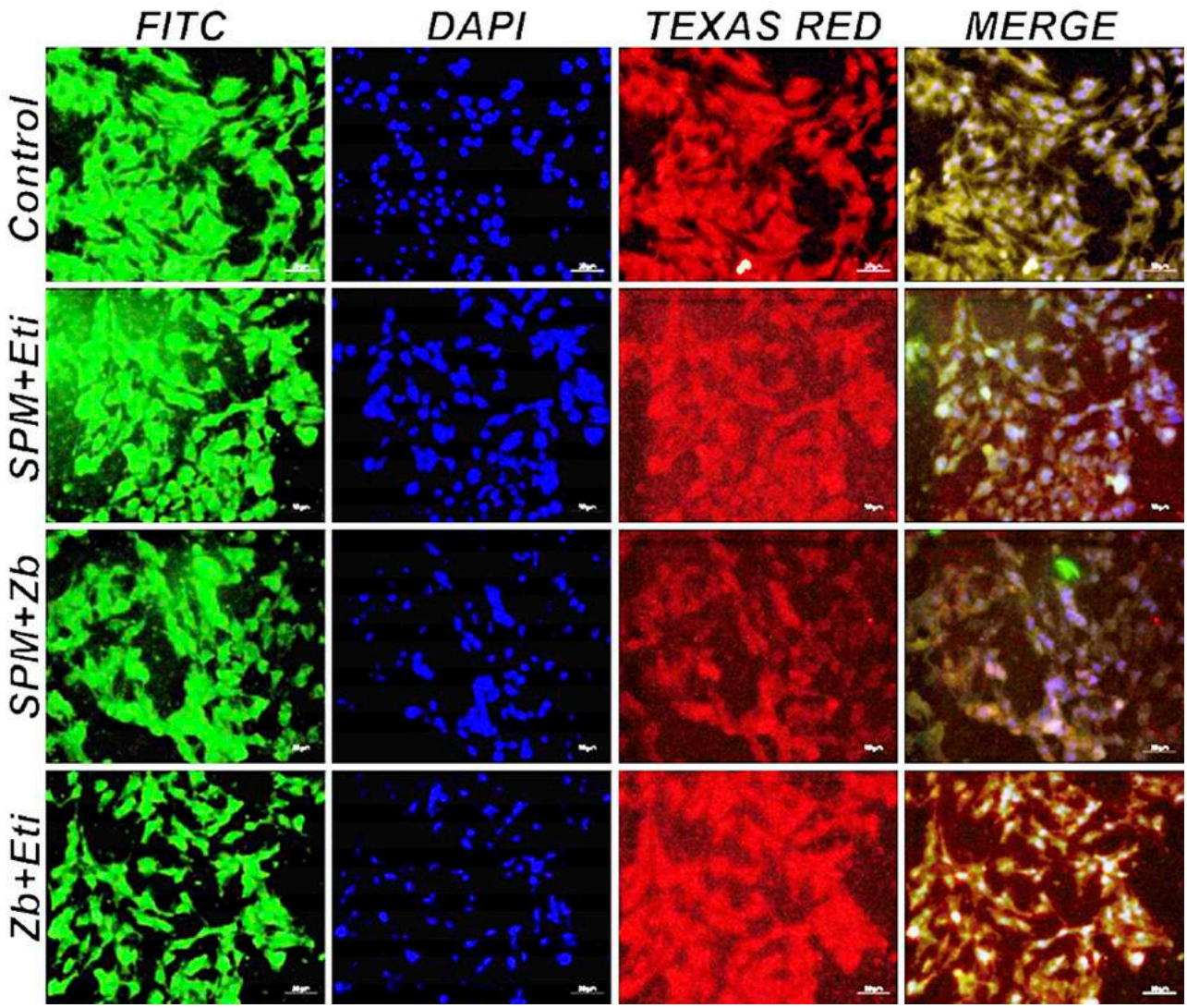

Figure 4. *Cell lines, 8-OHdG expression (FITC), and H2A. X expression (Texas Red), IF, Bar: 50 µm.*

Table 2. *Statistical analysis of immunofluorescent staining findings*

Groups	8-OHdG	H2A.X
Control	24.84±5.42[a]	18.43±4.38[a]
SPM + Etidote	42.86±6.18[b]	33.54±4.58[b]
SPM + ZB	32.45±5.18[ab]	26.18±5.69[ab]
ZB + Etidote	39.76±4.16[b]	31.44±3.2[b]

a, b, c: different letters in the same column were considered statistically significant differences (P<0.05).

Discussion

Biofilm-forming and multidrug-resistant (MDR) microorganisms speak to a worldwide well-being issue. Over the past decade, growing interest in alternative therapies has expanded the pool of potential candidates for antibacterial agents. Numerous analysts have explored boron compounds' anti-inflammatory, antifungal, and antibacterial viability. Be that as it may, the adequacy of these compounds on microorganisms has been examined *in vitro*, and cell viability and cytotoxicity are constrained[20,21]. In this consideration, we examined the impact of boron compounds on the human HepG-2 cell line amid disease with *E.coli*. Tetraacetylethylenediamine and sodium perborate, constituents of boron compounds, find application as endodontic disinfectants owing to their antimicrobial efficacy against various bacterial species, particularly at elevated concentrations. It is additionally used in numerous ponders to decrease the adequacy of thick bacterial biofilms, which may be related to the development of microbial species within the endodontic environment, expanded resistance to antimicrobial operators, and safe periapical periodontitis[22–25].

Shakouie et al.[26] decided on the antimicrobial movement of tetraacetylethylenediamine sodium perborate versus sodium hypochlorite against *Enterococcus faecalis*. Tetraacetylethylenediamine sodium perborate and 5% sodium hypochloride had comparative antibacterial action against *Enterococcus faecalis*, but tetraacetylethylenediamine sodium perborate, 2% had more prominent antibacterial action than five sodium hypochlorite. In our thinking, the least inhibitory concentrations (MIC) of the compounds were decided for 32 μg/mL SPM + 128 μg/mL Etidote, for 32 μg/mL SPM +64 μg/mL ZB, and 64 μg/mL ZB + 128 μg/mL Etidote. Sayin et al.[14] decided the antibacterial and antibiofilm impacts of boron on different microbes, and it was found that the MICs of boric corrosive and etidote extended from 0.77–3.09 mg/mL and 0.644–10.312 mg/mL, separately. *Pseudomonas aeruginosa* and a clinical confine of *Lactococcus garvieae* were more likely to make a biofilm than others when the microplate strategy was used. In their consideration, Celebi et al.[16] decided on the inhibitor concentration, fragmentary inhibitor concentration, and optical thickness of the biofilm of boron compounds against *Klebsiella pneumonia*. HepG2 cells within the measurement ranges decided. The non-toxic dosage extension was chosen for the line, and immunofluorescence recoloring was performed, appeared, and evaluated. The boron components for sodium perborate monohydrate and etidote have moo and tall least inhibitory concentrations, individually. In expansion, sodium perborate monohydrate was viable on biofilm arrangement. It appears that boron compounds are combined. They were more effective when utilized within the HepG2 cell line. Within the harmfulness demonstration, it was found that the cytotoxic impact of boron compounds diminished due to their antibacterial effects. In our consideration, the most elevated biofilm impact was observed at the concentrations of 128 μg/mL Etidote + 512 μg/mL Spm, 512 μg/mL Etidote + 1024 μg/mL ZB, and 512 μg/mL ZB + 128 μg/mL SPM. Simbula et al.[27] compared the cytotoxicity of tetraalkyldiamine sodium perborate and sodium hypochlorite within the L929 fibroblast cell line. Both compounds cause a dose-dependent misfortune of cell practicality; it was found that tetraacetylethylenediamine sodium perborate was less cytotoxic comes about than sodium hypochloride beneath all test conditions tried. The most punctual harmful impact supporting the known cytotoxic impact of sodium hypochloride on refined fibroblasts was illustrated by the MTT test, where a cell misfortune of 60% was watched 2 hours after treatment within the nearness of sodium hypochlorite concentrations ≥0.025%. In expansion, a dynamic diminishing in cell practicality was observed at all sodium hypochloride concentrations tried at 4, 6, and 24 hours, but for the 0.0025% measurements, which did not influence cell practicality compared with untreated cells. Concurring to our MTT and immunofluorescence comes about. Combining ZB and Etidote diminished bacterial movement, ensured HepG-2 cells, and diminished DNA fracture. In any case, SPM + Etidote and SPM + ZB did not successfully secure against *E.coli* disease (P <0.05). We did not know why the SPM combination did not impact *Escherichia coli* microbes. Be that as it may, it might depend on the Gram-negative properties of the bacteria.

Conclusion

The MICs of all combinations are distinctive; be that as it may, the FIC values are the same, appearing to have a synergistic impact with no noteworthy contrasts. All combinations anticipate the biofilm arrangement of *E.coli*. The ZB + Etidote combination diminished bacterial movement, secured HepG2 cells, and diminished DNA fracture. Zinc borate + Etidote may be a compelling combination against *E.coli* infections in HepG-2 cells.

Limitation of the thought: The end of the ponder should assess the quality expression level of miRNA. Moreover, apoptosis state and oxidative stretch levels distinguish which component is more viable for the antimicrobial action of the boron component and body cell security.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Hepatitis C Genotype Assay and Sequence Analysis at a University Hospital in Eastern Türkiye

Türkiyenin Doğusunda Bir Üniversite Hastanesinde Hepatit C Genotip Testi ve Sekans Analizi

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ABSTRACT

Aim: *Hepatitis C virus (HCV) is a virus with many genotypes and subtypes that can cause cirrhosis, liver failure, and cancer worldwide. Countries must also identify their genotypes and subtypes to determine the treatment process for HCV infections. This study aimed to determine the HCV genotype among 71 patients who visited Kafkas University Hospital and tested positive for anti-HCV via macro-ELISA over three years.*

Material and Method: *71 samples collected from patients admitted to our hospital and identified as anti-HCV positive using the ELISA method were included in our study. The 5'untranslated region (5'UTR) region of HCV was amplified and sequenced by PCR. 5'-ctgtgaggaactactgtctt-3' and 5'-atactcgaggtgcacggtctacgagacct-3' primers were used for 5'UTR region. Sequence reactions were conducted on an ABI Prism 3130xl DNA sequencer, and sequence analysis was performed. The resulting sequence was screened in HCV Databank to detect genotype.*

Results: *The HCV genotype distribution of 56 samples was as follows: 27 (48%) patients were male and 29 (51%) were female. Genotype1a, 6 (8.5%); Genotype1b, 40 (71%); Genotype3a, 7 (12%); Genotype4, 3 (6%). According to our results, Type1b, the most common species in Türkiye, was also found to be the highest in our city.*

Conclusion: *We hope this study provides the regional distribution of the procedures to be followed in the formation of treatments for HCV-positive patients in Türkiye since it is the first study to determine HCV genotype in our region.*

Keywords: *HCV; genotype; subtypes; sequence analysis; polymerase chain reaction*

ÖZET

Amaç: *Hepatit C virüsü (HCV), dünya çapında siroz, karaciğer yetmezliği ve kansere neden olabilen birçok genotip ve alt tipi olan bir virüstür. HCV enfeksiyonlarının tedavi sürecini belirlemek için ülkelerin HCV genotiplerini ve alt tiplerini belirlemeleri de önemlidir. Bu çalışmada üç yıldır Kafkas Üniversitesi Hastanesi'ne gelen ve makro-ELISA'da anti-HCV pozitif bulunan 71 hastada HCV genotipini belirlemeyi amaçladık.*

Materyal ve Metot: *Hastanemize başvuran ve ELISA yöntemi ile anti-HCV pozitif saptanan hastalardan alınan 71 örnek çalışmamıza dâhil edildi. HCV genomunun 5'çevrilmemiş bölgesi (5'UTR) bölgesi, PCR ile amplifiye edildi ve sekanslandı. 5'UTR bölgesi için 5'-ctgtgaggaactactgtctt-3' ve 5'-atactcgaggtgcacggtctacgagacct-3' primerleri kullanıldı. Sekans reaksiyonları, bir ABI Prism 3130xl DNA sekanslayıcı üzerinde gerçekleştirildi ve sekans analizi yapıldı. Ortaya çıkan sekans, genotipi tespit etmek için HCV Veri Bankasında tarandı.*

Bulgular: *Elli altı örneğin HCV genotip dağılımı şu şekildeydi; Hastaların 27'si (%48) erkek, 29'u (%51) kadındı. Genotip la, altı (%8,5); Genotip 1b, kırk (%71); Genotip 3a, yedi (%12); Genotip 4, üç (%6). Türkiye'de en yaygın tür olan Type1b, sonuçlarımıza göre ilimizde de en yüksek tür olarak bulundu.*

Sonuç: *HCV genotipini belirlemeye yönelik ilk çalışma olması nedeniyle bölgemizin Türkiye'de HCV pozitif hastalara uygulanacak tedavilerin oluşturulmasında izlenecek prosedürlerin bölgesel dağılımına katkı sağlayacağını umuyoruz.*

Anahtar kelimeler: *HCV; genotip; alt tipler; sekans analizi; polimeraz zincir reaksiyonu*

Introduction

HCV virus is an RNA virus that can cause disease in acute and chronic form, encoding ten proteins (5'-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3') in the Flaviviridae family, hepacivirus genus, with a single strand, positive sense RNA enveloped genome of 30–60 nm (1). 70% of HCV-positive patients are at risk of developing the virus into a chronic form, and 15–30% of patients in the chronic patient group are at risk of developing cirrhosis and liver cancer on average[1]. HCV infections are generally known to be transmitted in developing countries with poor

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sanitation conditions by intravenous drug use, non-sterile blood transfusion, unprotected sexual intercourse, and even rarely mother-to-baby transplacental or mother-to-child transmission (MTCT) invasive interventions during childbirth[2]. Almost 1.75 million new cases are encountered every year, according to WHO's report published in 2015. It is known that approximately 1.25 million of these patients are at risk of the chronic form of HCV. There are also around 360.000 cases of cirrhosis and cancer considering the rates[3]. It is known that the number of HCV-related liver diseases varies according to country. At the same time, HCV is expected to decrease or disappear in the next 15–20 years, considering the current situation. Although these data indicate a general decrease in HCV infection, especially in Western Europe, a recent modelization has estimated how the numbers of HCV– mortality will increase in the following decades.

According to this model, in the period 2013–2030, the number of decompensated cirrhosis, the prevalence of HCC in the general population, and the liver-related morbidity rate will increase in Europe by 80 %, 75 %, and 65 %, respectively[4].

The creation of the treatment protocol for the virus depends on the detection of genotypes, especially in terms of countries since DAA (interferon and/or ribavirin with the new direct-acting antiviral therapies) used in its treatment depends on the genotype of HCV, for this reason[5]. Today, 30%-35% of HCV nucleotides have eight different genotypes (G1, G2, G3, G4, G5, G6, G7, G8) and again <15% difference in terms of nucleotides; 67 of them have known, 20 investigated subtypes[4,6,7]. The gold standard for genotyping is whole-genome analysis. Still, since 5'UTR, NS3, NS5A, NS5B, and core antigen tests are new-generation tests, they are very expensive[8]. Genotypes 1a, 1b, 2a, and 3a, referred to as "epidemic subtypes" in the worldwide distribution of HCV, are also common in developing South Africa, South Asia, Africa, and Southeast Asia. Genotype 7 was first detected in a person who migrated from Africa to Canada. Genotype 8 was first reported in India[9]. Epidemiological studies have shown that the genotype of the virus in blood donors also changes according to geographical areas[10]. For example, Genotype 1 is more common worldwide than Genotype 2, whereas Genotype 3 is more common in South Asia, Australia, and Iran; Genotype 4 is in Central Asia and northern Africa. Genotype 5a is the most common species in South Africa, and Genotype 6a is the most common species in Hong Kong and Vietnam; Genotype 1b is the most common species in South and Northern Europe in South and Eastern Europe; Genotype 1a is the most common species in North America and Europe[10,11]. There is a gradual increase in the number of Genotype 3 and Genotype 4. In contrast, Genotype 1b is the most common genotype when Türkiye's data is examined in some studies[2]. This study, conducted for the first time in Türkiye, aims to compare the results of genotype determination and sequence analysis in serum samples of patients admitted to our hospital for any reason and diagnosed with HCV-positivity with Türkiye and the world data.

Materials and Methods

Patient Samples, Genotyping, and Sequencing

Plasma samples of 71 patients who were found to be anti-HCV positive with Biomerieux (France) Architect 1000 device by macro-ELISA method from the patient blood sent to Kafkas University Medical Microbiology Laboratory for routine examinations were stored at -80°C in our study. It was performed in an HCV RNA EZ1 advanced (Qiagen, Germany) automated extraction device using the EZ1 Virus Mini Kit v2.0 (Qiagen, Germany). The extracts obtained were amplified using 5'NCR, and the core gene region was amplified using 5'-ctgtgaggaactactgtctt-3'and 5'-atactcgaggtgcacggtctacgagacct-3'primers with HCV RNA Rt-quantitative 2.0 (NLM, Italy) kit in Rotorgene 6000 real-time polymerase chain reaction (Rt-PCR) (Qiagen, Germany) device. Sequence reactions were conducted on an ABI Prism 3130xl DNA sequencer in the remaining 56 patients, and sequence analysis software was performed with version 5.4 (Applied Biosystems, Foster City, CA, USA). Fifteen patients were observed to be negative. The resulting sequence was screened in the Hepatitis C Virus Databank to detect HCV genotype[13].

This study was carried out with the approval of the Kafkas University Faculty of Medicine Ethics Committee (Date: 30.01.2019 and Decision no: 80576354–050–99/47).

Statistical Data were analyzed using Windows IBM Statistical Package for Social Sciences (SPSS) program, version 22.0 (IBM Inc., Chicago, Illinois, USA), and the results were presented as median values ± standard deviations (SD), number, and percentage. The chi-square test was used to compare the groups; $p<0.05$ was considered statistically significant.

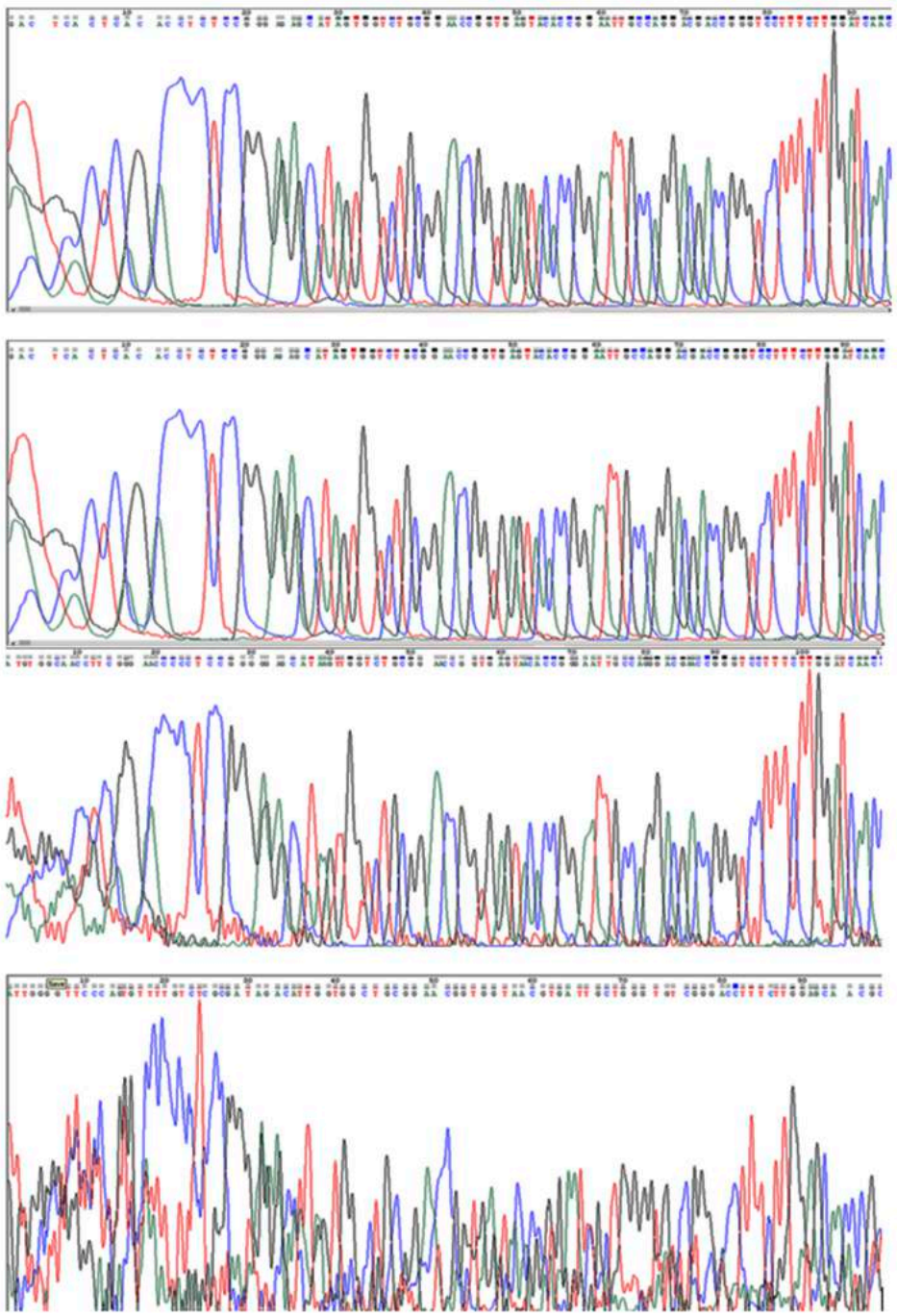

Figure 1. The results of four different sequence analyses were 1a, 1b, 3a, and 4, respectively.

Results

The HCV results of 56 samples were as follows: 27 (48%) patients were male and 29 (51%) were female. Genotype 1a, 6 (8.5%); Genotype 1b, 40 (71%); Genotype 3a, 7 (12%); Genotype 4, 3 (6%). No other genotypes were found (Figure 1) (Table 1).

Discussion

HCV is a viral disease that has almost 71 million chronic patients worldwide and is rarely transmitted maternally by blood transfusion or non-sterile injection, causing liver diseases, cirrhosis, and hepatocellular carcinoma in some patients[2,3].

Table 1. *Data from our study*

Number(n) / Gender	G1	G2	G3	G4	G5	G6
n: 56 M: 27(48%) F: 29(51%)	1a; 6(8.5%) 1b; 40(71%)	0	3a; 7(12%)	3(6%)	0	0

Table 2. *HCV genotype distribution by countries*

Country	Year	Method	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6
China[21]	2011-2019	PCR	2.94% 1a, 45.1% 1b	14.7% 2a	13.7% 3a	-	-	12.7% 6a
Czechia[22,23]	2001-2009	PCR	79%	1%	19.7%	-	-	-
France[24,25]	1995-2005	PCR	57%	9.3%	20.8%	8.9%	2.7%	0.9%
Germany[26,27]	1996-2002	PCR	61.7%	6.9%	28%	3.2%	-	-
Greece[28]	2011	PCR	47%	8.3%	20.8%	8.9%	2.7%	0.2%
Hungary[29]	2010	PCR	94.1%	0.8%	3.4%	1.7%	-	-
Italy[30]	2002	PCR	62%	27%	7%	0.8%	-	0.4%
Poland[31]	2008	PCR	57.5%		31.3%	4.8%	-	-
Norway[32]	2003	PCR	61.5%	1.5%	28%		-	-
Israel[33]	2010	PCR	70%	8%	20%	3%	-	-
S. Africa[34]	2020	PCR	21.8%	-	15.4%	10.3%	60.3%	-
Iranian[35]	2020	PCR	12.7%	-	26%	-	8%	8%
Georgia[36]	2014	PCR	45%	1.6%	50.3%	-	-	-
Bulgaria[37]	2019	PCR	86.9%	0.9%	11.3%	0.9%	-	-

Almost 2–3% of the world's population is known to be infected with HCV, totaling approximately 130–170 million people. Studies have shown that the frequency of HCV is generally higher in the Middle East and African countries and less frequent in Europe and America, indicating that these rates may vary between <1% and >10% according to countries[14]. The prediction suggests that the situation might change based on country-specific conditions, social factors, accessibility to healthcare facilities, and various other epidemiological conditions, despite the World Health Organization's set target to decrease the disease by 30% and fatalities by 10% for HCV by 2020 and to eradicate the disease in nine countries by 2030[15].

These treatments cannot be applied sufficiently in developing countries due to the inability to diagnose the disease in the acute phase, the failure to perform genotype determination, limited treatment infrastructure, and high costs, even though HCV is one of the promising therapeutic interventions for clinicians[16,] Serological tests such as ELISA are the first recommended tests for detecting the disease. Nucleic acid tests (NAT) and HCV ribonucleic acid detection (RNA) are advised to confirm the disease. Aminotransferase/platelet ratio index (APRI) and FIB-4 tests are used to show hepatic fibrosis to detect liver damage[17]. The non-structural (NS) 5b-encoded RNA-dependent RNA polymerase enzyme found in the HCV genome is an enzyme with no proofreading mechanism. It is known that different genotypes and subtypes of HCV emerge with mutations for this reason. There are thoughts that even diverse microbiota of the countries in this variety may be effective in the chronicity and mutation of HCV[18]. It aims to create new steps in treating this virus by sequence analysis of HCV with this different genetic distribution among countries[17]. This indicates that Genotype 1b in our province is positioned at the midpoint of the scale for 1b, ranging from 37% to 80% across the cities presented in Table 3, with a value of 71%. Even though the HCV genotype distribution within the countries is so different, it is relatively normal to see this diversity among the nations. According to our results, our genotype distribution is similar to some developing or developed countries, as shown in Table 2. This emphasizes the need for global genotyping studies and the development of treatment options[17,18].

The dose and duration of administration of the drugs used in the treatment of HCV vary according to genotype, acute or chronic occurrence of the disease, and the presence of cirrhosis. Clinical resistance to sofosbuvir (SOF) and mericitabine (MCB),

Table 3. *HCV genotype distribution in Türkiye*

Researcher	Location	Year	Method	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6
Özbek E, et al.[38]	Diyarbakır	2009	Inno-Lipa	4.1% 87.8% 1b	2.7%	2.7% 2.7% 3a	-	-	-
Şanlıdağ T, et al.[39]	Manisa	2009	Real-Time	2% 1a 90% 1b	2% 2a		5%	-	-
Çelik C, et al.[40]	Sivas	2010	HCV-PM BİO (LİPA)	8.9% 1a 88.2% 1b	1.2% 2a	1.7%	-	-	-
Kalaycı R, et al.[41]	Afyon	2010	Sequence Analysis	20% 1a 63.3% 1b	-	-	13.3% 4a	-	-
Aktaş E, et al.[42]	Zonguldak	2010	Versant HCV Gen. Assay	2.6% 1a 97.4% 1b	-	-	-	-	-
Karslıgil T, et al.[43]	Gaziantep	2011	Sequence Analysis	9.8% 1a 78.4% 1b	7.8% 2a	2% 3a	-	-	-
Tezcan S, et al.[44]	Mersin	2012	Inno-Lipa	3.8% 1.7% 1a 84.7% 1b 2.1% 1a/1b	0.4% 1.3% 2b	4.2% 3a	0.8% 4a	-	0.4%
Buruk CK, et al.[45]	Trabzon	2013	Real-Time	5.3% 1a 87.5% 1b	1.6%	4.9%	0.7%	-	-
Sağlık İ, et al.[46]	Antalya	2014	Real-Time	14.7% 1a 63.3% 1b	3.5%	11.1%	1.6%	-	-
Çalışkan A, et al.[47]	Kahramanmaraş	2015	Real-Time	51.7%	1.3%	46%	1%	-	-
Çetin Duran A, et al.[48]	Adana	2016	Real-Time	12.6% 1a 58.8% 1b	7.6%	16.8%	3.4%	0.8%	-
Tüzüner U, et al.[49]	Central Anatolia	2018	Reverse hybridization	1.9% 3.1% 1a 3.5% 1b 0.8% 1a/1b	1.3% 1% 2b 0.6% 2a/2c	0.3% 2.9% 3a	1.7% 0.2% 4a	0.2% 5a	-
Karabulut N. et al.[50]	İstanbul	2018	Real-Time	6.3% 38.8% 1a 37.4% 1b	4.6%	10.7%	2.2%	-	-
Çetin Duran A. et al.[51]	Coastal Aegean	2019	Real-Time	36.9% 7.4% 1a 44.1% 1b	2.1%	5.3%	2.9%	0.3%	-
Süntur et al.[52]	Adana	2020	Real-Time	1.3% 8% 1a 43.1% 1b	11.3%	28.6%	4.1%	0.8%	-

two of the medications used in the treatment, are associated with mutations in NS5B-S283 in some studies. The non-response rate was around 50% in Genotype 1. In contrast, the success rate of the combination of pegylated interferon alfa and ribavirin used to treat HCV infection was approximately 80% in Genotypes 2 and 3. The response to combined treatment is around 35%, and treatment difficulties are mentioned in Genotype 4 patients. These studies also show the importance of genetic studies in the treatment and eradication stage. Treatment failure in the disease is mainly associated with decreased susceptibility to DAAs and resistance-associated substitutions (RAS), also known to be caused by the high cost of treatments. The fact that genotype and sequencing are of great importance in eradicating the disease is known about the subject[17,19].

HCV virus has eight main genotypes currently known due to its variable genome structure[18]. The difficulty in identifying these genotypes in each patient stands as a solid barrier to the eradication of the disease. Studies show that Genotype 1 and Genotype 3 are more common in these eight main genotypes, with a rate of 46% and 30%, respectively. In addition, Genotypes 4, 5, and 6 are common in Egypt, the Middle East, and the central region of Africa.

Genotypes 1a, 3a, and 1b are common in South Africa, Asia, and Iran[20]. In this case, our study data is more similar to the Middle East and the Far East from developing countries. Genotype distribution of HCV infection in countries is shown in Table 2.

Genotype 1b was high in many studies conducted in Türkiye, as in our study. A limited number of studies

also undertaken sequence analysis even though PCR was used in most studies[42,49]. The first four genotypes and their subtypes were detected throughout Türkiye, including our research. Still, Genotype 5 was encountered in Adana, Central Anatolia, and Coastal Aegean, and Genotype 6 was encountered in Mersin when the genotype distributions were examined in the studies[48–52]. 13.4% suggest researching the genotype source by pointing to a regional prevalence in Afyon. Genotype 7 and Genotype 8 have not yet been reported in Türkiye[41]. According to our study's data, age and gender differences had no effect on HCV genotype distribution ($p>0.05$).

In conclusion, our study sheds light on the possibility of various problems in the treatment of HCV in the region due to the resistance of 1b (71%) and four genotypes (2%) to treatment response, as well as being the first study on the determination of HCV genotype in Türkiye. It is a known fact that the importance of regional genotype determination in treatment options that may be a beacon of hope in HCV treatment is indisputable in line with the studies conducted on this subject.

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Authors' Contributions

CEBB and AG Conceived and designed research. CEBB and AG Performed experiments; CEBB, AY, and AG Wrote the manuscript; AY and CEBB Analyzed data; AG and AY Interpreted results of experiments; CEBB and AY Prepared figures; CEBB and AG Edited and revised the manuscript; CEBB, AG, and AY Approved final version of the manuscript.

Conflict of Interest

We declare that there is no conflict of interest. The funding bodies had no role in the study's design, collection/ analysis/ interpretation of data, writing of the manuscript, or the decision to publish the results.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Outpatient Clinic Satisfaction Survey: Tertiary Center Experience

Poliklinik Memnuniyet Anketi: Üçüncü Basamak Merkez Deneyimi

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ABSTRACT

Aim: *Patient satisfaction surveys stand as one of the most effective methods for assessing the quality of healthcare services, aimed at gathering feedback from patient opinions and recognizing the service provider's pivotal role. This study aims to measure the satisfaction of the patients receiving outpatient services in a tertiary center with the quality of these services and to prepare an infrastructure that can create policies that can respond to the needs and wishes of the people as a hospital.*

Material and Method: *This study was conducted on 780 outpatients who applied obstetrics and gynecology services at Kafkas University Medical Faculty Training and Research Hospital, a tertiary public hospital, between November 2021 and February 2022." The questionnaire consisted of two parts, asking 15 questions about the service they received from the institution and six questions about their sociodemographic characteristics.*

Results: *The highest Yes rate was 96.4% (752 people) as "The doctor who examined me was interested". The highest No rate was 31.0% (242 people) with the expression "I was informed about the waiting times in the hospital".*

While the lowest yes rate was 51.5% (402 people), the statement "The hospital was clean in general", the lowest no rate was again the statement "The doctor who examined me was interested". 87.1% of the participants (679 people) stated that the waiting time for registration was long, 52.7% said it was difficult to reach the hospital, and 88.8% (693 people) said they would recommend the hospital to their friends and family. Patients reported that they were generally satisfied with the services provided.

Conclusion: *Similar to every sector, the pursuit of quality in delivering healthcare services is a prevalent goal in today's healthcare sector. Patient satisfaction surveys are essential in terms of measuring these services.*

Keywords: *healthcare management; health services; gynecology and obstetrics clinic; outpatient services; patient satisfaction; university hospital*

ÖZET

Amaç: *Sağlık hizmetlerinin kalitesini değerlendirmenin en etkili yöntemlerinden biri, hizmet sağlayıcının belirleyici olduğu gerçeğine dayanarak hasta görüşleri ve geri bildirim almak amacıyla hazırlanan memnuniyet anketleridir. Bu çalışma, bir hastane olarak halkın ihtiyaçlarına ve isteklerine yanıt verebilecek politikalar oluşturabilen bir altyapı hazırlamak amacıyla üçüncü basamak bir merkezde poliklinik hizmeti alan hastaların memnuniyetini ölçmeyi ve bu hizmetlerin kalitesini değerlendirmeyi amaçlamaktadır.*

Materyal ve Metot: *Bu çalışma, Kasım 2021 ile Şubat 2022 tarihleri arasında Kamu Hastanesi olarak üçüncü basamak bir merkez olan Kafkas Üniversitesi Tıp Fakültesi Eğitim ve Araştırma Hastanesi Kadın Hastalıkları ve Doğum Polikliniği'ne başvuran 780 poliklinik hastası üzerinde gerçekleştirildi. Anket, kurumdan aldıkları hizmetle ilgili 15 soru ve sosyodemografik özelliklerine dair altı soru içeren iki bölümden oluşmaktadır.*

Bulgular: *En yüksek "Evet" oranı, "Muayene eden doktor ilgiliydi" ifadesi ile %96,4 (752 kişi) olarak belirlendi. En yüksek "Hayır" oranı, %31,0 (242 kişi) ile "Hastanede bekleme süreleri hakkında bilgilendirildim" ifadesi ile kaydedildi. En düşük "Evet" oranı %51,5 (402 kişi) ile "Hastane genel olarak temizdi" ifadesiyle, en düşük "Hayır" oranı ise yine "Muayene eden doktor ilgiliydi" ifadesiyle oldu. Katılımcıların %87,1'i (679 kişi) kayıt için bekleme süresinin uzun olduğunu belirtirken, %52,7'si hastaneye ulaşmanın zor olduğunu, ve %88,8'i (693 kişi) hastaneyi arkadaşlarına ve ailelerine önerdiklerini belirtti. Hastalar, genel olarak sunulan hizmetlerden memnun olduklarını bildirdiler.*

Sonuç: *Her sektörde olduğu gibi, bugün sağlık sektöründe de sağlık hizmeti sunumunda kalite aranmaktadır. Bu hizmetleri ölçme açısından hasta memnuniyet anketleri önemli bir rol oynamaktadır. Hasta memnuniyet anketlerinin periyodik olarak gerçekleştirilmesi ve bu anketlerden elde edilecek sonuçların değerlendirilmesi, sağlık yöneticilerinin sağlık hizmetlerinin kalitesini artırmak için atacakları adımlarda kılavuz olabileceği düşünülmektedir.*

Anahtar kelimeler: *hasta memnuniyeti; kadın hastalıkları ve doğum kliniği; poliklinik hizmetleri; sağlık hizmetleri; sağlık yönetimi; üniversite hastanesi*

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Introduction

Medical care aims not only to improve the state of health but also to respond to the needs and wishes of the patient and to ensure their satisfaction with care. Since hospitals provide health services, the main thing here is to protect their assets while rehabilitating patients, which can only be possible by increasing their power in a fair competition environment[1]. The only way to do this is to provide quality service that will provide patient satisfaction by accurately analyzing the expectations of patients and their relatives and employing quality staff while following the current technology. As a general definition, quality is the sum of the characteristics of a product or service based on its ability to meet specified or potential needs[2]. Although there is a subjective perception to increase the quality of the service provided, there is a need for patient satisfaction inquiries based on the logic of 'you cannot fix the variable you cannot measure' [3]. Therefore, patient satisfaction is considered an essential criterion of service quality. Patient satisfaction: The service delivery depends on many factors, such as the interaction of the patient and the service providers, the service's continuity, the service providers' adequacy, and communication skills[4]. Although subjective satisfaction results from health services and indicates service quality. While subjective satisfaction reflects the quality of health services, patients themselves are the primary authority to gauge the fulfillment of their expectations regarding patient satisfaction[5]. Patient satisfaction is a relevant indicator of care quality; nevertheless, the measurement of patient satisfaction has faced criticism[6].

When assessing the available information, conducting regular satisfaction surveys with patients serves to determine both the service quality and the institution's overall performance. With the data obtained this way, the institution will be a guide to increasing service quality and meeting society's expectations. At the same time, the institution must achieve the success it aims for and identify the missing areas on this path. The responsiveness of the national healthcare system and the effective implementation of strategic changes are crucial factors in achieving patient satisfaction. However, the real challenge lies in communicating significant improvements to the general public[7]. Obstetrics and Gynecology is an exclusive specialty in which the foremost and primary objective of care is the overall health of the mother and child[8].

Our study aimed to measure the satisfaction level of the patients who applied to the Obstetrics and Gynecology outpatient clinics of the Kafkas University Faculty of Medicine and to determine the deficiencies and strengths of our institution by examining the factors affecting patient satisfaction. The data obtained will be used to develop appropriate policies for management staff to address and eliminate the identified shortcomings.

Materials and Methods

Ethics committee approval was obtained from Kafkas University Faculty of Medicine Ethics Committee with the number 80576354–050–99/238. Our descriptive study was conducted with outpatients who applied to the obstetrics and gynecology outpatient services of Kafkas University Medical Faculty Training and Research Hospital, a tertiary public hospital, between November 2021 and February 2022, with the ethics committee's approval. Seven hundred eighty patients over 18, literate, able to communicate with each other, and volunteering to answer all the questions in the questionnaire participated in our study.

The questionnaire method served as the primary data collection tool. Exclusions from the study encompassed children under the age of 18, patients with communication limitations due to mental retardation or mental disorders, individuals with severe comorbidities like cancer or chronic pain conditions such as fibromyalgia, and those who declined to answer specific questions in the questionnaire. Patients who refused to fill out the questionnaire due to dissatisfaction with the hospital were considered dissatisfied overall, and their dissatisfaction was reflected in all questionnaire questions. The general 'Outpatient Satisfaction' questionnaire, organized by the Ministry of Health Health Services, was administered to patients to assess their overall satisfaction with the healthcare institutions. Patients completed the questionnaire independently in a designated waiting area following their treatment and receipt of prescriptions. There were no health personnel present who could potentially influence their responses positively or negatively concerning the physician. The questionnaire comprised 15 questions aiming to evaluate the services received from the institution, along with six questions concerning the patients' sociodemographic characteristics (Tables 1, 2, 3). Four questions related to the institution required Yes/No responses, while the remaining 11 used a 3-point Likert-type scale (Yes/Partly/

No). For evaluation purposes, responses were scored, assigning zero points for 'No' , one for 'Partly' , and two for 'Yes' . Each individual's satisfaction level with these propositions was calculated based on their scores. Statistical analyses of the study were performed in the IBM Statistical Package for Social Sciences (SPSS) program version 21.0 package program. Categorical variables were represented by number and percentage, and continuous numerical variables were described by center and prevalence measures such as mean, standard deviation, and minimum and maximum values. The normal distribution conformity of the variables was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The Pearson Chi-square test was employed to compare categorical variables among groups. The Mann-Whitney U test was utilized to compare independent two-group continuous variables that did not adhere to normal distribution. Meanwhile, the Kruskal-Wallis test was applied for independent continuous variables among groups that did not conform to normal distribution, with Pairwise Comparison determining specific group differences. A statistical significance level of $p < 0.05$ was accepted.

Results

Seven hundred eighty female patients were included in the study, and the mean age was 35.52±10.99 years. 38.3% (299 people) of the patients were 30 years or younger, 84.1% (656 people) were married, 83.5% (651 people) had children, 47.9% (374 people)) had a secondary school or below education level, 56.8% (443 people) were working, and 97.3% (759 people) had social insurance.

Upon examining the patient's responses to the survey questions, the statement with the highest yes rate was "The doctor who examined me showed interest," with a rate of 96.4% (752 people). The highest no rate was 31.0% (242 people) in the statement "I was informed about the waiting times in the hospital"." was seen in the expression. It was determined that the lowest yes option was 51.5% (402 people) with the statement "The hospital was generally clean", and the lowest no option was "The doctor who examined me was interested".

72.7% (567 people) of the participants did not choose the doctor they would be examined by themselves, 87.1% (679 people) had a long waiting time to register, 52.7% said it was difficult to reach the hospital, 88.8% 693 people stated that they would recommend the hospital to their friends and family.

Table 1. *Sociodemographic characteristics of the patients*

	n	%
Age		
30 years and under	299	38.3
30–40 years	257	32.9
40 years and older	224	28.7
Marital status		
Married	656	84.1
Single	124	15.9
State of having children		
None	129	16.5
Yes	651	83.5
Educational status		
Middle school and below	374	47.9
High school	204	26.2
University	202	25.9
Working status		
Working	443	56.8
Not working	337	43.2
Social insurance		
Yes	759	97.3
No	21	2.7
Total	780	100.0

Table 2. *Patients' answers to survey questions*

	Yes n (%)	Partly n (%)	No n (%)
I was informed about the waiting times at the hospital.	404 (51.8)	134 (17.2)	242 (31.0)
Consultation and referral services were generally good.	596 (76.4)	155 (19.9)	29 (3.7)
Waiting rooms were comfortable.	425 (54.5)	228 (29.2)	127 (16.3)
I was examined within the specified time.	590 (75.6)	122 (15.6)	68 (8.7)
The doctor who examined me gave me information about my disease and took time.	739 (94.7)	24 (3.1)	17 (2.2)
The doctor who examined me was concerned.	752 (96.4)	20 (2.6)	8 (1.0)
Another staff was concerned about me.	680 (87.2)	66 (8.5)	34 (4.4)
The hospital staff took care of my privacy.	711 (91.2)	50 (6.4)	19 (2.4)
I had my examinations done within the time given to me.	641 (82.2)	97 (12.4)	42 (5.4)
The hospital was generally clean.	402 (51.5)	228 (29.2)	150 (19.2)
The service provided at the hospital was generally good.	628 (80.5)	126 (16.2)	26 (3.3)

Table 3. *Patients' answers to other survey questions*

	Yes n (%)	No n (%)
I chose the doctor, and I would be examined by myself.	213 (27.3)	567 (72.7)
The waiting time was too long to register	679 (87.1)	101 (12.9)
Was it difficult for you to reach the hospital?	411 (52.7)	369 (47.3)
I would recommend this hospital to my family and friends	693 (88.8)	87 (11.2)

Table 4. *Satisfaction level scores according to the sociodemographic characteristics of the patients*

	Median (min-max)	p
Age		
30 years and under	19 (9–22) [a]	0.001
30–40 years	18 (8–22) [a]	
40 years and older	20 (8–22) [b]	
Marital status		
Married	19 (8–22)	0.150
Single	19 (9–22)	
State of having children		
None	18 (9–22)	0.155
Yes	19 (8–22)	
Educational status		
Middle school and below	20 (8–22) [a]	<0.001
High school	19 (9–22) [a]	
University	18 (9–22) [b]	
Working status		
Working	19 (8–22)	0.006
Not working	20 (8–22)	
Social insurance		
Yes	19 (8–22)	0.802
No	19 (13–22)	

p value: Mann-Whitney U testi, Kruskal-Wallis test; a, b: Different characters indicate the difference between groups.

A significant difference was found in the satisfaction level scores between the age groups of the patients (p=0.001). It was shown that the group aged 40 and over had higher satisfaction levels than those aged 30 and below (p=0.012) and those aged 30–40 (p=0.001).

A significant difference was found in the satisfaction level scores between the education status groups of the participants (p<0.001). The satisfaction level score of the group with university or higher education level was significantly lower than the group with high school education (p=0.016) and secondary school and below (p<0.001).

When examined regarding working status, a significant difference was found between the groups' satisfaction levels (p=0.006). It was found that the non-working group had a higher satisfaction level score than the working group.

Discussion

Among the sociodemographic variables used in our study, there was a statistically significant positive correlation between patient satisfaction and age; a statistically significant negative correlation was found between patient satisfaction, educational status, and working status. Publications that found higher satisfaction rates with increasing age, like our study, interpreted as people becoming more mature and tolerant or that older people are treated more kindly[9].

Despite that, some studies have concluded that sociodemographic variables do not seriously affect patient satisfaction [3]. On the contrary, Widjaja et al. identified a significant relationship between sociodemographic characteristics and the level of patient satisfaction in their study[10].

In their studies, some researchers reported that age was not associated with patient satisfaction[11]. In contrast, Akinlusi et al. found that age and education were significantly associated with outpatient satisfaction[12].

Different results regarding the relationship between age and satisfaction outcomes were attributed to the fact that the content of the questionnaire applied may have been created differently or the method used for the questionnaire was different. Our study determined that the patient's satisfaction with the institution decreased as their education level increased. This result can be interpreted as people with higher education levels may have higher expectations from the institution. Higher education levels can also explain the higher the person's standards, the experience of different alternative institutions, and having a more critical perspective. Unlike the results of our study, Önsüz et al., In their study, however, could not establish a relationship between educational status and satisfaction level[9].

A Scandinavian study reported no significant difference between age and institution satisfaction. With the increasing education level, the patient's expectations and perspectives on the services differed, and patient satisfaction decreased[11]. In the case of low educational status, the possible cause of dissatisfaction is communication problems with the patients. However, the practical satisfaction level may be the low level of expectation in these patients.

The intersection of the working group with the higher education group explained the decreased institution satisfaction in the working patient group in our study. In our study, no significant relationship was found between social security and the general satisfaction level of the patients. This data is an expected result since patients with or without health insurance under the umbrella of the social state receive the same standard and quality service.

Although more studies on waiting times are needed to evaluate our country's standards, it is known that waiting time for examination affects satisfaction[13]. Tehrani et al. suggested in their study that the availability of healthcare providers for longer consultation durations could enhance patient satisfaction[14].

In their study conducted at the university hospital's obstetrics and gynecology outpatient clinic, Güney et al. found that the most significant satisfaction determinant was the examination waiting time. At the same time, the understanding of the information provided by the doctor was influenced by education level, age, the quality of the information provided, and the duration of the examination[15].

Our study determined that the patients who stated the waiting time as long had a high rate of dissatisfaction. Kabaroglu et al. reported from a different perspective that the perceived waiting time of the patients was longer than the actual waiting time in their study[1].

In our study, 87.1% of the patients answered, "I waited too long for the registration process," and 82.2% answered, "I had my tests done within the time I was informed." The patient group, who thinks that they do not wait too long for their examinations, thinks that they wait too long for the registration.

Patients see the long waiting times for the outpatient clinic because of our hospital's registration system. Appointments cannot be made online or by phone at our university. Our hospital provides service to many rural areas besides the city center. Considering the physical conditions of the city and the distance from the villages, people have to leave their homes much earlier than the outpatient clinic starting time, for the appointment process begins early in the morning. The fact that the patients come to the hospital long before the opening hours of the outpatient clinic to get a queue number and include this time in the waiting time of the outpatient clinic reveals dissatisfaction with the waiting times.

In addition, agriculture and livestock are an essential source of livelihood in our province. Since they are engaged in animal husbandry, many of our patients expect their examinations to be completed before noon and to return to their villages at the earliest hour by traveling a long distance with village cars, which have no alternative and are extremely rigid regarding hours. The preference for morning appointments leads to overcrowding in the outpatient clinics, significantly reducing our hospital's service quality.

Unfortunately, there are serious problems, such as patients who need help getting a queue number even though they enter the registration queue very early and have to return to their villages without getting outpatient clinic service, which creates a severe satisfaction problem for our institution. The questionnaire utilized in our study unveiled issues within the appointment scheduling system. It can cause patients and their relatives to be in constant conflict-filled communication with the doctor to get an examination or enter it earlier. At the same time, it may cause communication disorders that will damage the patient-physician trust relationship. Failure to establish proper communication with the patient from the beginning will also reduce the physician's motivation.

Therefore, we believe that implementing an online or telephone appointment system at our hospital and extending the allotted examination time for each patient by appropriately managing the number of patients would effectively reduce the perceived waiting time. Consequently, this approach would also enhance patient satisfaction.

Fifty-four percent of patients answered, "The waiting rooms were comfortable." This outcome underscores the advantage of reorganizing the patients' seating, waiting, and resting areas within our hospital. Additionally, it was noted that overall patient satisfaction stood at 51%. The most significant factor contributing to this decline in satisfaction was cleanliness issues and inadequacies concerning public restrooms.

As revealed in the publications, there is a positive interaction between patient satisfaction and the attitudes of health personnel[1]. Rather than the physical conditions of the institution, the patients are affected mainly by the smiling face, friendly attitudes, interest, listening to the patient, and the sincere answers they see from the physician, and by the friendliness and skills of other healthcare professionals such as nurses[16]. According to the study conducted by Rui, the incorporation of humanistic care into outpatient nursing practices in obstetrics and gynecology demonstrates a significant enhancement in patients' perception of service quality provided by community hospitals. This outcome highlights the practicality and importance of implementing and promoting such an approach[17]. In their study, Sanad et al. evaluated the parameters of timing,

nursing care, physician care, surrounding environment, and overall satisfaction. They identified waiting time, nurses' directions, physician communication, and the surrounding area influencing patient satisfaction. In conclusion, there is a clear need for continuous improvement in the quality of care within the healthcare setting, particularly to enhance patient satisfaction[18].

The trust and loyalty of the physician and the institution will increase when the patients can find answers to their questions in a simple way that they can understand, apart from their medical jargon about their diseases, and intelligibly for the parts that do not come to their minds.

According to our study, 96.4% of the patients were satisfied with the doctor's behavior, 87.2% of the staff members, and 94.7% were satisfied when the physician gave information about the patient's disease. 91.2% of patients were generally satisfied with the attention paid to their privacy (such as removing the door, curtain, or screen).

In our study, transport to the hospital is complex, with a result of 52.7%, and the related reason is that most patients come from distant villages. In the study, the vast majority of our outpatients, 88.8%, stated that they were satisfied with the hospital by saying they could recommend the hospital to their families and friends.

Taneja et al. emphasized in their study evaluating outpatient services that patients are the backbone of the healthcare system. Therefore, they should be prioritized to enhance the efficiency and effectiveness of the healthcare system[19].

We think that our hospital is the only university hospital in our province, and the physical conditions are better than the state hospital in the region and other institutions in the vicinity. The high quality of service of the doctors and staff is influential in forming this level of satisfaction.

Future research should shift its focus toward aspects related to clinical trial participation and explore beyond the conventional expectations of patients regarding healthcare accessibility, facilities, clinical skills of the healthcare team, and attentiveness toward patients' concerns[20].

Hospital administration should prioritize addressing the various drawbacks or deficiencies patients and attendants highlight. By doing so, improvements can be made to hospital services, leading to enhanced patient satisfaction[21].

Conclusion

As in every sector, quality is sought in providing health services in the health sector today. Patient satisfaction survey studies are essential in terms of measuring these services. Such studies will benefit health policies and planning to be established nationwide.

The logic that you cannot fix the variable you cannot measure is indisputably true. Patient satisfaction is assessing how satisfied a patient is with their healthcare. Therefore, it is one of the most important indicators of the success of the health institution.

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It's crucial to remember that healthcare institutions also function as commercial organizations. For the survival of the institutions, fair competition between the institutions and the more preferred and the survival of that health institution, hospital administrators should continuously measure patient satisfaction with questionnaires. This approach helps to identify the hospital's shortcomings; medical care should enhance the patient's health status and improve their overall well-being. In addition, it should act on the fact that responding to their needs and desires is too essential to be underestimated in ensuring patient satisfaction.

If hospital managers can use their financial resources rationally, they can increase the hospital's preference. While the number and quality of personnel within the outpatient clinic significantly impact satisfaction levels, these factors were not evaluated in our study, representing a missing aspect of our research. As a result, it was determined that the satisfaction level of the patients who applied to our hospital's obstetrics and gynecology outpatient clinic was high in general. The physician's attention was the highest satisfaction, while the hospital's general cleanliness was the lowest. The most common complaint was the length of waiting times for registration. The findings suggest that enhancing the quality of health services, reducing waiting times, and conducting assessments of waiting rooms and general service areas would be advantageous.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Satisfaction Evaluation of Patients Hospitalized in University Hospital Gynecology and Obstetrics Service

Üniversite Hastanesi Kadın Hastalıkları ve Doğum Servisi'nde Yatan Hastaların Memnuniyet Değerlendirmesi

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ABSTRACT

Aim: *A quality health service is not independent of patient satisfaction. Patient satisfaction research is one of the primary instruments for better quality service. With this study, we aimed to evaluate the satisfaction levels and expectations of the patients hospitalized in the Department of Gynecology and Obstetrics of the Kafkas University Training and Research Hospital to determine whether the satisfaction level differs according to socio-demographic characteristics and to restructure the light of the positive and negative data obtained.*

Material and Method: *Between May 2021 and September 2022, 655 patients who received inpatient services at the Department of Gynecology and Obstetrics at Kafkas University Training and Research Hospital were surveyed.*

The questionnaire included a section about socio-demographic characteristics and 14 questions to determine their views on satisfaction. The questions were answered as 'yes', 'a little', and 'no'.

Results: *In our study, the highest level of satisfaction was for the nurses giving information about the treatment and care to the patient and the doctor's interest in the patient. According to the results of the multivariate linear regression analysis, it was determined that age, education status, and length of stay affected the satisfaction level independently of other variables (p<0.001). It was observed that working status did not affect satisfaction (p=0.434).*

Conclusion: *Our study highlighted that hospital cleanliness significantly impacted the overall satisfaction levels, indicating a need for readjustments by hospital management.*

Keywords: *gynecology and obstetric department; healthcare management; healthcare services; inpatient services; university hospital; patient satisfaction*

ÖZET

Amaç: *Kaliteli bir sağlık hizmeti, hasta memnuniyetinden bağımsız düşünülemez. Hizmet kalitesini artırabilmek için hasta memnuniyeti araştırması temel araçlardan biridir. Bu çalışma ile Kafkas Üniversitesi Eğitim ve Araştırma Hastanesi Kadın Hastalıkları ve Doğum Kliniği'nde yatan hastaların memnuniyet düzeylerini ve beklentilerini değerlendirmeyi amaçladık. Ayrıca, memnuniyet düzeyinin sosyodemografik özelliklere göre farklılık gösterip göstermediğini belirlemeyi ve elde edilen olumlu ve olumsuz veriler ışığında sağlık hizmetlerinin yeniden gözden geçirilmesini hedefledik.*

Materyal ve Metot: *Mayıs 2021 ile Eylül 2022 tarihleri arasında Kafkas Üniversitesi Eğitim ve Araştırma Hastanesi Kadın Hastalıkları ve Doğum Kliniği'nde yatan 655 hasta soruşturuldu. Anket, sosyodemografik özellikleri belirlemek için bir bölüm ve memnuniyetlerini değerlendirmek için 14 soru içeren bir diğer bölüm olarak oluşturuldu. Sorular, 'evet', 'biraz' ve 'hayır' olarak cevaplandı.*

Bulgular: *Çalışmamızda, hasta ve doktor arasındaki iletişimde hemşirelerin bilgi verme konusundaki performansı değerlendirilmiş ve en yüksek memnuniyet düzeyine ulaşılmıştır.*

Çoklu değişkenli lineer regresyon analizi sonuçlarına göre, yaş, eğitim durumu ve kalış süresinin diğer değişkenlerden bağımsız olarak memnuniyet düzeyini etkilediği belirlendi (p<0,001). Hastanın çalışma hayatı ile ilgili durumunun memnuniyeti etkilemediği gözlemlendi (p=0,434).

Sonuç: *Çalışmamızda, hastanenin temizliğinin genel memnuniyet düzeyini düşürdüğü tespit edildi. Sağlık hizmetleri yöneticileri tarafından temizlik hizmetlerinin hasta memnuniyetini artıracak şekilde yeniden düzenlenmesi gerekliliği ortaya konmuştur. Hasta memnuniyet değerlendirmelerinin periyodik olarak yapılmasının sağlık hizmetlerinin kalitesini artıracağına inanılmaktadır.*

Anahtar kelimeler: *hasta memnuniyeti; kadın hastalıkları ve doğum servisi; sağlık hizmetleri; sağlık yönetimi; üniversite hastanesi; yatan hasta hizmetleri*

Introduction

Patient satisfaction is one of the essential factors that can be measured in evaluating health services[1]. Patient satisfaction is "the primary criterion that gives information about the level of satisfying the values and expectations of the patient for the leading authority is the patient shows the quality of care"[2]. For quality healthcare services, it's essential to efficiently utilize resources, maintain fairness in their allocation, deliver services effectively, and ensure satisfaction among

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service users during and after service delivery[3]. Low- and middle-income countries exhibit the highest prevalence of poor-quality health care[4].

Patient satisfaction, one of the quality determinants of health institutions, can generally be defined as "meeting the wishes and expectations of the patients or providing services above these requests and expectations"[3]. Patient satisfaction is a product of the quality that patients perceive and expect. According to this statement, a patient has some expectations before receiving the service and has certain perceptions based on his experience as a result of the service he receives. The patient decides his satisfaction by comparing the perceived and expected quality[5]. As evident here, patient satisfaction is determined by the patient's expectations and perception of the received service. The first factor, expectations, encompasses the features sought or desired by patients from healthcare institutions. These expectations vary based on the patient's age, gender, education level, socio-cultural characteristics, health services, and previous experiences with healthcare institutions. The second factor is measured based on the services individuals receive and their opinions or evaluations about this process. The perception factor varies according to the characteristics of the patients and their experiences with health institutions[3]. Since patient satisfaction is considered a cognitive response influenced by many factors, its subjective perception makes it difficult to compare results[1]. The behavior of doctors, nurses, and other health professionals, ease of access to the hospital or examination room, general cleanliness of the hospital, staff cleanliness, patient privacy, and trust between the patient and patient-physician are the factors that affect patient satisfaction the most[5].

Although patient satisfaction is a difficult perception that can vary between individuals, it is essential in shaping and managing the service provided. In recent years, the interest in patient satisfaction in health institutions has increased, and measurement studies have become widespread[3]. Patient satisfaction is gaining increasing prominence in medicine; however, there needs to be more knowledge regarding patient satisfaction in women's health and other specialties. The most effective way to learn about the patient's satisfaction with the health service they receive is to use the evaluation tools prepared to determine their thoughts. Questionnaires designed to measure patient satisfaction also serve this purpose[6].

This study aimed to evaluate the satisfaction levels and expectations of the patients hospitalized in the Department of Gynecology and Obstetrics of the Kafkas University Training and Research Hospital to determine whether the satisfaction level differs according to socio-demographic characteristics and to restructure the light of the positive and negative data obtained.

Materials and Methods

This study used face-to-face and under-observation survey methods on 655 patients who received inpatient service in the Department of Gynecology and Obstetrics of the Kafkas University Training and Research Hospital between May 2021 and September 2022. The Ethics Committee of Kafkas University Training and Research Hospital obtained written permission for the study. The study sample comprised patients over 18 hospitalized in the Gynecology and Obstetrics Department. The participants could understand what they read, communicate with each other, and volunteered to participate in the study. Patients in the intensive care unit were excluded from the study.

In addition to the independent variables in the questionnaire used in the study, age, marital status, education level, length of stay, working, and having children, there is a section with 14 questions to determine their views on satisfaction. The questions about satisfaction included questions about the patient's room, hospital food, the hospital's and staff's hygiene status, the hospital staff's attitude, treatment, and discharge. The questions were answered as 'yes' , 'a little' , and 'no' .

The data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) program version 21.0 program. The normal distribution of variables was assessed via Kolmogorov-Smirnov and Shapiro-Wilk tests. Categorical variable comparison between groups was conducted using the Pearson Chi-square test. The Mann-Whitney U test was utilized for independent two-group continuous variables that did not follow a normal distribution. For continuous variables that did not adhere to normal distribution, the Kruskal-Wallis test was applied, with Pairwise Comparison determining differences between groups. Multiple Linear Regression analysis was carried out in the study to identify independent risk factors influencing the satisfaction level. The significance level was accepted as $p<0.05$.

Results

Six hundred fifty-five patients received inpatient treatment in the Department of Obstetrics and Gynecology of Kafkas University Training and Research Hospital participated. Some socio-demographic characteristics of the participating patients are given in Table 1. The mean age of the patients was 38.63±8.61. 40.9% (268 people) of the patients were under 35 years old, 85.2% (558 people) were married, 98.8% (647 people) had children, 64.3% (421 people) had a secondary school education level and below, and 50.4% (330 people) were not working. It was determined that 67.8% (444) of the participants were treated in the gynecology service, and 35.7% (234) were hospitalized for three days or more.

Participants of the research, Patient room, hospital food, hospital hygiene, staff attitudes toward hygiene, doctors, nurses, and other personnel, and satisfaction levels regarding treatment and discharge are given in Table 2. The comparison of the satisfaction levels of the participants with their demographic characteristics is shown in Table 3.

Upon analyzing the patients' responses to the survey questions, the highest yes rate was 90.5% (593 people), and "I was informed about my treatment and care by the nurses." and "My doctor was interested in me." Their statement, "The hospital was clean in general," with the lowest satisfaction rate of 12.2% (80 people) found in the statement. "The hospital was generally clean," with 67.9% (445 people) of the lowest yes option. None of the participants "I was informed about my treatment and care by the nurses.", "My doctor was interested in me." and "The service at the hospital was generally good." did not tick the no option to their statements.

There was a significant difference in satisfaction level scores between the age groups of the patients (p=0.028). It was demonstrated that the group aged 35 and below showed higher satisfaction compared to those aged 45 and over (p=0.024).

A significant difference was found in the satisfaction level scores among the groups categorized by the education status of the participants (p<0.001). The satisfaction level score of the group whose education level is a university and above is significantly lower than the group with high school (p<0.001) and secondary school and below (p<0.001).

Table 1. *Socio-demographic characteristics and hospitalization status of the patients*

	n	%
Age		
35 years and under	268	40.9
35–45 years	242	36.9
45 years and older	145	22.1
Marital status		
Married	558	85.2
Single	97	14.8
Status of having children		
No	8	1.2
There is	647	98.8
Education status		
Middle school and below	421	64.3
High school	130	19.8
University and above	104	15.9
Working status		
Working	309	47.2
Not working	330	50.4
Retired	16	2.4
Service		
Gynecology	444	67.8
Obstetrics	211	32.2
Length of stay		
1 day	188	28.7
2 days	233	35.6
3 days or more	234	35.7
Total	655	100.0

Table 2. *Patients' answers to survey questions*

	Yes n (%)	A little n (%)	No n (%)
The patient room and its surroundings provided a quiet and calm environment.	479 (73,1)	172 (26,3)	4 (0,6)
The furniture in the room was in working condition.	543 (82,9)	89 (13,6)	23 (3,5)
The staff distributing food acted according to the hygiene rules	541 (82,6)	81 (12,4)	33 (5,0)
The temperature of the food was appropriate.	542 (82,7)	96 (14,7)	17 (2,6)
I was informed about my disease by my doctor.	589 (89,9)	65 (9,9)	1 (0,2)
I was informed about my treatment and care by the nurses.	593 (90,5)	62 (9,5)	0 (0,0)
My doctor was interested in me.	593 (90,5)	62 (9,5)	0 (0,0)
Nurses were concerned about me.	589 (89,9)	63 (9,6)	3 (0,5)
The cleaning staff was concerned about me.	548 (83,7)	43 (6,6)	64 (9,8)
The hospital staff took care of my privacy.	557 (85,0)	91 (13,9)	7 (1,1)
The hospital was generally clean.	445 (67,9)	130 (19,8)	80 (12,2)
I reached my doctor when I needed it regarding my illness.	577 (88,1)	18 (2,7)	60 (9,2)
The service provided at the hospital was generally good.	563 (86,0)	92 (14,0)	0 (0,0)
While leaving the hospital, I was informed about the post-discharge process.	564 (86,1)	73 (11,1)	18 (2,7)

Table 3. *Satisfaction scores according to patients' socio-demographic characteristics and hospitalization status*

	Median (min-max)	p
Age		
35 years and under	28 (17–28) [a]	**0.028**
35–45 years	27 (11–28)	
45 years and older	27 (12–28) [b]	
Marital status		
Married	28 (11–28)	0.354
Single	27 (11–28)	
Status of having children		
No	28 (20–28)	0.557
There is	28 (11–28)	
Education status		
Middle school and below	28 (11–28) [a]	**<0.001**
High school	27 (19–28) [a]	
University and above	22 (12–28) [b]	
Working status		
Working	27 (11–28) [a]	**<0.001**
Not working	28 (11–28) [b]	
Retired	27 (12–28)	
Service		
Gynecology	28 (11–28)	0.101
Obstetrics	27 (12–28)	
Length of stay		
1 day	28 (22–28) [a]	**<0.001**
2 days	27 (17–28) [b]	
3 days or more	27 (11–28) [b]	

p-value: Mann-Whitney U test, Kruskal-Wallis test; a, b: Different characters indicate the difference between groups.

When Table 4 is reviewed, it is observed that there is a significant difference in satisfaction levels among the groups when analyzed according to their employment status ($p<0.001$). The satisfaction level score of the employed group was determined to be lower than that of the non-employed group ($p<0.001$).

A significant difference was found in the satisfaction level scores between the groups determined according to the length of hospitalization of the participants ($p<0.001$). The satisfaction level score of those hospitalized for 1 day was significantly higher than the group hospitalized for 2 days ($p<0.001$) and hospitalized for 3 days or more ($p<0.001$).

There was no significant difference in the median scores of the satisfaction level in the groups determined regarding marital status, having children, and the service they were hospitalized ($p>0.05$).

According to the results of the multivariate linear regression analysis, which included the parameters affecting the satisfaction level of hospitalized patients according to the univariate analysis, it was determined that age, education status, and length of stay affected the satisfaction level independently of other variables ($p<0.001$). It was observed that working status did not affect satisfaction ($p=0.434$).

Discussion

Using performance indicators to evaluate these aspects is a means of assessing the quality of the healthcare system. It serves as a roadmap for future actions. The healthcare sector, particularly institutions like hospitals, plays a crucial role in ensuring the widespread availability of high-quality healthcare services[7].

A quality health service cannot be considered independent of patient satisfaction. For this reason, patient satisfaction research is one of the basic needs to provide better quality service in hospitals. The academic hospitals express satisfaction with the feasibility of their proposal for a concise, dependable, valid, and discriminatory questionnaire to measure patient satisfaction[8].

Omotayo et al. observed in their study that patients' satisfaction was reflected in their favorable remarks concerning various aspects of healthcare delivery, including the overall facility, doctor's consultations, and the efficiency of the healthcare system. These findings indicate a positive perception and contentment among patients regarding the services they receive. Additionally, the majority's consistent and regular utilization of healthcare services contributes to patient satisfaction[9].

Our study's general satisfaction level with hospital services was 86.1%. Many studies have been done on

Table 4. *Factors affecting the level of satisfaction in patients, multiple linear regression analysis*

	Not standardized β	Standard error	Standardized β	t	p	%95 confidence interval for β	
	33.009	0.915		36.08	<0.001	31.212	34.805
Age	-0.568	0.211	-0.097	-2.699	0.007	-0.982	-0.155
Education	-1.999	0.248	-0.334	-8.070	<0.001	-2.486	-1.513
Working status	0.274	0.349	0.033	0.783	0.434	-0.412	0.960
Length of stay	-1.959	0.198	-0.348	-9.873	<0.001	-2.348	-1.569

Dependent variable: Inpatient satisfaction level score; R2=0.251; F=54.527; p<0.001.

patient satisfaction before. In their study, Kaya et al. demonstrated varying overall client satisfaction with health services[10].

In their study, Marama et al. found that respondents who lived in rural areas had a hospital stay of less than four days, were admitted for the first time, and reported satisfaction with privacy assurance were more likely to express satisfaction than their counterparts[11].

In their study, Akinlusi et al. assessed patients' satisfaction with gynecological services at a tertiary hospital to facilitate improvements in health service delivery. The study involved face-to-face interviews using closed-ended questionnaires. The participants had a mean age of 37.8±10.9 years, with 63.8% having tertiary education. Findings revealed that 20.8% of respondents experienced delayed retrieval of medical records, 22.2% expressed dissatisfaction with hospital meals, and 31.6% were displeased with waiting time[12].

In their study, Patel and colleagues have found that although several factors influence patient satisfaction, adopting factors such as reducing waiting times, ensuring effective patient-physician communication, and involving patients in the decision-making process might aid physicians in achieving optimal results[13].

In their study, Widjaja demonstrated a significant correlation between socio-demographic factors and patient satisfaction. The findings revealed that most satisfied respondents were between 18–25 and were single. Additionally, most were college graduates, unemployed, and from the low-middle socioeconomic class[14].

Mahfouzet et al. emphasized the need to assess patient satisfaction regarding the quality of care across all aspects of hospitals, from admission to discharge. In their study, the overall satisfaction with general medical care was found to be 44.3%, and most patients were satisfied with admission, toilets, and the food provided to them[15].

In the study conducted at Marmara University Medical Faculty Hospital, the satisfaction level of hospitalized patients was found to be 64.5%[3]. Similarly, in the study conducted at Dicle University Medical Faculty Hospital, the satisfaction level in the inpatient wards was reported as 91.8%[16]. Furthermore, in the Gynecology and Obstetrics Service of Süleyman Demirel University Medical Faculty Hospital, the inpatients' satisfaction level was 90.2%[17]. However, in the study conducted at Silvan State Hospital, the satisfaction level was found to be 76%[18].

No cut-off value for patient satisfaction has been determined. Still, the literature has stated that values of 70% and above reflect satisfaction[3]. In our study, when we compare this ratio, the level of satisfaction is high. The high satisfaction may be because there is only one university hospital in our city and no private hospital. Hence, people compare the university hospitals with state hospitals. We think that university hospitals have academic staff and more technological facilities, that their employees serve in this direction, and that the services provided are carried out as scientifically as possible, an essential reason for patient preference and satisfaction.

In the study of Önsüz et al., the average age of the group that stated that they were generally satisfied with the service they received was higher than the average age of the group that said that they were not satisfied[3]. In our study, a significant difference was found between the age groups and satisfaction levels of the patients. It has been shown that the level of satisfaction is higher, especially in the group aged 35 and below, compared to the group aged 45 and above. This result may be because of the prolongation of the treatment due to chronic disease and the comorbidities of the elderly group. In our study, chronic diseases were not considered, but the length of hospital stay was evaluated. Participants' hospitalization times were assessed, and the satisfaction levels of those who received hospitalization for 1 day were higher than those who received hospital treatment for 2 or more days.

In the study of Önsüz et al., no relationship was found between education level and general satisfaction levels [3]. In the study of Uzuntarla et al., a significant difference was found between the education level of the participants and their satisfaction level. It was observed that those who graduated from primary or secondary school were more satisfied than those who were illiterate[6]. Our study found a significant difference in the satisfaction level among the participants' educational background groups. The satisfaction level of the group whose education level is at a university and above is significantly lower than those whose education level is at high school and secondary school and below. A significant difference was found between the groups' satisfaction levels regarding working status. The satisfaction level of the working group was lower than that of the non-working group. This difference may be because as

the level of education and participation in social life increases, people's standards rise, and the level of satisfaction becomes more difficult due to increased expectations due to more information about alternatives.

No significant difference was found in the satisfaction level. The median scores of the groups were determined based on marital status and having children. The service they were hospitalized in indicates that these factors may not significantly affect patient satisfaction in our study.

In our study, the highest level of satisfaction was for the nurses giving information about the treatment and care to the patient and the doctor's interest in the patient. Providing information makes patients feel valued and establishes interpersonal trust[19]. Research shows that the quality of communication between doctors and patients contributes to patient satisfaction. Good self-expression of patients provides physicians with a more accurate diagnosis and a more effective treatment plan. It is observed that when doctors can communicate clearly with their patients about diagnosis, treatment options, and treatment, patients are more likely to comply with the treatment plan thanks to a stronger patient-physician trust relationship, increasing patient satisfactio[n3].

In their study, Chang et al. also discovered that the highest increase in overall medical treatment satisfaction was associated with improved satisfaction with medical professionals. However, the most negligible impact was observed concerning service personnel in the general and gynecology clinics[20].

In their studies, Aksakal and Bilgili found that the satisfaction level of informing in nursing services was low[21]. Some studies have stated that satisfaction with nurse services is more important than overall satisfaction. This result may be related to the fact that inpatients communicate with nurses the most, reach them quickly, and receive more support from them[3]. As a result, physicians and nurses have adequately informed patients about their current diseases and procedures, positively affecting patient satisfaction. Jha et al. found that hospitals with high nurse staffing levels are associated with better patient experiences, indicating areas that require improvement. Their study also proves hospitals can deliver high-quality clinical care and a positive patient experience[22].

In their study, Hussain and colleagues found that 90.9% of patients expressed satisfaction with their treatment. In comparison, 89% of patients had confidence in the competency of their doctors. However, only 60% of patients considered the treatment affordable, and cleanliness was satisfactory for only 20%. Additionally, how doctors greeted their patients satisfied only 17% of patients. The study suggests an urgent need to improve doctors' communication skills and hospital cleanliness. Effective communication with patients regarding their illness and treatment is crucial in reducing anxiety, dispelling misconceptions, and promoting treatment compliance. By achieving these good health standards, patients can develop confidence in the healthcare system[23]. Yen and Eryn's research suggests that by combining previously successful methods of improving patient satisfaction and enabling physicians to utilize individualized patient-centered communication, patient satisfaction can be further enhanced[24].

Patient satisfaction affects the attitudes and behaviors of physicians, nurses, and other personnel toward patients, as well as the physical conditions of the hospital. In our study, while the general satisfaction level was found to be high with the patient rooms and their belongings, the general satisfaction level regarding the cleanliness of the hospital was found to be low. This situation reveals that Kafkas University Training and Research Hospital should give more importance to cleaning.

Some factors may affect satisfaction but were not evaluated in our study. These include the number of personnel working, job satisfaction of working personnel, chronic disease history of patients receiving health services, transportation to the hospital, canteen services, companion satisfaction, reasons for choosing the hospital and re-preferring. As well as measuring patient satisfaction, ensuring the continuity of satisfaction is also important. It is important to repeat the studies at regular intervals to try to eliminate the deficiencies revealed as a result of the studies and to determine the needs and wishes of the patients. Ensuring the employees' satisfaction is one of the crucial factors in patient satisfaction[19].

Conclusion

As a result, the general satisfaction levels among patients hospitalized in the Department of Gynecology and Obstetrics at Kafkas University Training and Research Hospital were high. It was concluded that a statistically significant difference existed based on socio-demographic characteristics, including age,

educational status, working status, and length of stay. In the general cleaning of the hospital, where the satisfaction level of the patients is the lowest, addressing the cause of dissatisfaction as personnel error, lack of personnel, and lack of material and making improvements will be beneficial in terms of patient satisfaction and service quality. Assessing patient satisfaction with healthcare services, identifying the reasons for patient dissatisfaction, and addressing them will enable opportunities for improvement. Through such studies, significant contributions can be made to enhance national health policies and hospital standards.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Examination of Healthcare Workers' Knowledge, Attitudes, and Behaviors Regarding the Pandemic

Sağlık Çalışanlarının Pandemiye Yönelik Bilgi, Tutum ve Davranışlarının İncelenmesi

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ABSTRACT

Aim: *This study examined healthcare workers' knowledge, attitudes, and behaviors towards the pandemic.*

Material and Method: *This descriptive study involved 546 healthcare workers, with data collected through an information form.*

Results: *The results indicated that most participants engaged in training and information sessions related to the COVID-19 pandemic, utilized trustworthy information sources, and did not consistently receive the influenza vaccine. 41.8% of the participants worked in the health sector during the influenza A (H1N1) pandemic. It was statistically significant that the participants had children getting influenza vaccinations and worked actively during the influenza A (H1N1) pandemic. Those who worked in the healthcare sector during the influenza A (H1N1) pandemic saw themselves as competent in terms of the risks of influenza and COVID-19 pandemics, ways of protection, patient management, had less anxiety during patient management, and influenza vaccine and antiviral treatment statistically significantly reduced anxiety. It was determined that they defined the lack of effective vaccine or antiviral treatment for COVID-19 as a factor that increased their stress during the study.*

Conclusion: *It was determined that the knowledge, attitudes, and behaviors of the participants towards the pandemic were at an adequate level and that past pandemics, having children, and pandemic treatment were factors affecting the level of knowledge, attitudes, and behaviors. In the pandemic, the continuity of education and supporting the strengths of healthcare workers are recommended, so more comprehensive and qualitative studies on the subject are recommended. Also, It is advised to incorporate pandemic-related topics into training programs.*

Keywords: *pandemic; healthcare workers; education; knowledge*

ÖZET

Amaç: *Bu araştırmada, sağlık çalışanlarının pandemiye yönelik bilgi, tutum ve davranışlarının incelenmesi amaçlanmıştır.*

Materyal ve Metot: *Tanımlayıcı olarak planlanan çalışma, bir eğitim araştırma hastanesinde çalışan 546 sağlık çalışanı ile gerçekleştirilmiştir. Veri toplama aracı olarak sosyodemografik özellikleri ve pandemiye yönelik bilgi, tutum ve davranışları belirlemek amacıyla hazırlanan bilgi formu kullanılmıştır.*

Bulgular: *Katılımcıların çoğunluğunun COVID-19 Pandemisi'ne yönelik eğitim ve bilgilendirme faaliyetlerine katıldığı, güvenilir bilgi kaynaklarından yararlandıkları, influenza aşısını düzenli yaptırmadığı, katılımcıların %41,8'inin İnfluenza A (H1N1) 2009 Pandemisi'nde sağlık sektöründe çalıştığı saptanmıştır. Çalışmaya katılan sağlık çalışanlarının influenza aşısı yaptırma davranışında, çocuk sahibi olması ve İnfluenza A (H1N1) Pandemisi'nde aktif olarak çalışmış olmaları istatistiksel olarak anlamlı bulunmuştur. İnfluenza A (H1N1) Pandemisi'nde sağlık sektöründe çalışmış olanların, İnfluenza ve COVID-19 Pandemilerinin riskleri, korunma yolları, hasta yönetimi konusunda istatistiksel olarak anlamlı bir biçimde kendilerini yeterli gördükleri, hasta yönetimi esnasında daha az kaygı duydukları, influenza aşısı ve antiviral tedavinin istatistiksel olarak anlamlı biçimde kaygı duymalarını engelleyen birer faktör olarak tanımladıkları, çalışmanın yapıldığı dönemde COVID-19 için henüz etkili aşı ya da antiviral tedavi bulunamamasını kaygı duymalarını artıran faktör olarak tanımladıkları belirlenmiştir.*

Sonuç: *Katılımcıların pandemiye yönelik bilgi, tutum ve davranışlarının yeterli düzeyde olduğu, geçmiş pandemilerde görev almanın, çocuk sahibi olmanın ve pandemiye yönelik tedavinin bilgi, tutum ve davranış düzeyini etkileyen faktörler olduğu saptanmıştır. Bir afet olarak tanımlanan pandemi yönetiminde eğitimin sürekliliği ile sağlık çalışanlarının güçlü yönlerinin desteklenmesi ve zayıf yönlerinin iyileştirilmesi, konuya ilişkin daha geniş katılımlı ve niteliksel çalışmaların yapılması ve eğitim programlarına pandemiye ilişkin konuların eklenmesi önerilmektedir.*

Anahtar kelimeler: *pandemi; sağlık çalışanları; eğitim; bilgi*

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Introduction

Throughout history, pandemics have had significant regional, national, and global effects, bringing about crucial changes and developments in various aspects of life, including social, educational, economic, and healthcare systems. Pandemics such as Influenza A (H1N1) and COVID-19, known as recent pandemic examples, exhibit essential differences in mortality and fatality rates and rapid global spread, emphasizing the dynamic nature of these events[1,2]. Predicting when, from which source, or in what form pandemics will emerge is challenging[3]. Consequently, it is anticipated that a critical characteristic for individuals and professionals following all these pandemics will be their ability to cope with pandemics, as facing pandemics will continue to be a part of the "pandemic future," necessitating learning to live with it. The experiences from past pandemics, the current situation, and forecasts particularly compel preventive measures and practices to ensure the sustainability of the healthcare system[4,5].

For the successful management of the pandemic process, not only does multidisciplinary collaboration play a crucial role, but healthcare workers' competence is also paramount. The effective management of the COVID-19 Pandemic, described as an "indefinite and prolonged complex emergency," requires standard solutions and practices outlined by the World Health Organization (WHO) and our country's Pandemic Influenza Preparedness Plan and COVID-19-related guidelines[5–6]. Among the most effective strategies is the importance of accurate information management and current, sound education[8]. Reviewing relevant literature reveals that healthcare workers, described as effective crisis managers during pandemics, carry significant risks. It is emphasized that their knowledge, attitudes, and behaviors towards pandemics are crucial. Possessing adequate information about pandemics will support positive perceptions and attitudes. It will also effectively reduce stress, anxiety, or levels of concern [9–13]. Despite the importance of this issue, some studies indicate that healthcare workers may not have sufficient knowledge about pandemics[14–16]. Furthermore, it is noted that research on pandemics will not only guide preventive measures and strategies but also contribute to preparing for future anticipated pandemics[17].

Therefore, with the focal point of healthcare services being the "individual," the individual and professional responsibilities of healthcare services and healthcare workers gain even more significance during this process. Despite the increasing research on COVID-19 in all fields, studies on pandemics, in general, are limited, and the conducted studies often separate influenza and COVID-19 for individual examination[9,18–20]. Considering these factors, it is believed that this study on the awareness levels of healthcare workers regarding pandemics will be crucial due to its biopsychosocial effects and critical role in quality care. It is expected to enhance awareness in the fight against pandemics and shed light on the literature.

Materials and Methods

Study Design

This research, designed as a descriptive and cross-sectional study, aimed to investigate healthcare workers' knowledge, attitudes, and behaviors regarding the pandemic. The research questions are as follows:

1. What are the knowledge levels of healthcare workers regarding the pandemic?

2. What are the attitudes of healthcare workers towards the pandemic?

3. What are the behaviors of healthcare workers related to the pandemic?

4. What factors influence healthcare workers' knowledge, attitudes, and behaviors towards the pandemic?

Ethical Considerations

Necessary written permissions were obtained from the Clinical Research Ethics Committee (dated 12.06.2020, no. 2397), the Ministry of Health Scientific Research Platform, and the relevant hospital's Scientific Committee (dated 13.05.2020, no. 248-05). The principles of the Helsinki Declaration were used to conduct the research. Participants in the study read the informed consent form at the beginning of the questionnaire, stating, "I have been informed about the research and agree to participate," by selecting "yes" and indicating whether they volunteered.

Population and Sample of the Study

The study population consisted of employees (N: 1, 008) actively involved in Yedikule Chest Disease and Thoracic Surgery Training and Research Hospital during the COVID-19 Pandemic, similar to past pandemics. The study did not use sampling; it included healthcare workers who met the inclusion criteria.

Inclusion Criteria: Individuals actively employed at the hospital willing to participate in the study.

Exclusion Criteria: Individuals who decline participation in the study, are illiterate, do not use a smartphone, are on leave, or have a medical report during the study period. The research was completed with 52.6% (n=546) of healthcare workers actively working at the hospital.

Data Collection Tools and Collection of Data:

A questionnaire consisting of 28 questions, developed by the researchers in the light of literature[9,17–18,21] to determine the sociodemographic characteristics and pandemic-related knowledge, attitudes, and behaviors of the study group, was utilized. Before sharing the form, a pilot study was conducted with 20 healthcare workers from internal and external institutions representing different healthcare professions to test the usability and clarity of the questions. Data from the pilot study were not included in the research. After the pilot study, necessary changes were made, and the final version of the questionnaire was provided through Google Forms. The participants filled out the form between June and September 2020 using snowball sampling via email or social media. The completion of the form took approximately 7–8 minutes. IP and cookie controls were implemented to prevent repeated entries from the same device, and data collection ended when there was no further increase in responses.

Data Analysis and Evaluation

The data obtained was analyzed using the IBM Statistical Package for Social Sciences (SPSS) program version 16. The distribution of qualitative characteristics was assessed in percentages, while numerical data was evaluated using means and standard deviations. Multiple arrangements were compared using chi-square tests. A statistical significance level of $p<0.05$ was considered. Moreover, three individuals who did not provide age data were excluded from calculating the average age.

Findings

A total of 546 healthcare workers participated in the study. The mean age of the participants was 35.5±9.3 (min: 20, max: 64), and the mean working period was 10.5±8.7 years (min: 2, max: 24). Sociodemographic characteristics of the participants are presented in Table 1. According to Table 1, 55.3% of the participants were

Table 1. *Distribution of sociodemographic characteristics of the participants (n=546)*

Sociodemographic Characteristics	Average	
Age	35.5±9.3 years (min: 20, max: 64)	
Duration of working	10.5±8.7 years (min: 2, max: 24)	
Sex	n	%
Male	244	44.7
Female	**302**	**55.3**
Marital Status		
Single	237	43.5
Married	**309**	**56.5**
Spouse's Employment Status in the Health Sector		
Yes	116	37.5
No	**193**	**62.5**
Parenthood Status		
Yes	**277**	**50.7**
No	269	49.3
Number of Children		
4	3	1.1
3	27	9.8
1	112	40.2
2	**135**	**48.9**
Having a relative with a chronic disease and/or over the age of 65 living together		
Yes	153	28.0
No	**393**	**72.0**
Occupation		
Other	25	4.6
Security personnel	27	4.9
Health technician	43	7.9
Medical secretary/Data entry	61	11.2
Cleaning staff	102	18.7
Physician	104	19.0
Nurse	184	33.7
Graduation		
Literate	1	0.2
Secondary School Graduate	18	3.3
Primary School Graduate	31	5.7
High School Graduate	90	16.5
Specialization/PhD/Master's degree	104	19.0
Associate degree	118	21.6
Bachelor's degree	184	33.7
Unit of Work		
Operating room	27	4.9
Lab	31	5.7
Intensive care	43	7.9
Policlinic	74	13.6
Emergency room	84	15.4
Other	130	23.8
Inpatient treatment unit	**188**	**34.4**
Unit Change During the Pandemic Process		
Yes	195	35.7
No	**351**	**64.3**
The unit worked during the change of unit during the COVID-19 Pandemic		
Policlinic	9	1.6
Intensive care	24	4.4
Emergency room	33	6.0
Other	29	5.3
Clinic	**102**	**18.7**
Vaccination status with influenza (flu) vaccine		
I have it done regularly every year.	67	12.3
I've had it done before, although not regularly.	113	20.7
I've never had it done.	**365**	**66.7**

*More than one option is marked.

Table 2. *Sociodemographic characteristics of the participants and their behaviors regarding influenza vaccine administration (n=546)*

	I get the influenza vaccine every year, regularly or irregularly		I did not get the influenza vaccine		p-value
	n	%	n	%	
Sex					
Male	70	28.7	174	71.3	0.056
Female	110	36.4	192	63.6	
Parenthood					
Yes	119	43	158	57	**0.001**
No	61	22.7	208	77.3	
Spouse's Employment Status in the Healthcare Sector					
Yes	46	39.7	70	60.3	0.084
No	134	31.2	296	68.8	
Having a relative with a chronic disease and/or over the age of 65 living together					
Yes	54	35.3	99	64.7	0.470
No	126	32.1	267	67.9	
Working in the healthcare sector during the Influenza A (H1N1) Pandemic					
Yes	124	54.4	104	45.6	**0.001**
No	56	17.6	262	82.4	
Graduation					
Primary/middle school	43	30.9	96	69.1	0.579
Higher education	136	33.5	270	66.5	

* p<0.05; Chi-square test

female, 56.5% were married, 62.5% had spouses not working in the healthcare sector, 50.7% had children, 89.1% had 1 or 2 children, 72% did not have chronic illnesses or relatives over 65 years old living with them, 33.7% were nurses, 33.7% had a bachelor's degree, and 34.4% worked in inpatient care units. It was determined that 64.3% did not change their unit during the pandemic. Among those who changed units, 18.7% worked in inpatient care units. Only 12.3% of the participants received regular influenza vaccination every year, while 66.7% did not receive influenza vaccination at all.

The comparison of sociodemographic characteristics with vaccination behavior is given in Table 2. In the participants' behavior of receiving influenza vaccination regularly or irregularly, it was observed that having children and actively working during the Influenza A (H1N1) Pandemic had a statistically significant effect (p=0.001).

The distribution of education and information processes during the COVID-19 Pandemic is presented in Table 3. It was found that 60.8% of healthcare workers obtained information about the COVID-19

Table 3. *Distribution of education and information processes during the COVID-19 Pandemic (n=546)*

	n	%
Information Sources Used for COVID-19 Pandemic*		
Social media (Instagram, WhatsApp, etc.)	39	7.1
Social environment	141	25.8
Official websites (Public Health, Ministry of Health, CDC, WHO, etc.)	183	33.5
Mass media (TV, radio, etc.)	237	43.7
Relevant teams within the institution (such as Infection Control Committee, Occupational Health and Safety)	**332**	**60.8**
Frequency of Following Current Information		
None	18	3.3
Occasionally	184	33.7
Constantly	**344**	**63.0**
Received Training and/or Information About the COVID-19 Pandemic		
No	12	2.2
Yes	**534**	**97.8**
Educator from whom Training and Information Received*		
Training nurse	184	33.7
Distance education lecturer	271	49.6
Infection control committee	**511**	93.6
Training and Information Type*		
Distance Education System (HES)	290	53.1
Face-to-face education	**413**	**75.6**
Training and Information Techniques*		
Practical training	116	21.2
Written information	161	29.5
Verbal information	**503**	**92.1**
Monitoring of Official Letters and Information Published on the In-house Web		
None	110	20.1
Constantly	118	21.6
Occasionally	**318**	**58.2**
Opinions Regarding Sufficiency of Training and Information		
Insufficient	66	12.1
Partially Sufficient	226	41.4
Sufficient	**242**	**44.3**
The Effectiveness Degree of Poster, Video, Brochure, Voice Announcement Applications for Healthcare Professionals		
Not effective at all	98	17.9
Partially effective	140	25.6
Very effective	**308**	**56.4**
Effectiveness Degree of Poster, Video, Brochure, and Voice Announcement Applications for Patients/Patient Relatives		
Not effective at all	111	20.3
Partially effective	308	56.4
Very effective	127	23.3
Working in the Health Sector During the Influenza A (H1N1) Pandemic		
Yes	228	41.8
No	**318**	**58.2**
Taking an Active Role in the Influenza Pandemic Process		
Yes	113	20.7
No	**433**	**79.3**

*More than one option is marked.

Pandemic from internal relevant teams (infection control committee, occupational health, and safety, etc.), 63% continuously followed current details on the COVID-19 Pandemic, 97.8% received education and/or information about the COVID-19 Pandemic, and 93.6% received education and information from the Infection Control Committee. Additionally, 75.6% received face-to-face training, and 92.1% benefited from verbal information techniques. Internal training and information processes were followed by 58.2%, and 85.7% found these processes to be sufficient or partially sufficient. While 56.4% of the participants defined posters, videos, brochures, and voice announcements within the institution as very effective, the same participants described these applications as less effective for patients or their relatives. It was observed that 41.8% of the participants had worked in the healthcare sector during the Influenza A (H1N1) 2009 Pandemic, and 20.7% had worked in areas related to patients infected with the Influenza A (H1N1) virus.

Statistically, those who had worked in the healthcare sector during the Influenza A (H1N1) 2009 Pandemic considered themselves adequately informed about the risks, preventive measures, and patient management of the Influenza Pandemic (p=0.001). Additionally, they reported experiencing less anxiety during patient management (p=0.002).

Those who had worked in the healthcare sector during the Influenza A (H1N1) 2009 Pandemic defined influenza vaccination and antiviral treatment as factors significantly reducing their anxiety levels (p=0.001) (Table 4).

Those who had worked in the healthcare sector during the Influenza A (H1N1) 2009 Pandemic statistically considered themselves adequately informed about the risks, preventive measures, and patient management of the COVID-19 Pandemic (p=0.001) and reported experiencing less anxiety during patient management (p=0.006). It was found that both those who had and had not worked in the healthcare sector during the Influenza A (H1N1) 2009 Pandemic identified the lack of effective vaccine (p=0.742) or antiviral treatment (p=0.606) for COVID-19 during the study period as factors increasing their concerns (Table 5).

Table 4. *Comparison of knowledge, attitudes, and behaviors regarding the Influenza A (H1N1) Pandemic process according to working status in the healthcare sector during the Influenza A (H1N1) Pandemic*

Working status in the healthcare sector during the Influenza A (H1N1) Pandemic		Yes	No	Yes	No	Yes	No	
Influenza A (H1N1) Pandemic Process		Always / Mostly	Always / Mostly	Occasionally / Rarely	Occasionally / Rarely	Never	Never	p-value
1. I am aware of the risks posed by the influenza pandemic to employees and patients.	n	183	192	37	111	8	15	**0.001***
	%	80.3	60.4	16.2	34.9	3.5	4.7	
2. I know how to protect myself and my patients from influenza pandemics.	n	191	202	30	104	7	12	**0.001***
	%	83.8	63.5	13.2	32.7	3.1	3.8	
3. I consider myself competent in service/care/treatment for patients with influenza infection.	n	166	187	48	116	14	21	**0.002***
	%	72.8	58.8	21.1	34.6	6.1	6.6	
4. I do not feel anxious when providing service/care/treatment to patients with influenza infection.	n	147	175	64	128	17	15	0.010
	%	64.5	55	28.1	40.3	7.5	4.7	
5. The fact that influenza is a vaccine-treated disease prevents me from feeling anxious when providing patient service/care/treatment.	n	164	182	49	119	15	17	**0.001***
	%	71.9	57.2	21.5	37.4	6.6	5.3	
6. The fact that there is a known effective drug treatment for influenza prevents me from feeling anxious while providing service/care/treatment to patients.	n	171	188	44	116	13	14	**0.001***
	%	75	59.1	19.3	36.5	5.7	4.4	

* p<0.05; Chi-square test in multi-well layouts.

Discussion

In the effective execution of qualified health services during a pandemic, the protection and empowerment of healthcare workers and the identification of risks are essential. Undoubtedly, healthcare workers' knowledge, skills, and attitudes play a crucial role in addressing this need[17]. Additionally, the uncertainties associated with each pandemic from the past to the present, coupled with the ongoing spread of COVID-19, make it challenging to predict when it will end, emphasizing the increasing importance of this situation.

The majority of participants were found to be in the young or middle-aged group, female, married, with multiple children, nurses, and graduates. In the research

Table 5. *Comparison of knowledge, attitudes, and behaviors regarding the COVID-19 Pandemic process according to working status in the healthcare sector during the Influenza A (H1N1) Pandemic*

Working status in the healthcare sector during the Influenza A (H1N1) Pandemic		Yes	No	Yes	No	Yes	No	
Covid-19 Pandemic Process		Always / Mostly	Always / Mostly	Occasionally / Rarely	Occasionally / Rarely	Never	Never	p-value
1. I am aware of the risks posed by the Covid-19 pandemic to employees and patients.	n	197	224	23	81	8	13	0.001*
	%	86.4	70.4	10.1	25.5	3.5	4.1	
2. I know how to protect myself and my patients from the Covid-19 pandemic.	n	192	212	30	92	6	14	0.001*
	%	84.2	66.7	13.2	28.9	2.6	4.4	
3. I consider myself competent in service/care/ treatment for patients with Covid-19 infection.	n	149	154	62	142	17	22	0.001*
	%	65.4	48.4	27.2	44.7	7.5	6.9	
4. I do not feel anxious when providing service/care/ treatment to patients with Covid-19 infection.	n	123	146	73	143	32	29	0.006
	%	53.9	45.9	32	45	14	9.1	
5. The fact that there is no known vaccine for Covid-19 yet makes me feel anxious when providing service/care/treatment to patients. **	n	176	244	40	61	12	13	0.742
	%	77.2	76.7	17.5	19.2	5.3	4.1	
6. The lack of a known effective drug treatment for Covid-19 makes me feel anxious when providing service/care/treatment to patients. **	n	180	247	35	57	13	14	0.606
	%	78.9	77.7	15.4	17.9	5.7	4.4	

* p<0.05; Chi-square test in multi-well layouts; ** Questions 5 and 6 relate to the period in which the study was conducted.

conducted by Orhan and Gumus in 2021[20], aiming to examine the knowledge, practices, and stress levels of healthcare workers during the COVID-19 pandemic, it was found that most participants were women, nurses, and the young or middle-aged group. Similarly, in Yilmazer's 2021[23] study, which aimed to evaluate healthcare workers' knowledge, perception, and behaviors regarding COVID-19, most participants were identified as women, nurses, and in the young age group. In the study by Cui et al. in 2021[24], which aimed to determine the psychological effects of COVID-19 and related factors, the majority were women, nurses, graduates, and the young age group. Gender, parental status, and education were identified as associated factors in these studies. These findings, while showing parallels with the results of our research, highlight the influential factors in behavioral protection levels during pandemics, especially for women, married individuals, and those with children (such as the use of personal protective equipment, hand hygiene, etc.). Notably, these sociodemographic characteristics constitute most of our study. They are essential for research findings, positively influencing knowledge, attitudes, and behaviors.

When examined regarding education and information sources about the COVID-19 pandemic, it was determined that most healthcare workers received training, primarily benefited from relevant in-house teams, and most frequently received education from the Infection Control Committee. However, it was also found that most participants continuously follow up-to-date information about the COVID-19 pandemic. In-house applications such as posters, videos, brochures, and voice announcements are perceived as highly effective for healthcare workers but less effective for patients or their relatives. A study conducted in Vietnam on healthcare workers' knowledge levels about COVID-19 revealed that the majority had received a good education, with the top three sources being institutional websites, social media, and television (TV)[25]. Another study in China by Zhou et al. in 2020[16] indicated insufficient knowledge levels among healthcare workers. Yilmazer's study in 2021[23] found that most participants received education and used reliable information sources such as the Ministry of Health and the World Health Organization. Studies with non-healthcare worker groups also identified sources of obtaining information about COVID-19 as social media, the internet, and TV[26,27]. A study by Genc in 2021[28] revealed that individuals who frequently use traditional media and highly trust social media have a more positive attitude toward the COVID-19 vaccine. Salman et al.'s[29] research determined that most participants obtained information about COVID-19 from official institutions. A study by Ceyhan and Uzuntarla[30] in 2020,

which aimed to determine the knowledge, attitudes, and behaviors of academic staff regarding COVID-19, found that academics had sufficient levels of expertise, the number of family members influenced attitudes in terms of knowledge and behaviors were influenced by gender, age, and status. These findings are in parallel with the results of our study. The definition of "pandemic education," considered one of the most effective components in pandemic management, as sufficient and healthcare workers accessing reliable sources of information positively affecting knowledge, attitudes, and behaviors, suggests that being a pandemic hospital strengthens this finding. However, it is also considered necessary to provide more support for individuals outside the healthcare profession, such as patients or their relatives, in the correct use of information sources.

The majority of participants were found not to have received the influenza vaccine. However, it was observed that healthcare workers who received the influenza vaccine were significantly influenced by factors such as having children and actively working during the Influenza A (H1N1) pandemic. Although this aligns with the existing literature, showing similarities with the findings of our study, it is well-known that the influenza vaccination rate among healthcare workers is low[31–33]. The importance of vaccination as a significant health-protective intervention and the influential role of factors such as having children and active involvement during the Influenza A (H1N1) 2009 pandemic in influencing healthcare workers' vaccination behaviors are believed to enhance protective behaviors. Considering the individual and professional dimensions of healthcare workers' vaccination status, it is anticipated that activities to increase vaccination rates should be intensified.

The findings of our study reveal that individuals who had experience working in the health sector during the Influenza A (H1N1) 2009 Pandemic considered their experiences as factors that made them feel more competent in understanding the risks, prevention methods, and patient management related to both Influenza and COVID-19 Pandemics. However, during our research, ongoing studies on effective vaccines or antiviral treatments for COVID-19 increased concerns among those who had and had not worked in the health sector during the Influenza A (H1N1) 2009 Pandemic. Research by Imai (2020)[34] conducted in Japan on voluntary participation in COVID-19-related work indicated that 28.4% of participants were strongly motivated, while 14.7% expressed significant hesitation to work. Those hesitant about working cited concerns about infection, potential loss of income in case of disease, and the fear of isolation. Aghili and Arbabi (2020) [35] compiled a study exploring what COVID-19 meant psychologically for healthcare workers, particularly frontline workers like nurses, reporting increased anxiety and stress symptoms. They suggested that such conditions would impact during and after the pandemic. However, they did not predict a permanent situation and recommended necessary improvements. However, research on the SARS epidemic has shown that psychological effects are not always short-lived and can lead to severe and persistent mental health issues[36]. Roy et al. (2020)[18] noted the similarity between Influenza A (H1N1) and COVID-19, emphasizing that each pandemic has unique features. Mishra et al. (2016)[9] reported that healthcare professionals with a more positive attitude and awareness toward pandemics experienced lower anxiety. All these findings suggest that while previous pandemic experiences among healthcare workers may be effective in managing the process, the uncertainties and inevitable anxiety or stress associated with each pandemic highlight the importance of psychological support.

Our study found that despite healthcare workers having sufficient knowledge and practical levels to combat COVID-19, they still experienced anxiety. Zhong et al. (2020)[37] also mentioned that past pandemic experiences were related to effective management and that knowledge and attitude toward the pandemic were associated with psychosocial conditions such as panic and fear. In a study conducted by Roy et al. (2020)[18] on Indian healthcare workers during the COVID-19 pandemic, participants were found to have moderate knowledge about COVID-19, but their anxiety levels were high. Orhan and Gumus (2021)[20] similarly discovered that while healthcare workers mostly had sufficient knowledge about COVID-19 management, stress levels were high among them. Barello et al. (2020)[19] conducted a systematic review of 36 studies, highlighting that healthcare workers frequently worried about their health, feared transmitting the infection to family, friends, and colleagues, and reported issues such as social isolation, uncertainty, fear of stigma, and work reluctance or inability to come to work. They emphasized that high levels of stress, anxiety, and depression symptoms were widespread among healthcare workers. Afzal et al. (2021)[38] conducted a study in Pakistan with the general public, revealing that knowledge about COVID-19 was

positively associated with behavioral levels. Devkota et al. (2020)[39] also reported positive but weak linear correlations between behavior, knowledge, and attitude. Fikri et al. (2022)[40] found that while doctors worked diligently in all aspects of the pandemic, they could not update themselves with sufficient in-service knowledge in a rapidly changing situation. They suggested that in-service knowledge flow is crucial in a pandemic with rapidly changing treatment recommendations. Abebe et al.'s (2016)[41] study aimed to determine healthcare workers' knowledge, attitudes, and perceptions about the Ebola virus. The findings revealed that most healthcare workers had insufficient expertise and erroneous beliefs, emphasizing the vital importance of this issue and recommending an intensive education program to deliver quality healthcare services. While these findings are in parallel with the results of our study, they suggest that education and information are supportive factors not only for the effective management of the pandemic but also for individual and professional psychosocial resilience.

The limitations of our study include its single-hospital setting, potential recall bias due to the past occurrence of the Influenza Pandemic, and the unavailability of information regarding whether participants had experienced the relevant infections.

Conclusion

In conclusion, a pandemic, considered a disaster, profoundly affects individuals and society in all dimensions. Pandemics not only entail negative impacts but also contain potential opportunities that can be defined with concepts such as change, empowerment, and post-traumatic growth. The success of this struggle significantly depends on healthcare workers' knowledge, attitudes, and behaviors. It is also known that the emotions of healthcare workers, who are effective crisis managers, can influence and be reflected in the service during this process. Despite the limitations of our study, it provides data on the knowledge, attitudes, and behaviors of healthcare workers in two different pandemic periods and their post-pandemic effects.

Supporting the strengths and improving the weaknesses of healthcare workers, ensuring the continuity of education before, during, and after pandemics, conducting more extensive and qualitative studies on the subject, adding specific training programs to secondary and higher education curricula, especially considering the anticipated future pandemics, are necessary and vital.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Dynamic Contrast-Enhanced Breast MRI Findings Predictive of Malignancy in MRI-Only Breast Lesions Examined by MR-Guided Biopsy: A Single-Center Experience

MRG'de Saptanabilen ve MR Eşliğinde Biyopsi Yapılan Meme Lezyonlarında Maligniteyi Öngören Dinamik Kontrastlı Meme MRG Bulguları: Tek Merkez Deneyimi

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ABSTRACT

Aim: *It was aimed to investigate the association between dynamic contrast-enhanced magnetic resonance imaging (MRI) findings and malignancy in suspicious MRI-only breast lesions performed by MR-guided biopsy.*

Material and Method: *Between December 2014 and December 2020, 57 suspicious MRI-only lesions were identified. Among these, 46 lesions underwent MR-guided wire localization and excisional biopsy, while 11 underwent MR-guided core biopsy. Clinical data, MRI findings, biopsy results, and information about the MR-guided biopsy procedures were collected. The predictive value of MRI findings in determining malignancy was analyzed.*

Results: *According to biopsy results, 14 (24.6%) of the 57 lesions were malignant, and 43 (75.4%) were benign. The malignancy rate was significantly higher in lesions in the lower inner quadrant (55.5%, p=0.040) and those with a washout kinetic curve than those with persistent and plateau curves (p=0.034). Malignancy was detected in 83.3% of the lesions classified as BI-RADS-MRI category 5 versus only 17.6% of those classified as BI-RADS-MRI category 4 (p=0.006).*

Conclusion: *Kinetic curve type and BI-RADS-MRI category were identified as MRI findings predicting the malignancy of lesions assessed by MR-guided biopsy. According to MR-guided biopsy, BI-RADS-MRI category 5 lesions and those with washout kinetic curves were significantly more likely to be malignant.*

Key words: *image-guided biopsy; magnetic resonance imaging; breast cancer; breast imaging reporting and data system*

ÖZET

Amaç: *MR kılavuzluğunda biyopsi yapılan, yalnızca MRG'de izlenebilen şüpheli meme lezyonlarının dinamik kontrastlı manyetik rezonans görüntüleme (MRG) bulguları ile malignite arasındaki ilişkiyi araştırmak amaçlandı.*

Materyal ve Metot: *Aralık 2014 ile Aralık 2020 tarihleri arasında yalnızca MRG'de izlenebilen 57 şüpheli meme lezyonu tespit edildi; bunların 46'sına MR kılavuzluğunda tel lokalizasyonu ve eksizyonel biyopsi, 11'ine MR kılavuzluğunda kor biyopsi uygulandı. Hastaların klinik verileri, MR bulguları, biyopsi sonuçları ve MR eşliğinde biyopsi işlemlerine ilişkin veriler kaydedildi. Lezyonun MR bulgularının, malignite açısından öngörü değeri analiz edildi.*

Bulgular: *Biyopsi sonuçlarına göre 57 lezyonun 14'ü (%24,6) malign, 43'ü (%75,4) benigndi. Alt iç kadranda yerleşen lezyonlarda (%55,5, p=0,040) ve wash-out kinetik eğrisi olanlarda, persistan ve plato eğriye sahip olanlara göre (p=0,034) malignite oranı anlamlı olarak daha yüksekti. BI-RADS-MRI kategori 5 olarak sınıflandırılan lezyonların %83,3'ünde malignite tespit edilirken, BI-RADS-MRI kategori 4 olarak sınıflandırılan lezyonların sadece %17,6'sında malignite tespit edildi (p=0,006).*

Sonuç: *Kinetik eğri tipi ve BI-RADS-MRI kategorisi, MR eşliğinde biyopsi ile değerlendirilen lezyonların malignitesini öngören MR bulguları olarak belirlendi. BI-RADS-MRI kategori 5 lezyonlar ve wash-out kinetik eğrisi olan lezyonların MR kılavuzluğunda yapılan biyopsi sonrası elde edilen patoloji sonucunda malign raporlanma olasılıkları anlamlı derecede yüksekti.*

Anahtar kelimeler: *görüntü kılavuzluğunda biyopsi; manyetik rezonans görüntüleme; meme kanseri; meme görüntüleme raporlama ve veri sistemi*

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Introduction

Dynamic contrast-enhanced MRI is widely used in the evaluation of breast lesions[1,2]. Magnetic resonance imaging is more sensitive than mammography (MG) or ultrasound (US) in detecting breast lesions[3,4]. The sensitivity of MRI for breast cancer can reach up to 100%[5,6]. As a result of this high sensitivity, MRI can reveal lesions that are occult on MG and US, which are termed MRI-only lesions[3]. The American Cancer Society (ACR) and European Society of Breast Imaging (EUSOBI) guidelines recommend MR-guided tissue sampling for MRI-only lesions suspected of being malignant according to evaluation with the Breast Imaging Reporting and Data Analysis (BI-RADS) MRI lexicon[4,7]. Tissue sampling of these lesions uses MR-guided wire localization (MGWL) followed by excisional biopsy or MR-guided core biopsy (MGCB). MR-guided wire localization is relatively easy to perform and yields good results in complete excision because it is followed by excisional biopsy. However, the surgery performed after wire localization is invasive, costly, and carries the risk of perioperative complications[4,8]. Percutaneous MGCB is a safe, less invasive, faster, and cheaper procedure that reduces open biopsies in benign lesions[4,8]. Unfortunately, this method is challenging with small lesions and those in specific locations (superficial, medial, in the anterior retroareolar region, or near the chest wall)[9,10]. Additionally, many centers in Türkiye do not perform MR-guided interventional methods because they lack the technological or logistical capacity or experience to perform the procedures.

Normal breast parenchyma and many benign lesions can mimic malignancy in terms of morphological and contrast enhancement features on MRI, thereby reducing the specificity of breast MRI[11]. This situation may result in unnecessary MR-guided biopsies, which is a time-consuming and expensive procedure. Improving the differentiation of benign and malignant lesions in pre-biopsy MRI images is vital in determining the need for biopsy and evaluating the concordance of pathological and radiological findings[12].

In this study, we aimed to present our clinical experience with MGWL and MGCB procedures performed in our center and to investigate the predictive value of initial MRI findings in detecting malignancy in suspicious MRI-only lesions sampled by MGWL and MGCB.

Material and Methods

Our institute's Scientific Research Ethics Committee approved this retrospective study (2022–08/130), and the participating patients provided written informed consent.

Patients

We retrospectively reviewed the data of 64 breast lesions in 62 women who underwent MGWL and excisional biopsy or MGCB in our center between December 2014 and December 2020. Three women were lost to follow-up before undergoing the recommended biopsy procedure. Four lesions in two women were excluded from the study because a core biopsy couldn't be conducted due to their inability to be visualized on MRI during the procedure. (Fig. 1). As a result, 46 women who underwent MGWL (age range: 27–75 years, mean age: 52 years) and 11 women who underwent MGCB (age range: 19–67 years, mean age: 47 years) were included in the study. The mean age of the study group was 49 years (range: 19–75 years).

Data Collection

Medical records in the hospital electronic data archive were reviewed for patient age, physical examination findings, family history of breast cancer, MRI indication, radiology and pathology reports, and clinical follow-up results. Pre-biopsy MRI images were retrieved from the electronic data archive of the radiology department and reviewed together with MG or US findings. The type of biopsy performed and post-biopsy complications were determined from the patients' medical records. Procedure time was calculated as the difference between the timestamp of the last MR image acquired after wire or clip localization and the first localizer MR image in the electronic archive.

Magnetic Resonance İmaging Technique

Magnetic resonance imaging images were acquired with patients in the prone position using a dedicated 1.5-Tesla (T) MR scanner (SignaHDx; GE Healthcare, Wisconsin, USA) and breast array coil. Imaging parameters consisted of axial T1-weighted (T1W) images (repetition time/echo time [TR/TE]: 400/8.8, field of view (FOV): 320 mm, matrix: 448×224, number of excitations (NEX): 1, and slice thickness (ST): 5 mm); axial short tau inversion recovery (STIR) (TR/TE: 6500/45, inversion time [TI]: 150 ms, FOV: 320 mm, matrix: 416×224, NEX: 1, and ST: 5 mm); dynamic

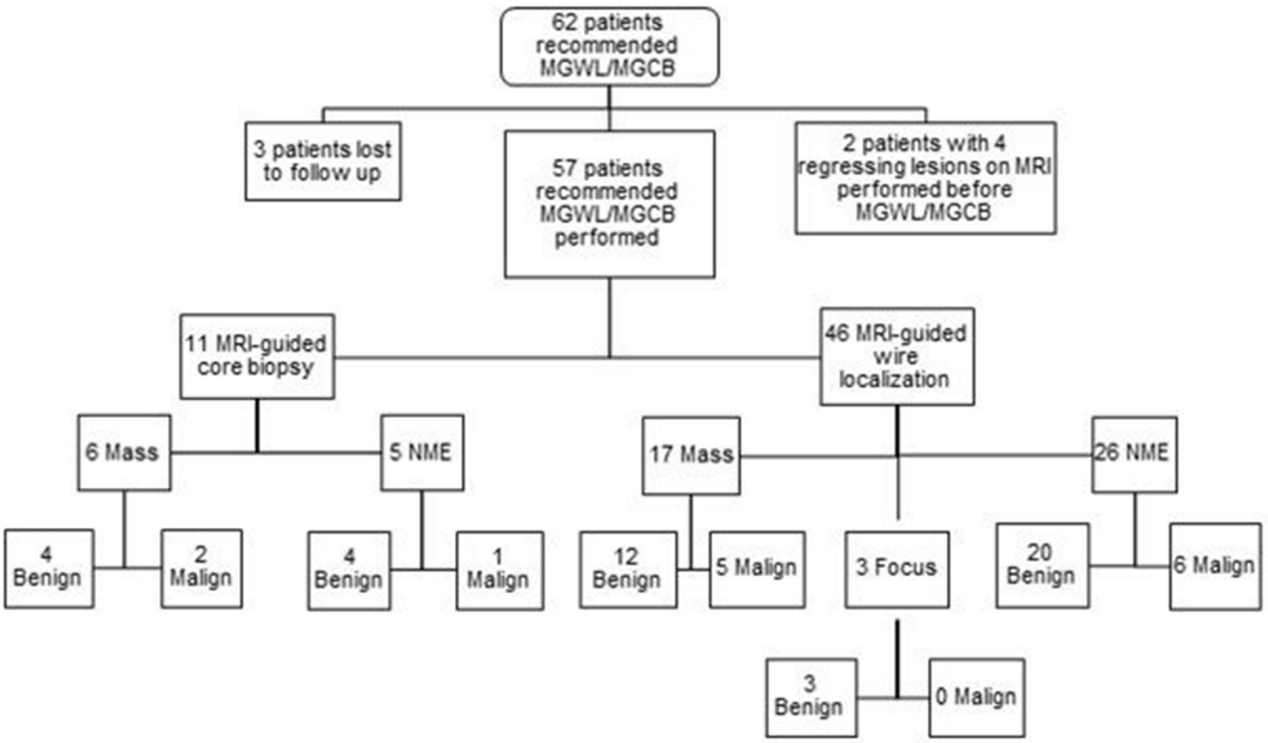

Figure 1. *Inclusion flowchart. MGWL, MR-guided wire localization; MGCB, MR-guided core biopsy; NME, non-mass enhancement.*

axial fat-saturated (FS) T1W images (before and after contrast injection) (TR/TE: 4/1.5, flip angle: 10°, FOV: 320 mm, matrix: 350×350, NEX: 1, and ST: 2.8 mm). An intravenous injection of 0.1 mmol/kg gadobutrol/gadopentetate dimeglumine was administered as a contrast material, followed by a saline bolus. Precontrast images were obtained once, and post-contrast images were obtained five times at intervals of 80 seconds. Standard subtraction images, maximum-intensity projection (MIP), and multiplanar reconstruction (MPR) were rendered automatically on a workstation. Kinetic curves of the lesions were constructed automatically by the device using a time-intensity curve.

Magnetic Resonance İmaging Findings

In our center, dynamic contrast-enhanced breast MRI was performed on all patients before MGWL or MGCB. Magnetic resonance imaging images were interpreted retrospectively on one workstation by three radiologists experienced in breast imaging. The radiologists were informed about the clinical course of the patients and their MG and US imaging findings. Each case was evaluated through a consensus between these three radiologists by interpreting the imaging findings according to the BI-RADS-MRI descriptors (American College of Radiology, 2013). All lesions were classified as BI-RADS category 4 or 5 based on MRI findings.

The location of the target lesions in the breast was determined by examining dynamic breast MRI images and categorized as central, upper inner, upper outer, lower inner, and lower outer quadrant. Breast density (BD) and background parenchymal enhancement (BPE) were categorized according to the BI-RADS lexicon[13]. Breast density was classified as predominantly fatty, scattered, heterogeneously dense, or extremely dense based on the ratio of fibroglandular tissue in the breast on T1W images. Background parenchymal enhancement was determined by assessment of the intensity of fibroglandular tissue contrast enhancement in the early phase of post-contrast T1W FS sequence and was interpreted as minimal, mild, moderate, and marked[13]. The largest lesion dimension was measured from post-contrast T1W FS, MIP, and MPR images.

Morphology of the lesions was classified as mass, non-mass enhancement (NME), and focus as defined in the BI-RADS lexicon. The shape of the mass/focus (circumscribed or irregular), distribution of NME (linear, segmental, focal, and regional), and internal enhancement pattern of each lesion (homogeneous and heterogeneous,

as well as ring pattern for masses and foci) were analyzed on post-contrast subtraction, MIP, and sagittal MPR images[13,14]. The kinetic enhancement pattern of the lesions was characterized as washout, plateau, or persistent according to the kinetic curves obtained.

Indications for MR-Guided Wire Localization or MR-Guided Core Biopsy

MR-guided wire localization or MGCB was performed on a single lesion in each patient. Fifty-seven suspicious lesions were detected only on MRI and were occult on MG and US. Therefore, MGWL and excisional biopsy or MGCB were recommended for the pathological diagnosis of BI-RADS-MRI 4 and 5 lesions. MR-guided wire localization was preferred in 30 lesions with difficult locations for MGCB (anterior or subareolar, far posterior, or superficial [<2 cm from the skin]), eight lesions scheduled for surgical excision, and eight patients with small breasts. MR-guided core biopsy was performed for the remaining lesions (n=11).

MR-Guided Biopsy Procedures

All MR-guided biopsies were performed in a 1.5-T MR scanner (SignaHDx; GE Healthcare, Wisconsin, USA) with the patient in the prone position, using a breast array coil and grid-localization system. During interventional procedures for lesions in the inner quadrants, a cardboard barrier was used to prevent the contralateral breast from entering the coil for a medial approach. In the lateral approach, both breasts were in the coil. The grid plate was placed over the breast in the target lesion area according to the diagnostic MR image, and compression sufficient to stabilize the breast was applied. A pre-contrast image was obtained to confirm the breast was positioned with the target lesion within the area of accessibility. By examining the images, the estimated location of the lesion was marked externally by placing cotton soaked with gadolinium contrast agent on the skin. Gadobutrol/gadopentetate dimeglumine at a 0.1 mmol/kg dose was intravenously injected as a contrast agent and then flushed with saline. The lesion's exact location was confirmed and positioned according to the marker by evaluating post-contrast images. The distance of the lesion from the skin was noted. The skin entry site was prepared by cleaning with an antiseptic solution and anesthetizing with 1% lidocaine.

MR-Guided Wire Localization Technique

An MR-compatible 18-gauge needle and hook wire were inserted into the desired depth through the prepared skin entry site. Considering the calculated skin-lesion distance, the needle tip was inserted up to 1 cm beyond the target lesion. Sagittal and axial contrast-enhanced images were obtained to confirm the appropriate needle location. The needle appeared as a hypointense structure in these images because of the susceptibility artifact (Fig. 2). The wire was deployed when the needle was in the desired location. Contrast-enhanced T1W images and two-view MG were performed to document wire location.

MR-Guided Core Biopsy Technique

An MR-compatible 14-gauge coaxial system consisting of a needle and sheath was inserted through the skin to the calculated depth for the tip of the needle to reach the lesion margin. Axial sequence images were acquired to confirm the correct positioning of the system (Fig. 3). The needle was then removed, and a 14-gauge MRI-compatible automatic core biopsy needle was inserted through the sheath.

A minimum of five specimens were obtained from the lesion. After sampling, an MRI-safe marker clip was placed at the biopsy site via the sheath. The coaxial system was then removed. Compression was applied manually to the skin entry and needle sampling sites to achieve hemostasis. After the procedure, a two-view MG was performed to verify the clip location.

Pathological Data Evaluation

The pathology results of the biopsied lesions were obtained from our center's electronic data archive. The results were classified as benign or malignant and recorded. The radiologists evaluated the concordance between pathology and radiology using dynamic contrast-enhanced MRI findings.

Statistical Analysis

Statistical analyses were performed using the IBM IBM Statistical Package for Social Sciences (SPSS) package program version 20.0 (IBM Corporation, Armonk, NY, USA). Data were grouped according to benign and malignant lesions. Categorical data such as family history, MRI indication, MR-guided biopsy procedure, pathological diagnosis, and distribution of MRI findings were expressed as numbers and percentages. Continuous variables, including patient age and lesion size, were expressed as mean ± standard deviation (minimum-maximum values) or median (25–75% percentiles). Normality analyses of continuous

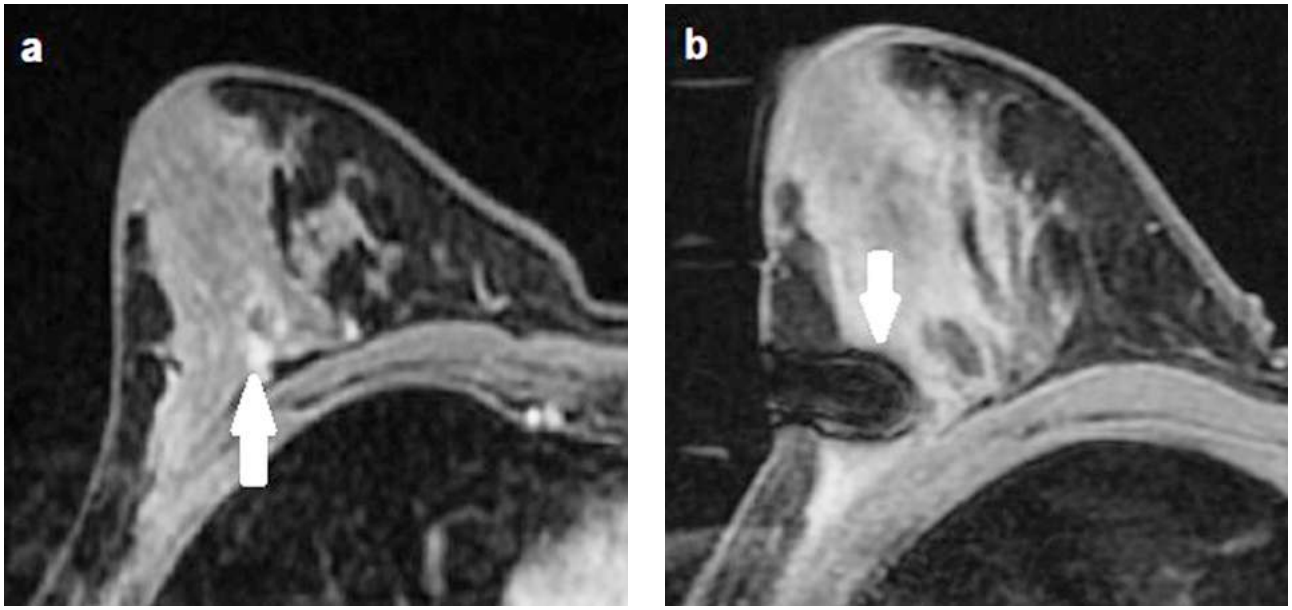

Figure 2. *a–b. A 34-year-old woman with known breast cancer in the right breast undergoing imaging to investigate the extent of disease. Axial post-contrast T1-weighted fat-suppressed MR shows a focus suspicious for malignancy located near the chest wall in right breast (arrow)(a). Low signal intensity due to susceptibility artifact of the needle is seen in axial post-contrast T1-weighted fat-suppressed MR image obtained during MR-guided wire localization (arrow). The final pathological diagnosis was sclerosing adenosis (b).*

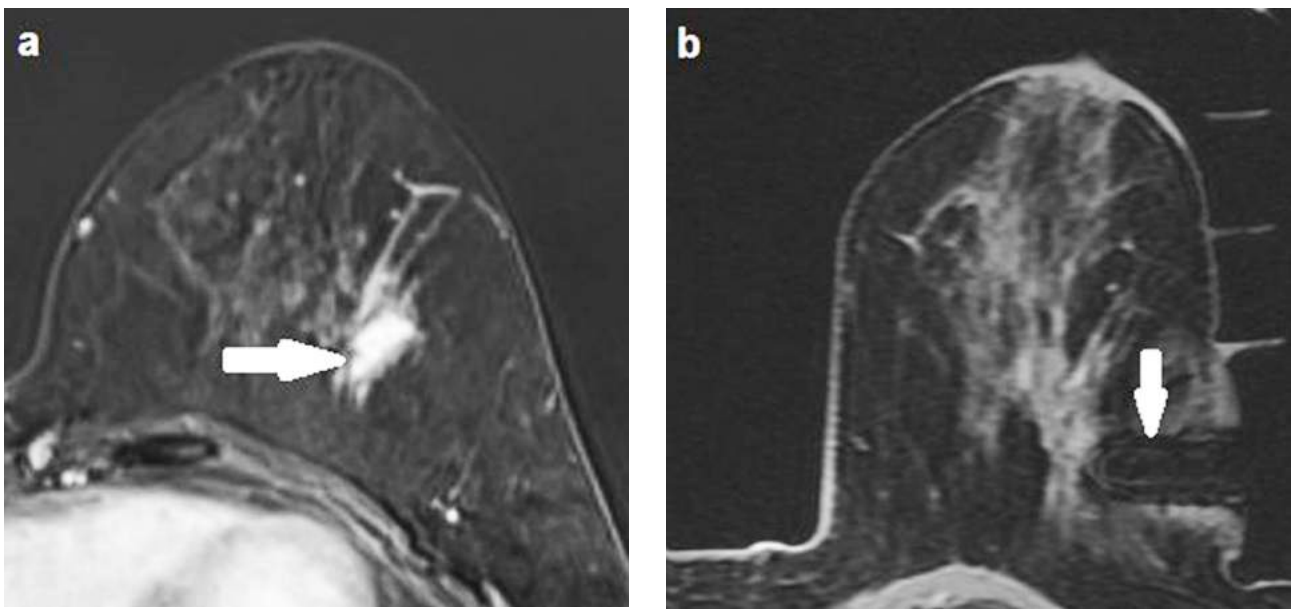

Figure 3. *a–b. A 49-year-old woman with equivocal sonographic findings in right breast. Axial post-contrast T1-weighted fat-suppressed MR subtraction image shows mass lesion with microlobulated margin and heterogenous internal enhancement pattern in the left breast (arrow)(a). Low signal intensity due to susceptibility artifact of the coaxial system is seen in axial post-contrast T1-weighted fat-suppressed MR image obtained during MR-guided core biopsy (arrow) (b).*

variables were performed using the Shapiro-Wilk test. In the statistical analysis between groups, the independent samples t-test was used for continuous variables if normally distributed, and the Mann-Whitney U test was used if non-normally distributed. Logistic regression analysis was performed using the categorical variables, including family history, MR indication category, MR-guided biopsy procedure, lesion location, BD, BPE, lesion morphology, lesion shape or distribution, lesion enhancement pattern, lesion kinetic curve type, and BI-RADS-MRI category to assess their association with malignancy. A p-value less than 0.05 was considered statistically significant.

Results

Lesion Histologic Results

Biopsy results indicated that 14 of 57 lesions (24.6%) were malignant and 43 (75.4%) were benign. The pathologic diagnoses obtained are shown in Table 1. The majority of malignant lesions (57.1%) had a pathological diagnosis of ductal carcinoma in situ (DCIS). The most frequently diagnosed benign lesion (18.6%) was sclerosing adenosis.

Breast MRI Indications

Indications for breast MRI included imaging for problem-solving (34/57, 59.6%), evaluation of disease extent in patients with known breast cancer (12/57, 21.1%), and screening of women at high/moderate risk (11/57, 19.3%). Of the high/moderate-risk women, 5 had a family history of breast cancer, 3 had a personal history of breast cancer, and 3 had a previously diagnosed lesion with atypia. Magnetic resonance imaging for the indication of problem-solving was performed in patients with equivocal MG or US findings (n=24), nipple discharge (n=9), or primary carcinoma of unknown origin with bone metastases (n=1).

The malignancy rate was higher in the group that underwent MRI for the investigation of breast cancer extent (41.7%) than for the indications of problem-solving (23.5%) and screening (9.1%). However, the groups had no statistically significant difference (p=0.222).

Malignancy was detected in 21.4% of the patients with positive family history and 25.6% of those without (p=0.754) (Table 2).

Magnetic Resonance Imaging Findings and Lesion Morphologic Features

Dynamic contrast-enhanced MRI findings were evaluated to determine the predictors of malignancy in patients undergoing MR-guided biopsy. The mean size of the benign lesions was 14 mm (range, 4–50 mm), whereas it was 18 (range, 4–50) mm in the malignant lesions, which was not a statistically significant difference (p=0.590).

According to the location of the lesions in the breast, malignancy rates were 55.5%, 28.6%, 21.4%, and 17.4% in the lower inner quadrant, central region, lower outer quadrant, and upper outer quadrants, respectively. Pathology results indicated all upper-inner quadrant lesions were benign. The lower inner quadrant location was significantly associated with malignancy (p=0.040), with 6-fold higher odds of detecting malignancy than the upper outer quadrant.

When evaluated according to BD, malignancy rates were 33.3% in predominantly fatty, 10% in scattered, 31.2% in heterogeneously dense, and 11.1% in highly dense lesions. Although the malignancy rate was highest for lesions in predominantly fatty breasts, the difference was not statistically significant (p=0.431).

Table 1. *The pathologic diagnoses of the lesions*

Pathology	No. of lesions n (percentage)	Pathology	No. of lesions n (percentage)
Malignant lesions	14 (24.6%)	Benign lesions	43 (75.4%)
DCIS	8 (14%)	Sclerosing adenosis	8 (14%)
IDC	5 (8.8%)	Fibroadenoma	7 (12.3%)
ILC	1 (1.8%)	Columnar cell hyperplasia	6 (10.5%)
		IDP	5 (8.8%)
		Fat necrosis	4 (7%)
		Fibrocystic changes	4 (7%)
		Hyalinized breast tissue	3 (5.3%)
		Epithelial hyperplasia	2 (3.5%)
		Adenosis	2 (3.5%)
		Lymph node	2 (3.5%)

DCIS: ductal carcinoma in-situ; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; IDP: intraductal papilloma.

Table 2. *Comparison of benign and malignant lesions in terms of patients' age, lesion size, family history for breast cancer of patients, indications of breast MR and biopsy procedure*

	Benign (n=43)	Malignant (n=14)	p-value	Odds ratio (95% CI)
Age (year) (mean±SD)	48.95±12.76	47.86±15.06	0.791	0.95 (0.42-2.02)
Lesion median size (mm) (25%-75% percentiles)	14 (9.00-20.00)	18 (9.75-20.50)	0.590	1.31 (0.87-2.50)
Family history, n (percentage)				
None	32 (56.1%)	11 (19.3%)	0.754	1.00
Positive	11 (19.3%)	3 (5.3%)		0.79 (0.18-3.37)
MRI indications, n (%)				
Breast cancer extent	7 (12.3%)	5 (8.8%)	0.222	1.00
Problem solving	26 (45.6%)	8 (14%)	0.237	0.43 (0.10-1.73)
High risk screening	10 (17.5%)	1 (1.8%)	0.102	0.14 (0.01-1.47)
Procedure, n (%)				
MR-guided core biopsy	8 (14%)	3 (5.3%)	0.147	1.00
MR-guided wire localization	35 (61.4%)	11 (19.3%)		0.37 (0.18-3.71)

CI: confidence interval; SD: standard deviation.

Table 3. *The distribution of MRI findings of the lesions according to MR-directed US non-correlated and correlate*

MRI findings	Benign, n (percentage)	Malignant, n (percentage)	p-value	Odds ratio (95% CI)
Location				
UOQ	19 (33.3%)	4 (7%)	0.335	1.00
UIQ	4 (7%)	0 (0%)	0.999	0.00
Central	5 (8.8%)	2 (3.5%)	0.522	1.90 (0.26–13.52)
LOQ	11 (19.3%)	3 (5.3%)	0.761	1.29 (0.24–6.88)
LIQ	4 (7%)	5 (8.8%)	0.040	5.93 (1.08–32.51)
Breast density				
Predominantly fatty	4 (7%)	2 (3.5%)	0.431	1.00
Scattered	9 (15.8%)	1 (1.8%)	0.270	0.22 (0.01–3.22)
Heterogeneously dense	22 (38.6%)	10 (17.5%)	0.920	0.90 (0.14–5.80)
Extremely dense	8 (14%)	1 (1.8%)	0.311	0.25 (0.01–3.66)
BPE				
Minimal	11 (19.3%)	4 (7%)	0.783	1.00
Mild	11 (19.3%)	6 (10.5%)	0.600	1.50 (0.32–6.83)
Moderate	16 (28.1%)	4 (7%)	0.643	0.68 (0.14–3.35)
Marked	5 (8.8%)	0 (0%)	0.999	0.00
Morphology				
Mass	16 (28%)	7 (12.3%)	0.810	1.00
NME	24 (42.1%)	7 (12.3%)	0.516	0.66 (0.19–2.26)
Focus	3 (5.3%)	0 (0%)	0.999	0.00
Mass/Focus (n=26)				
Shape				
Circumscribed	10 (17.5%)	3 (5.3%)	0.659	1.00
Irregular	9 (15.8%)	4 (7%)		1.48 (0.25–8.49)
Enhancement				
Homogeneous	10 (17.5%)	4 (7%)	0.997	1.00
Heterogeneous	8 (14%)	3 (5.3%)	0.943	0.93 (0.16–5.46)
Ring enhancement	1 (1.8%)	0 (0%)	1.000	0.00
NME				
Distribution				
Linear	8 (14%)	3 (5.3%)	0.985	1.00
Segmental	2 (3.5%)	1 (1.8%)	0.837	1.33 (0.86–20.70)
Focal	10 (17.5%)	3 (5.3%)	0.813	0.80 (0.12–5.09)
Regional	4 (7%)	0 (0%)	0.999	0.00
Enhancement				
Homogeneous	8 (14%)	14 (24.6%)	0.813	1.00
Heterogeneous	4 (7%)	5 (8.8%)		1.25 (0.19–7.92)
Kinetic curve type				
Persistent	10 (17.5%)	1 (1.8%)	0.004	1.00
Plateau	22 (38.6%)	1 (1.8%)	0.590	0.45 (0.02–8.02)
Washout	11 (19.3%)	12 (21%)	0.034	10.90 (1.19–99.68)
BI-RADS-MRI				
Category 4	42 (73.6%)	9 (15.8%)	0.006	1.00
Category 5	1 (1.8%)	5 (8.8%)		23.33 (2.42–224.61)

CI: confidence interval; UOQ: upper outer quadrant; UIQ: upper inner quadrant; LOQ: lower outer quadrant; LIQ: lower inner quadrant; BPE: background parenchymal enhancement; NME: non-mass enhancement.

Considering BPE, the malignancy rate was 26.7% in breasts with minimal BPE, 35.3% with mild BPE, and 20% with moderate BPE. All lesions in breasts with marked parenchymal enhancement were benign (Table 3).

Regarding lesion morphology, all three lesions assessed as focus lesions had benign pathology results. Although the malignancy rate was higher in mass lesions (30.4%) than in those with NME (22.6%), it was not statistically significant (p=0.810). In addition,

no meaningful relationship was found between the shape of mass and focused lesions and malignancy (p=0.659). Mass and focus lesions with homogeneous (28.6%) and heterogeneous (27.3%) enhancement had similar malignancy rates. Only one ring-enhanced mass lesion was diagnosed as benign. When the distribution of NME lesions on MRI was evaluated as linear, segmental, focal, and regional, the malignancy rates in these subgroups were 27.3%, 33.3%, 23.1%, and 0%, respectively. Malignancy was detected more in lesions with segmental distribution than those with linear and focal distribution. Still, it was not statistically significant (p=0.837). Furthermore, no statistically significant correlation was found between the rate of malignancy and the enhancement patterns of NME lesions (p=0.813).

When the distribution of malignant lesions according to the kinetic curve of all lesions was examined, the highest rate (52.2%) was found in lesions with a washout kinetic curve (p=0.034). Lesions with persistent and plateau kinetic curves had significantly lower rates of malignancy (9.1% and 4.3%, respectively; p=0.004).

BI-RADS Category

The malignancy rates detected in BI-RADS category 4 and 5 lesions were 17.6% and 83.3%, respectively (p=0.006). The odds of malignancy were 21 times higher for BI-RADS category 5 lesions than BI-RADS category 4 lesions.

MR-guided Biopsy Procedures

MR-guided core biopsy was performed in 11 of the 57 patients (19.3%), and MGWL was conducted in the other 46 patients (80.7%). When compared according to the type of biopsy procedure performed, no statistically significant difference was found in the detected malignancy rates (p=0.147).

Follow-up

There was radiologic-pathologic concordance in all lesions. A follow-up MRI was recommended six months later in cases with benign pathological diagnoses. Follow-up MRI was obtained from 43 patients 6, 12, and 24 months after biopsy. None of these patients exhibited findings suggestive of malignancy and were assessed as BI-RADS category 2 or 3.

Discussion

Breast MRI can detect lesions not seen on US and MG in 10–39% of examined patients[15]. MR-guided wire localization and MGCB biopsy are appropriate, safe, and accurate methods in the pathological diagnosis of clinically and mammographically occult breast lesions[9].

Unlike previous studies in the literature, we included patients who underwent MGWL and those who experienced MGCB in the present study. Instead of comparing procedures, we focused on evaluating the ability of MRI findings to predict the malignancy of lesions for which technically challenging MR-guided biopsy procedures are recommended for tissue diagnosis. Of the 61 lesions initially planned for MGCB, the procedure could not be performed in 4 cases (6.5%, all with NME) because the lesions could not be visualized. This finding is consistent with the literature[1,5,10]. Positioning, compression, or the phase of the patient's menstrual cycle are possible reasons why the lesion was not visible on images obtained on the day of the procedure[10]. Gao et al. found this rate to be 2%, which they attributed to the high rate of newly diagnosed breast cancer patients (62%) in their study[9].

Complications such as significant bleeding, infection, vasovagal reaction, contralateral skin puncture, and wire dislocation were not observed during or after any of the procedures, indicating that these MR-guided procedures are safe. Complication rates reported in the literature range between 0% and 3%, consistent with our study[1,10,11].

In our study, the mean procedural time per lesion for MGWL and MGCB was 30 minutes and 45 minutes, respectively, similar to the literature[9,10,11].

Previous studies have mentioned challenges in the application of MR-guided techniques, such as limited access to the lesion (e.g., those located near the skin, axillary tail, or chest wall or in the inner quadrants or retroareolar region of the breast), lesions with washout enhancement kinetics, marked breast parenchymal enhancement, and lesions in fatty breasts were mentioned in previously published articles[3,5,16]. All MR-guided biopsy procedures performed in our study resulted in technical success, as reported in the literature[11,16].

The positive predictive value (PPV) of MR-guided biopsy procedures in this study was 24.5%, consistent with the values stated in previous studies in the literature[2,10,12,17]. Schneider et al. and Constance et al. found

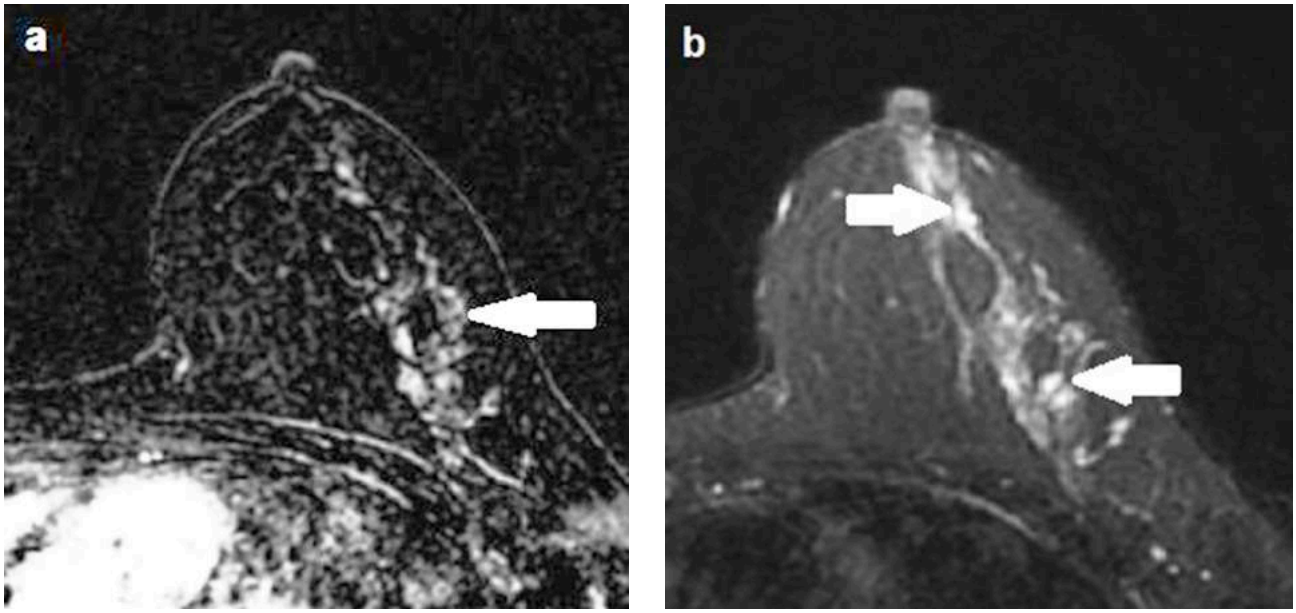

Figure 4. *a–b. A 52-year-old woman with known familial high risk for breast cancer. Axial post-contrast T1-weighted fat-suppressed MR subtraction image shows non-mass enhancement with heterogeneous internal pattern and segmental distribution in the left breast (arrow) (a). Axial STIR MR image shows several millimeter-sized cystic lesions (arrows) in the non-mass enhancement area. After MR-guided needle localization and excisional biopsy, the pathological diagnosis was fibrocystic disease (b).*

PPVs of 40%, which is considerably higher than in our study. These differences may be due to the low number of cases in their studies and the higher proportions of high-risk patients and patients with diagnosed breast cancer in the study populations compared to ours[15,18]. In our research, malignancy detection rates were similar for MGWL/excisional biopsy (23.9%) and MR-guided core biopsy (27.3%). In the study by Taneja et al., both biopsy techniques were used. Still, we could not find any data in the literature comparing the PPVs of the procedures[11]. However, the individual PPV values of both methods were consistent with those in the literature[8,19].

As expected, we found a higher PPV (41.7%) in patients who underwent MR-guided biopsy to investigate the extent of cancer as compared to other indications. This rate was similar to some studies in the literature. It showed that preoperative breast MRI is an essential guide in the treatment management of patients with breast cancer[9,10,20].

Of the malignant lesions in our study, 57.7% were diagnosed as ductal carcinoma in situ (DCIS), 13.8% as invasive ductal carcinoma, and 7.2% as invasive lobular carcinoma. In the studies by Morris et al. and Meucci et al., DCIS rates were 54.5% and 61.5%, respectively, and were higher than for invasive carcinoma, similar to our study[7,10]. As mentioned in the literature, clinically, US-and MG-occult breast cancers are primarily early stage and can be visualized on MRI[6,10,21,22]. Therefore, we attribute the higher rate of DCIS to our study sample, which is comprised exclusively of MRI-only lesions[10].

As stated in the literature, sclerosing adenosis can mimic malignancy by showing suspicious features on breast MRI more often than fibroadenoma and fibrocystic disease[23,24]. Therefore, in our study, including BI-RADS-MRI 4 and 5 lesions, the most common pathological diagnosis of benign lesions was sclerosing adenosis, which was an expected result.

The patient group selected for our study had MRI-only lesions for which an MR-guided biopsy was recommended. Little data in the literature regarding the diagnostic value of MRI findings in this patient group[9,12]. Many studies have shown that breast cancer most commonly occurs in the upper outer quadrant of the breast[25]. Surprisingly, we found approximately 6-fold higher odds of detecting a malignant lesion in the lower inner quadrant than in the upper outer quadrant. We believe the reason for this is that the MRI indication for the majority (55.5%) of patients with lower inner quadrant lesions was to evaluate the extent of breast cancer. However, Gao et al. found no relationship between lesion localization and malignancy[9].

MR-guided biopsy was recommended for all lesions included in this study due to MRI findings suspicious of malignancy. This could explain the absence of a significant difference in predicting malignancy when comparing the morphological features and enhancement patterns of the lesions between the groups. Dratwa et al. found that NME lesions were more benign, while mass lesions with irregular shapes and margins were more malignant[17]. However, unlike our study, BI-RADS category 3 lesions were also included in their research, which may explain the discrepancy between our results.

According to the results of our study, the kinetic curve and BI-RADS classification of the lesions were significant predictors of malignancy (Table 3). As expected, lesions in our study that showed enhancement with a washout kinetic curve were significantly more likely to be cancer than those with a persistent or plateau kinetic curve. Previous studies in the literature have obtained similar results[5,9,17].

Our study demonstrated that BI-RADS 5 lesions on MRI were more likely to be malignant than BI-RADS 4 lesions. This finding is similar to some studies in the literature[17]. Only one lesion that we classified as BI-RADS 5 was pathologically diagnosed as fibrocystic breast disease (Fig. 4). This lesion was segmental NME with a washout kinetic curve and heterogeneous internal enhancement. The patient was in the high-risk group for breast cancer. In the literature, the segmental distribution pattern of NME has been reported to have a significantly higher predictive rate for malignancy in the literature[26,29]. However, Morakkabati-Spitz et al. reported in their study that fibrocystic breast disease could present as segmental NME, as in our case[30].

As observed in the study by Dratwa et al., lesion size, BD, and BPE were not found to contribute to the prediction of malignancy[17].

Our study has several limitations. First, it is limited to a single-center experience with MGWL and MGCB. As a retrospective study, the sample size was small. Moreover, there was no comparative evaluation of the effects of MGWL and MGCB on cancer diagnosis and treatment management. Further studies may be designed to compare MGWL and MGCB in this regard. Thirdly, given that only patients who underwent MR-guided biopsy procedures were included in the study, the findings do not represent all the lesions for which MR-guided biopsy was recommended. Finally, we did not refer to diffusion-weighted imaging (DWI) sequences when evaluating the lesions in our study. Apparent diffusion coefficients obtained from DWI images could also help predict the malignancy of lesions.

Conclusion

In MRI-only lesions recommended for MR-guided biopsy, the lesions' BI-RADS-MRI category and kinetic curve type may help predict malignancy before biopsy. Our results showed that having a washout kinetic curve of enhancement and being BI-RADS-MRI category 5 were significant predictors of malignancy in these lesions.

Authors' Contributions

Conceptualization, methodology: ACB, HA; Formal analysis and investigation: ACB, HA, IEB; Writing—original draft preparation: ACB, HA; Writing—review and editing: ACB, IEB; Supervision: ACB, HA, IEB.

Conflict of İnterest

The authors declare that they have no conflict of interest.

Information Consent

This retrospective study was approved by the local institutional review board (Registration number: 2022–08/130), which waived the need for informed consent.

Ethical Statement

All procedures conducted in studies involving human participants adhered to the ethical standards set by the institutional and national research committee, following the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical standards.

Information Concerning Grants

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Distribution of Respiratory Pathogens After the COVID-19 Pandemic: A Single-Center Pediatric Study From Türkiye

COVID-19 Pandemisinden Sonra Solunum Patojenlerinin Dağılımı: Türkiye'den Tek Merkezli Bir Pediatrik Çalışma

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ABSTRACT

Introduction: *This study aimed to evaluate the frequency and typing of viral and bacterial pathogens in patients with a preliminary diagnosis of respiratory tract infection during the epidemic period in Türkiye.*

Material and Method: *The study included the respiratory pathogen analysis results of 215 patients aged 0–17 years admitted to the Pediatrics Department of Kütahya Health Sciences University Evliya Çelebi Training and Research Hospital between October 2022 and March 2023 with symptoms of respiratory tract infections. Twenty-three viruses and six bacteria in the respiratory panel were studied using multiplex real-time PCR. Samples were obtained from a nasopharyngeal swab in non-intubated patients and tracheal aspiration material in intubated patients.*

Results: *While 92.1% of the patients were positive for one or more agents, no agent was detected in 7.9%. Among the positive results, the rate of the single pathogen was 27.3% (n=54), while the rate of multiple pathogens was 72.7% (n=144). Respiratory syncytial virus was the most common viral agent with a rate of 23.3% and Adenovirus with a rate of 19.1%, while Haemophilus influenzae (48.8%) and Streptococcus pneumoniae (29.3%) were the most common bacterial agents.*

Conclusion: *In this study, viral and bacterial pathogens have been studied intensively in children. Simultaneous, rapid, and sensitive detection of these agents is essential in preventing unnecessary antibiotic use and infection control. This is also important in terms of reducing mortality and morbidity rates.*

Keywords: *COVID-19; pandemic; respiratory pathogens; respiratory syncytial viruses*

ÖZET

Giriş: *Bu çalışmanın amacı, Türkiye'de salgın döneminde solunum yolu enfeksiyonu ön tanısı alan hastalarda viral ve bakteriyel patojenlerin sıklığını ve tiplendirmesini değerlendirmektir.*

Materyal ve Metot: *Ekim 2022-Mart 2023 tarihleri arasında Kütahya Sağlık Bilimleri Üniversitesi Çelebıya Eğitim ve Araştırma Hastanesi Çocuk Sağlığı ve Hastalıkları Kliniğine solunum yolu enfeksiyonu semptomları ile başvuran 0–17 yaş arası 215 hastanın solunum yolu patojenleri analiz sonuçları çalışmaya dâhil edilmiştir. Solunum panelinde 23 virüs ve altı bakterinin varlığı multipleks gerçek zamanlı PCR kullanılarak çalışılmıştır. Örnekler entübe olmayan hastalarda nazofarengeal sürüntüden, entübe hastalarda ise trakeal aspirasyon materyalinden elde edilmiştir.*

Bulgular: *Hastaların %92,1'inde bir veya daha fazla etken pozitif bulunurken, %7,9'unda hiçbir etken saptanmamıştır. Pozitif sonuçlar arasında tek patojen oranı %27,3 (n=54) iken, çoklu patojen oranı %72,7 (n=144) idi. Respiratuvar sinsityal virüs %23,3 ve Adenovirüs %19,1 oranıyla en sık görülen viral etkenler olurken, Haemophilus influenzae (%48,8) ve Streptococcus pneumoniae (%29,3) en sık görülen bakteriyel etkenler olmuştur.*

Sonuç: *Bu çalışmada çocuklarda viral ve bakteriyel patojenler yoğun olarak saptanmıştır. Bu etkenlerin eş zamanlı, hızlı ve duyarlı bir şekilde saptanması gereksiz antibiyotik kullanımının önlenmesi ve enfeksiyon kontrolü açısından önemlidir. Bu da mortalite ve morbidite oranlarının azaltılması açısından önemlidir.*

Anahtar kelimeler: *COVID-19; pandemi; solunum patojenleri; respiratuvar sinsityal virüs*

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Introduction

Respiratory tract infections (RTIs) are the most common cause of both outpatient treatment and hospitalizations and antibiotic use in children. They are one of the leading causes of morbidity and mortality worldwide. The causative agents of these diseases may be of community or hospital origin[1]. The loss of labor force and treatment costs caused by RTIs show that they seriously burden national economies[2].

Although 60–80% of RTIs are reported as viral agents, the most common viruses are influenza virus, human rhinovirus (HRV), respiratory syncytial virus (RSV), human coronavirus (HCoV), and parainfluenza virus (PIV)[3]. The most common bacterial agents associated with RTI are *Streptococcus pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis,* and *Streptococcus pyogenes*[1,4].

In RTIs, it is difficult to distinguish whether the pathogen is viral or bacterial without laboratory diagnosis. Since clinical symptoms that may be pathogen-specific are scarce in RTIs, the diagnosis is primarily based on laboratory investigation[5]. Tests that can rapidly identify various viruses simultaneously may help initiation of appropriate treatment. Multiplex real-time PCR (MRT-PCR) is an in vitro test that qualitatively detects the viruses and bacteria causing RTIs[6]. Rapid and accurate identification of these infectious agents is of great importance in terms of timely initiation of antiviral treatment and prevention of unnecessary antibiotic use, as well as reducing the duration of hospitalization, risk of nosocomial transmission, and treatment costs.

Our country faces a rapidly spreading epidemic that has seriously affected society in recent days. People have complaints such as fever, weakness, sore throat, and joint pain, which do not put them to bed but make it difficult to stand. For this reason, the number of hospitalizations with respiratory infections, which are more common in the 0–17 age group, has increased recently. In this study, we aimed to evaluate the frequency and typing of viral and bacterial pathogens in patients with a preliminary diagnosis of upper and lower respiratory tract infection in the pediatric population, which causes an increase in the number of hospital admissions in many regions of our country.

Material and Method

Study Population and Data Collection

This study included all children aged 0 to 17 years who underwent MRT-PCR of nasopharyngeal swabs for respiratory pathogens at a tertiary university hospital (Kütahya, Türkiye) between October 2022 and March 2023. The International Classification of Diseases Code was used to identify cases. Data was collected from computer databases and electronic medical records. Each patient's demographic information, symptoms, and laboratory results were documented. Respiratory tract samples sent from patients with a preliminary diagnosis of acute respiratory tract infection were analyzed for viral and bacterial agents by MRT-PCR. Patients were divided into age groups: 0–2, 3–5, 6–9, and 10–17 years. The age classification is based on the school system. Children under two years of age are kept separately at home or attend kindergarten; children from 3 to 5 years old attend kindergarten; children from 6 to 9 years old attend primary school. After age 10, children go to middle school and high school. The multiplex PCR was performed on nasopharyngeal swabs from patients presenting with symptoms attributable to respiratory tract infection (fever, myalgia, chills, weakness, sore throat, shortness of breath, cough, chest pain, headache, abdominal pain, nausea/vomiting, diarrhea, loss of taste, loss of smell, rash, and conjunctivitis). The hospital's pediatric emergency and inpatient services requested the test. This study protocol was approved by the local ethics committee (2023/01-39).

Nasopharyngeal Swab Collection and Genetic Material Extraction

Nasopharyngeal swabs were obtained from patients presenting to pediatric departments with RTI. For this purpose, rayon-tipped swabs with a bendable rod were used. The swab was inserted into the nasopharynx by inserting it through the nostril. The sample was obtained by gently holding it for 5 seconds, then gently rotating it, and finally transporting it to the microbiology laboratory in a Viral Nucleic Acid Buffer (vNAT) transport medium (Bioeksen, Türkiye), adhering to the cold chain regulations. Nasopharyngeal/oropharyngeal swab samples from patients in vNAT transport medium nucleic acid extracts were not pretreated. The study was performed on the CFX96 real-time PCR detection system (CFX96; Bio-Rad, USA).

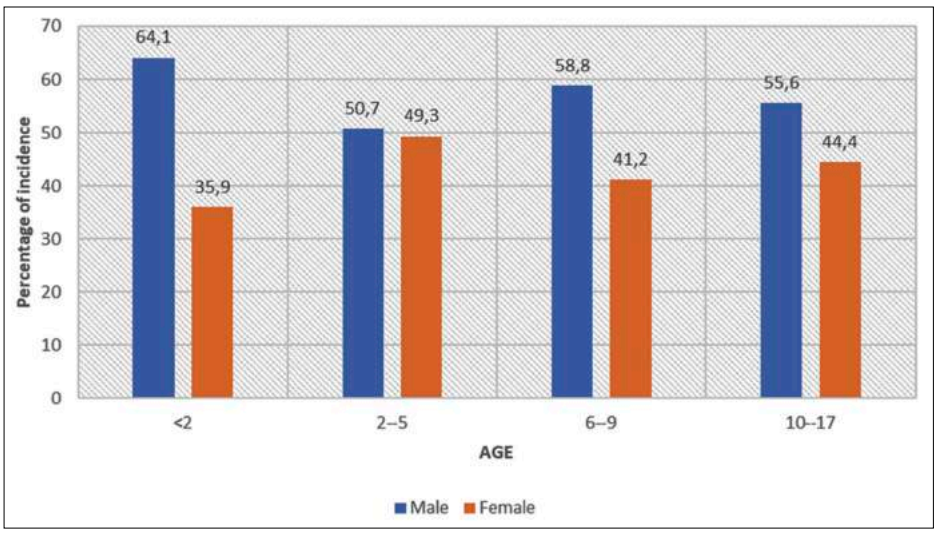

Figure 1. *Characteristics of children enrolled in the study.*

Multiplex PCR

Viral and bacterial nucleic acids (DNA or RNA) in respiratory tract samples were investigated with the Bio-Speedy® Respiratory Tract RT-qPCR MX-24S kit (Bioeksen, Turkiye). Based on the MRT-PCR technique, this system can simultaneously detect the presence of twenty-three viruses and six bacteria causative agents of respiratory tract infections. The agents that can be seen with the respiratory panel kit used are; Human bocavirus (HBOV), Adenovirus (AdV), Human coronavirus (HCOV-HKU1), Human coronavirus (HCOV-NL63), Human coronavirus (HCOV-OC43), Human coronavirus (HCOV-229E), Human enterovirus/Human rhino, Human metapneumovirus (HMPV A), Human parainfluenza virus 1–4, Human parechovirus (HPEV), Human rhinovirus (RV), Influenza A (FluA), Influenza A H1, Influenza A virus subtype (H1), Influenza A H3Nx, Influenza A H5Nx, Influenza A H37Nx, Influenza B (FluB), RSV, SARS-CoV-2, *Bordetella pneumoniae*, *Chlamydophila pneumoniae*, *H.influenzae, Legionella pneumophilia*, *Mycoplasma pneumoniae, S.pneumoniae*. *Streptococcus pyogenes*, the most common cause of RTI in children, was not evaluated as it was not included in the respiratory panel test list[1,4].

Data Analysis

Descriptive statistics, including mean and standard deviation, were provided for numerical variables exhibiting normal distributions. Percentage values and frequency tables were presented for specific variables. Statistical analyses were conducted using IBM Statistical Package for Social Sciences (SPSS) program version 23.0 (IBM Corp., Armonk, NY, USA). The study used the Phi correlation V test incorporating cross-table statistics to examine the relationship between positive and negative respiratory pathogens. The study defined the absolute value of the correlation coefficient as a "high correlation" between 0.7 and 1, a "moderate relationship" between 0.7 and 0.3, and a "low relationship" between 0.3 and 0. A significance level of $p<0.05$ was also considered statistically significant.

Results

Description of Cohort

From October 2022 to March 2023, 215 children, 125 (58.1%) boys and 90 (41.9%) girls, were tested for upper and lower respiratory tract pathogens. The demographic characteristics of the children included in the study are shown in Fig. 1.

The most common symptoms were fever (n=175, 81.4%), cough (n=168, 48.3%), weakness (n=131, 60.9%), and shortness of breath (n=121, 60.9%) (Table 1).

When respiratory pathogens were analyzed, more than one respiratory pathogen was found to be present at the same time. Among respiratory pathogens, it was determined that at least one pathogen was present in one child. In contrast, a maximum of 6 pathogens could be seen simultaneously. Among the positive results, the rate of the single pathogen was 27.3% (n=54), while the rate of multiple pathogens was 72.7% (n=144).

Table 1. *Clinical findings of patients*

	Age (years)				
	<2 n=92	2–5 n=71	6–9 n=34	10–17 n=18	Total Positive
Clinical Finding, N (%)					
Fever	81 (88)	58 (81.7)	27 (79.4)	9 (50)	175 (81.4)
Myalgia	0	13 (18.3)	5 (14.7)	5 (27.7)	23 (10.7)
Chills	0	27 (38)	13 (38.2)	5 (27.8)	45 (20.9)
Weakness	41 (44.6)	53 (74.6)	21 (61.8)	16 (88.9)	131 (60.9)
Sore throat	0	62 (87.3)	17 (50)	10 (55.6)	89 (41.4)
Shortness of breath	49 (53.3)	57 (80.3)	12 (35.3)	3 (16.7)	121 (56.3)
Cough	71 (77.2)	62 (87.3)	21 (61.8)	14 (77.8)	168 (78.1)
Chest pain	0	0	5 (14.7)	1 (5.6)	6 (2.8)
Runny nose	50 (54.3)	28 (39.4)	19 (55.9)	9 (50)	106 (49.3)
Headache	0	0	2 (5.9)	2 (11.1)	4 (1.9)
Abdominal pain	0	0	6 (17.6)	0	6 (2.8)
Nausea/vomiting	19 (20.7)	12 (16.9)	0	1 (5.6)	32 (14.9)
Diarrhea	7 (7.6)	16 (22.5)	0	0	23 (10.7)
Loss of taste	0	0	0	0	0
Loss of smell	0	0	0	0	0
Rash	9 (9.8)	3 (4.2)	0	0	11 (5.1)
Conjunctivitis	0	2 (2.8)	0	0	2 (0.9)

The most common concurrent pathogens were SARS-CoV-2 and *H.influenzae*, seen simultaneously in 14% (n=30) of children.

There was no significant correlation between the incidence of related respiratory pathogens according to gender ($p>0.05$). When the difference in the prevalence of pathogens between age groups was compared, a significant difference was found between AdV, *H.influenzae*, FluA, Human enterovirus/Human rhino, Influenza A virus Subtype H1, Influenza A H3Nx, and RSV according to age ($p<0.05$). No statistically significant difference was found between the frequency of other pathogens according to age groups ($p>0.05$).

The most common viruses were RSV (23.3%) and AdV (19.1%), while *H.influenzae* (48.8%) and *S.pneumoniae* (29.3%) were the most common bacteria. The pathogenicity of children divided according to age groups is given in Table 1, and the most frequent positivity was observed in the <2 years age group. This was followed by the 2–5, 6–9, and 10–17 age groups, respectively (Table 2).

Since the *H. influenza* and *S. pneumonia* agents we detected can normally colonize, it is thought that these bacteria may be the causative agents of the disease in 13 patients with only *H. influenza* positivity, six patients with only *S. pneumonia* positivity, and eight patients with both positive, in whom no other agents were detected in patients with clinical signs of respiratory tract infection.

Of the patients, 64 (29.8%) were hospitalized in the ward, and 11 (5.1%) were hospitalized in the pediatric intensive care unit (PICU). The mean duration of hospitalization in the ward was 2.3 days, and ten days in the PICU.

Finally, 51 (23.7%) patients received respiratory support. Of these, 43 (84.3%) received free-flow oxygen therapy, 5 (9.8%) high-flow nasal cannula (HFNC) oxygen therapy, 2 (3.9%) noninvasive ventilation therapy, and 1 (1.9%) endotracheal intubation.

Discussion

The COVID-19 pandemic has evolved rapidly and caused health problems worldwide. There was reportedly a decline in pediatric emergency department visits and visits due to RTIs during the pandemic period[7] and a significant decrease in the frequency of seasonal respiratory infections in the pediatric population during quarantine periods compared to previous years[8]. It has been observed that the number of applications to emergency and pediatric clinics with complaints such as fever, cough, sore throat, fatigue, joint pain, etc., in the pediatric population in our hospital between October 2022 and March 2023 increased excessively. We investigated the identification and incidence of

Table 2. *Distribution of respiratory pathogens by age groups*

	Age (years)					
	<2 n=92	2–5 n=71	6–9 n=34	10–17 n=18	Total Positive	p-value
Pathogens detected, N(%)						
AdV	7 (7.6)	19 (26.8)	13 (38.2)	2 (11.1)	41 (19.1)	**<0.001**
Bordetella pneumoniae	0	0	1 (2.9)	0	1 (0.5)	0.294
Haemophilus influenzae	37 (40.2)	45 (63.4)	18 (52.9)	5 (27.8)	105 (48.8)	**0.006**
HBOV	1 (1.1)	5 (7)	1 (2.9)	1 (5.6)	8 (3.7)	0.221
HCOV-NL63	2 (2.2)	2 (2.8)	0	0	4 (1.9)	0.511
HCOV-OC43	10 (10.9)	4 (5.6)	5 (14.7)	1 (5.6)	20 (9.3)	0.404
HCOV-229E	0	0	0	1 (5.6)	1 (0.5)	0.171
Human enterovirus/Human rhino	12 (13)	5 (7)	0	2 (11.1)	19 (8.8)	**0.037**
HMPV A	2 (2.2)	1 (1.4)	0	1 (5.6)	4 (1.9)	0.514
Human parainfluenza virus 3	15 (16.3)	5 (7)	1 (2.9)	1 (5.6)	2 (10.2)	0.065
Human parainfluenza virus 4	3 (3.3)	0	0	0	3 (1.4)	0.161
HPEV	1 (1.1)	0	0	0	1 (0.5)	0.636
FluA	5 (5.4)	19 (26.8)	8 (23.5)	2 (11.1)	34 (15.8)	**<0.001**
Influenza A H1	4 (4.3)	12 (16.9)	4 (11.8)	1 (5.6)	21 (9.8)	0.050
Influenza A virus subtype (H1)	5 (5.4)	13 (18.3)	4 (11.8)	0	22 (10.2)	**0.012**
Influenza A H3Nx	1 (1.1)	6 (8.5)	4 (11.8)	1 (5.6)	12 (5.6)	**0.045**
FluB	7 (7.6)	6 (8.5)	0	0	13 (6)	0.058
Streptococcus pneumoniae	29 (31.5)	21 (29.6)	7 (20.6)	6 (33.3)	63 (29.3)	0.639
RSV	38 (41.3)	10 (14.1)	1 (2.9)	1 (5.6)	50 (23.3)	**<0.001**
SARS-CoV-2	3 (3.3)	0	0	1 (5.6)	4 (1.9)	0.131
HCOV-HKU1	0	0	0	0	0	0
Chlamydophila pneumoniae	0	0	0	0	0	0
Human parainfluenza virus 1	0	0	0	0	0	0
Human parainfluenza virus 2	0	0	0	0	0	0
Influenza A H5Nx	0	0	0	0	0	0
Leginella pneumophilia	0	0	0	0	0	0
Mycoplasma pneumoniae	0	0	0	0	0	0

AdV: Adenovirus; HBOV: Human bocavirus; HCOV-HKU1: Human coronavirus-HKU1; HCOV-NL63: Human coronavirus-NL63; HCOV-OC43: Human coronavirus-OC43; HCOV-229E: Human coronavirus-229E; HMPV A: Human metapneumovirus; HPEV: Human parechovirus; FluA: Influenza A; FluB: Influenza B; RSV: Respiratory sinsityal virus.

respiratory tract pathogens to determine the causative agents of the disease and to organize the treatment for the causative agent during these dates when the number of applications intensified nationwide.

Timely detection of respiratory tract pathogens and initiation of appropriate antimicrobial treatment significantly reduces morbidity and mortality. MRT-PCR methods, which have recently been widely used in the detection of respiratory tract pathogens and used as a diagnostic tool that can detect many pathogens at the same time with high sensitivity, are essential in the application of correct and effective treatment by differentiating viral and bacterial infections and provide a better understanding of epidemiologic data on the distribution of these agents[9–10].

In this study, respiratory pathogen agent (s) were detected with a rate of 92.1% in samples obtained from patients admitted to pediatric departments within six months. Some studies reported positivity rates of 17.5%, 44%, 67.8%, and 73.5%. This study found a higher positivity rate of 92.1% than in the literature[11–14]. We think the positivity rate was high in this study because children stayed away from public areas such as schools, kindergartens, playgrounds, etc., during the pandemic. The spread of microbial agents in an epidemic manner was less with the use of masks, paying attention to distance and hygiene rules in this process, and these rules were relaxed after the pandemic. At the same time, it is thought that the high positivity rate may be due to the low body resistance of children who did not intensively encounter microorganisms during the pandemic. The distribution of respiratory tract pathogens by gender was compatible with the literature, and no significant correlation was found between boys and girls ($p>0.05$)[12,14,15].

This study's most common viral agents were RSV (23.3%) and AdV (19.1%). Our data are compatible with the data of studies conducted worldwide[13,16–19]. In contrast to similar studies, one study reported that HRV, and another said that HCoV was detected at the highest rate[20,21]. In a large-scale study, it was reported that 66.000–160.000 children under five years of age died from RSV infection-related complications[22]. Therefore, knowing the clinical effects and epidemiology of RSV, early detection, and giving the necessary importance to vaccination in the risky population are of great importance in preventing a severe RSV epidemic in the future. AdV is responsible for at least 5 to 10% of infections that frequently infect the lower or upper respiratory tract, conjunctiva, and gastrointestinal system and can remain in the bodies of infected children for months and require hospitalization[23]. In a study on gastroenteritis agents in the same city, AdV was the second most common agent[24]. To prevent exposure to AdV, which causes mortality and morbidity and poses a risk for childhood, it is essential to comply with hygiene rules in all living areas, especially in public places, and to provide education on these rules.

As a result of studies conducted in our country, the *S.pneumoniae* pathogen was reported as the most frequently detected bacterial agent from respiratory tract samples[14,25,26]. The most commonly isolated bacterial agents in these studies were reported to be *S.pneumoniae*, *S.aureus,* and *C.pneumoniae,* respectively[14]. In this study, *H.influenzae* (48.8%) was the most common bacterial agent, followed by *S.pneumoniae* (29.3%). Early detection and agent-directed treatment are essential to prevent antibiotic resistance against *S.pneumoniae* and *H.influenzae*, which are the agents of community-acquired bacterial pneumonia.

In studies on the simultaneous presence of more than one pathogen in the respiratory tract sample, RSV was reported to be the most common pathogen, and association with AdV, HBoV, and INF-A pathogens was reported[27]. Different studies said the most common pathogens accompanying HCoVs were HRV, RSV, HRV, HRV, and *S.pneumoniae*[28–30]. In this study, although the results were similar to the literature data, 27.3% of the analyzed samples were associated with a single agent and 72.7% were associated with 2–6 agents. *S.pneumoniae*, human influenza viruses, and *S.pneumoniae* and RSV synergies significantly exacerbate pneumonia's morbidity and mortality[31,32]. Large-scale studies are needed to investigate the mechanisms underlying these synergies and bacterial, viral, and host immune factors that increase susceptibility to infection.

Studies have shown that children <2 years of age appear more susceptible to infections, similar to our research[33,34]. In particular, rhinovirus, enterovirus, SARS-CoV-2, other coronaviruses, and Adenovirus were reported more frequently. Current assumptions relate to the lack of prior immunity[35] and the failure of children under 6 to use masks after schools reopen[36].

In conclusion, although our study was conducted briefly and in a single center during a nationwide epidemic, a high positivity rate was detected, and multiple agents were frequently found in the same patient. Respiratory tract infections are one of the most common reasons for using antimicrobials. Widespread and inappropriate use of antimicrobials is one of the reasons for the emergence of multidrug resistance, which continues to increase alarmingly[37]. The use of broad-spectrum antibiotics leads to more resistant strains. As resistance increases, there are delays in initiating appropriate and effective antibiotic treatment, and the frequency of sepsis and mortality rates increase. Detection of respiratory tract pathogens is also important to avoid delaying antibacterial treatment because the causative agents are viral during epidemic periods. For this reason, it is vital to prevent unnecessary and incorrect treatments with early diagnosis by using the MRT-PCR method (fast, sensitive, cost-effective, and capable of studying many agents) in detecting respiratory tract infections and pathogens.

This study had many limitations. It was a single-center study, and the most common pathogen, S. pyogenesis in RTI, was not included in the respiratory panel tests. Another limitation was the lack of data before and during the pandemic. We could not fully collect these data due to systemic change.

In conclusion, our data showed a change in the typical epidemiology of pediatric respiratory tract pathogens according to the time of the pandemic. Although our study was conducted in a single center shortly after the nationwide pandemic, RSV and/or influenza viruses, which decreased during the pandemic, were found after the pandemic, with a high rate of positivity and often more than one agent in the same patient. We think this is due to the transition to the normalization process and children's entry into the social environment.

Authors' Contributions

EA, YD ve NA performed experiments. DPR designed experiments supervised the team during the study, and prepared the manuscript. GGY analyzed statistics. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Data Availability

The datasets generated during and analyzed during the current study are not publicly available due to the potential compromise of individual privacy. Still, they are available from the corresponding author on reasonable request.

Code Availability

Not applicable to this submission.

Ethical Approval

Ethics committee approval for the study was obtained from the non-interventional ethics committee of Kütahya Health Sciences University with the letter dated 11.01.2023 and decision number 2023/01-39.

Competing İnterests

The authors declare no competing interests.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Determination of the Effects of Docetaxel Chemotherapy on Surgical Results and Survival in High-Risk Locally Advanced Prostate Cancer Before Radical Prostatectomy

Yüksek Riskli Lokal İleri Prostat Kanserlerinde Radikal Prostatektomi Öncesi Dosetaksel Kemoterapisinin Cerrahi Sonuçlar ve Sağkalım Üzerine Etkilerinin Belirlenmesi

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ABSTRACT

Aim: *Determination of the effect of neo-adjuvant docetaxel chemotherapy combined with radical prostatectomy (RP) on surgical outcome and survival.*

Material and Method: *The data of 132 non-metastatic prostate cancer (PC) patients, considered high-risk according to the D'Amico Risk Stratification System and who underwent radical prostatectomy, among those who applied to the Hacettepe University Faculty of Medicine Urology Clinic between August 1987 and August 2017, were retrospectively evaluated. Data from 28 patients selected via pair matching from the group operated without chemotherapy and 14 patients identified to have received neoadjuvant androgen deprivation therapy (NADT) preoperatively were compared regarding biochemical recurrence, survival, surgical outcomes, and some additional variables.*

Results: *The findings of our study revealed that, while NADC is usually tolerated well by patients and can be administered without severe side effects, it has no statistically significant advantage on PSA values (p=0.145), Gleason scores, and pathologic stages (p=0.273, p=0.109), biochemical recurrence risk (p=0.040) and overall survival (p=0.527). It did not affect surgical complication rates, may have benefitted malignant involvement of lymph nodes, and prolonged biochemical relapse-free survival time.*

Conclusion: *In high-risk PC patients, the ineffectiveness of neoadjuvant androgen deprivation therapy in combination with RP suggests the presence of castration-resistant cell clones that exist at the time of the diagnosis and brings up treatment options that could be effective on castration-resistant clones as systemic treatments. As a neo-adjuvant treatment, combining docetaxel chemotherapy with RP can be beneficial.*

Keywords: *neoadjuvant; docetaxel; high-risk prostate cancer*

ÖZET

Amaç: *Neo-adjuvan dosetaksel kemoterapisinin (NADC) radikal prostatektomi (RP) ile kombine edilmesinin cerrahi sonuç ve sağkalım üzerine etkisinin belirlenmesi.*

Materyal ve Metot: *Ağustos 1987 ile Ağustos 2017 tarihleri arasında Hacettepe Üniversitesi Tıp Fakültesi Üroloji Kliniğine başvuran hastalar arasından D'Amico Risk Evreleme Sistemine göre yüksek riskli kabul edilen ve RP uygulanan 132 metastatik olmayan prostat kanseri (PK) hastasının verileri geriye dönük olarak değerlendirildi. Kemoterapi almadan ameliyat edilen hasta grubundan "pair match" ile seçilen 28 hasta ile ameliyat öncesi NADC verildiği tespit edilen 14 hastanın verileri biyokimyasal nüks, sağkalım, cerrahi sonuçlar ve bazı ek değişkenler açısından karşılaştırıldı.*

Bulgular: *Çalışmamızın bulguları, NADC'nin genellikle hastalar tarafından iyi tolere edildiği ve ciddi yan etkiler olmadan uygulanabildiğini ancak PSA değerleri (p=0,145), Gleason skorları ve patolojik evre (p=0,273, p=0,109), biyokimyasal nüks riski (p=0,040) ve genel sağkalım (p=0,527) üzerine istatistiksel olarak anlamlı bir avantajının olmadığını ortaya koydu. Cerrahi komplikasyon oranları üzerinde etki saptanamadı. NADC'nin lenf nodu tutulumunun azalmasında muhtemel olumlu etkileri olduğu ve biyokimyasal nükssüz sağkalım süresini uzattığı tespit edilmiştir.*

Sonuç: *Yüksek riskli PK hastalarında RP ile birlikte neoadjuvan androjen deprivasyon tedavisinin etkisiz olması, tanı anında var olan kastrasyona dirençli hücre klonlarının varlığını düşündürmekte ve kastrasyona etkili olabilecek tedavi seçeneklerini gündeme getirmektedir. Neo-adjuvan bir tedavi olarak, dosetaksel kemoterapisinin RP ile birleştirilmesi yüksek riskli prostat kanseri taşıyan hasta grubunda faydalı olabilir.*

Anahtar kelimeler: *neo-adjuvan; dosetaksel; yüksek riskli prostat kanseri*

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Introduction

Following lung cancer, prostate cancer (PC) is the second most prevalent form of cancer. When all men with a cancer diagnosis are examined, it can lead to significant morbidity and mortality[1]. After the diagnosis, the most critical issue is predicting the disease's prognosis and selecting the most appropriate treatment based on the patient's risk. D'Amico Risk Evolution, which evaluates patients as low, moderate, and high risk according to the level of serum prostate-specific antigen (PSA) at diagnosis, the clinical stage suggested by digital rectal examination (DRE), and Gleason Score (GS) that is obtained after biopsy has been widely accepted in the risk classification of patients and has been used in studies conducted on this field[2].

Although the diagnostic and treatment guidelines developed by multinational urological associations, such as the European Association of Urology (EAU) and the American Urological Association (AUA), clearly define treatments for various risk stages, discussions on treatment protocols for patients at high-risk groups are still in progress. High-risk PC patients are at increased risk for recurrence of PSA, need for secondary treatment, risk of metastasis, and death due to PC[3]. There are several studies in this group of patients claiming that castration-resistant cancer cells may be present in the tumor at the time of diagnosis, which may lead to a relapse of the disease in a relatively short period, the use of neo-adjuvant docetaxel chemotherapy (NADC) before radical prostatectomy (RP) could reduce the likelihood of recurrence and improve survival[4–8].

This study aimed to evaluate the effects of NADC applied before RP on surgical outcomes, recurrence, and survival in high-risk prostate cancer patients.

Material and methods

Study Protocol

Ethics committee approval was obtained for the thesis study from the Hacettepe University Ethics Committee with the number GO 17/715-40 on 24.08.2017. In this study, we retrospectively evaluated non-metastatic PC patients admitted to the Hacettepe University Faculty of Medicine, Urology Clinic between 1987 and 2017 who were over 18 years of age and had high risk according to the D'Amico Risk Staging System. All patients underwent RP. It aimed to compare the patients who received preoperative NADC (combination therapy) and those who underwent surgery without chemotherapy (monotherapy) in terms of biochemical recurrence, cancer-specific and overall survival, and some additional variables.

Among 1308 cases of local or locally advanced-stage PC patients who had undergone RP surgery between 01.08.1987 and 01.08.2017, 132 cases with high risk, according to the D'Amico Risk Stage, were recruited and screened retrospectively. When the data of these 132 patients were examined, it was found that 14 patients received four cycles of NADC (combination therapy) before RP. Considering the PSA value, Gleason score, and age at the diagnosis in terms of the comparison with this group, a group of RP (monotherapy) was formed with the "pair match" technique, which consists of 28 patients with similar characteristics at the time of diagnosis.

Before the treatment, all patients were examined with computed tomography and bone scintigraphy (BS), and metastasis was investigated. The study excluded patients who had metastatic disease at the diagnosis. Patients with uncontrolled comorbidities (including oncologic diseases) who had received chemotherapy for another reason or a history of pelvic radiotherapy, which may influence the survival and the treatment process, were also left out of the study. All patients' pre– and post-treatment specimens were examined and reported by the same uropathology team at Hacettepe University, Faculty of Medicine Department of Pathology.

In a total of fourteen patients who received combined therapy, docetaxel was started at a dose of 75 mg/m^2 every three weeks, and 8 mg oral dexamethasone was given to the patient twelve hours, three hours, and one hour before the infusion of chemotherapy (CTx). Patients were given prednisolone 2×5 mg throughout CTx. The blood tests for control purposes were made in the CTx sessions immediately before each treatment cycle. "Common Terminology Criteria for Adverse Events v 4.0" was used to evaluate adverse events during treatment[9].

All patients underwent "open retropubic radical prostatectomy and bilateral pelvic lymph node dissection" (RRP-BPLND) surgery by the Urooncology Team of the Urology Clinic of the Medical Faculty of Hacettepe University. The amount of bleeding during operation, operation time, and complications were noted separately and compared between the two study groups. The duration of postoperative hospital stay was also compared between the two study groups.

Table 1. *Characteristics of the groups at the diagnosis*

		Combined Therapy Group (n=14)	Monotherapy Group (n=28)	p
Age (Years)	(Median, Min-Max)	58 (46–73)	64 (52–77)	***0.007***
PSA levels at the diagnosis (ng/mL)	(Median, Min-Max)	20.61 (3.07–170)	17 (7.44–111.33)	0.208
ISUP Scores at the diagnosis	ISUP 3	1 (7.1%)	2 (7.1%)	
	ISUP 4	4 (28.6%)	8 (28.6%)	1.000
	ISUP 5	9 (64.3%)	18 (64.3%)	
Lymph node involvement at the diagnosis	Negative	9 (64.3%)	24 (85.7%)	0.133
	Positive	5 (35.7%)	4 (14.3%)	

*PSA: Prostate Spesific Antigen, ISUP: International Society of Urological Pathology

Table 2. *PSA values during chemotherapy cycles*

PSA (ng/mL)	Cycle 1	Cycle 2	Cycle 3	Cycle 4	p
Mean	24.17	26.29	16.47	21.95	
SD	22.16	24.04	15.15	21.25	0.145
Median	16.95	18.62	13.31	17.17	
Min-Max	0.72–61.98	2.58–72.84	1.50–48.49	1.11–68.67	

* PSA: Prostate Spesific Antigen, SD – Standart Deviation

The pathologic findings of the patients and pathology results obtained after RP were compared with each other in terms of ISUP (International Society of Urological Pathology) scores, clinical stages, and presence of lymph node involvement. Post-RP PSA values of the patients were first measured after one month of surgery, every three months for the first two years afterward, and every six months for the following periods. The biochemical recurrence (BCR) criteria were a PSA limit value of 0.2ng/mL; Patients with two PSA values of 0.2 ng/mL or greater, with at least one week between them, were considered BCR. Up-to-date EAU Treatment Guidelines have been considered while adjuvant therapies were being planned. Both groups of patients were evaluated for biochemical recurrence-free survival (BRFS) and patient survival (PS).

Statistical Analysis

In numerical variables, descriptive statistics included mean, standard deviation, and median (minimum-maximum), while categorical variables included numbers and percentages. When comparing the groups receiving and not receiving chemotherapy, the "significance test of the difference between the two means" was used for the normalized numerical variables, and the Chi-square test was used for the categorical variables. The Mann-Whitney U test was utilized to compare standard non-normally distributed numerical variables between two groups. The marginal homogeneity test was used in independent groups to compare variables with more than two categories, and the Mc Nemar test in variables with two categories. Cumulative survival probabilities and mean survival times were calculated using the Kaplan-Meier method. Survival curves were drawn and compared with the log-rank or Breslow tests according to the group factor. Comparisons were considered statistically significant when $p<0.05$. Analyses were performed on IBM Statistical Package for Social Sciences (SPSS) program version 23.0 (IBM Corp., Chicago, IL, USA).

Results

The study included a total of forty-two patients. Twenty-eight (66.6%) patients had only RP monotherapy for prostate cancer treatment, and 14 (33.3%) had combined therapy with four cycles of NADC before surgery. The median age of the 14 patients receiving combined therapy was 58, whereas the median age in the monotherapy group was 64 (p=0.007). The groups did not differ significantly regarding PSA values, ISUP groups, and lymph node involvement on CT at the diagnosis (Table 1).

There was no significant change in the PSA values of the patients due to docetaxel chemotherapy (p=0.145) (Table 2). None of the patients had grade 3 or more side effects of chemotherapy. When the complications related to surgical treatment were examined, it was found that 1 (7.14%) patient in the combined therapy group experienced bleeding that necessitated a blood transfusion (p=0.233). In contrast, seven patients (25%) in the monotherapy group experienced bleeding that necessitated a blood transfusion, 2 (7.14%) had intraoperative rectal injuries (p=0.545), 1 (3.57%)

Table 3. *Comparison of ISUP scores of diagnostic biopsy and final pathologies according to groups*

	Combined Therapy Group (n=14)		p	Monotherapy Group (n=28)		p
	Diagnostic Biopsy	RP Specimen		Diagnostic Biopsy	RP Specimen	
ISUP 1	*	1 (7.1%)		*	1 (3.6%)	
ISUP 2	*	*		*	1 (3.6%)	
ISUP 3	1 (7.1%)	3 (21.4%)	0.273	2 (7.1%)	4 (14.3%)	0.109
ISUP 4	4 (28.6%)	2 (14.3%)		8 (28.6%)	3 (10.7%)	
ISUP 5	9 (64.3%)	8 (51.7%)		18 (64.3%)	19 (67.9%)	

*ISUP: International Society of Urological Pathology, RP: Radical Prostatectomy

Table 4. *Comparison of lymph node involvement by groups*

	Combined Therapy Group (n=14)		p	Monotherapy Group (n=28)		p
	CT at the Diagnosis	Final Pathology		CT at the Diagnosis	Final Pathology	
LNI Positive	5 (35.7%)	8 (57.14%)	0.375	4 (14.3%)	11 (39.29%)	**0.039**
LNI Negative	9 (64.3%)	6 (42.86%)		24 (85.7%)	17 (60.71%)	

LNI: Lymph Node Involvement, CT: Computerized Tomography

Table 5. *Data of the groups during the follow-up period*

		Combined Therapy Group (n=14)	Monotherapy Group (n=28)	p
Follow-up Periods (Months)	(Median, Min-Max)	49.50 (18-106)	34.50 (12-199)	0.759
PSA Recurrence	Yes	11 (78.6%)	25 (89.3%)	0.383
	No	3 (21.4%)	3 (10.7%)	
Biochemical Recurrence-Free Survival (Months)	(Median, Min-Max)	18 (7-95)	7 (2-162)	**0.040**

*PSA: Prostate Spesific Antigen, SD – Standart Deviation

had ureter injury and repair (p=1.000), and one patient (3.57%) who had previously undergone diagnostic laparotomy experienced intestine injury due to adhesions (p=1.000). There were no statistically significant differences between the two groups regarding complications.

There was no significant difference between the ISUP scores of the diagnostic biopsies and the ISUP scores of the surgical specimen in the combined therapy group and the monotherapy group (p=0.273, p=0.109, respectively) (Table 3).

When patients were evaluated regarding lymph node positivity, there was no statistically significant difference between the rate of lymph node involvement detected in the preoperative CT scan and the rate of lymph node involvement seen in the pathology results of the combined therapy group (p=0, 375). However, in the monotherapy group, the rate of lymph node involvement in the pathology results was statistically significantly higher than in the preoperative CT scan (p=0.039) (Table 4).

The patients' median follow-up period was 49.5 months in the combined therapy group. In comparison, the median was 34.5 months in the monotherapy group. When the patients were assessed for risk of biochemical recurrence, PSA recurrence was encountered in 11 (78.6%) patients receiving combined therapy during the study period. In contrast, when the monotherapy group was examined, PSA recurrence was seen in 25 (89.3%) patients. No statistically significant difference was found between these two groups regarding the risk of PSA relapse (p=0.383) (Table 5). When patients were examined regarding BRFS, the median time in the group receiving combined therapy was 18 months (7–95). When the data of the monotherapy group were evaluated, the median BRFS was seven months (2–162). When the two groups were compared, a significant difference was found in favor of the combined therapy group regarding BRFS (p=0.040).

When survival analyses were performed, 36 (85.7%) of the patients included in the study were alive. Two (14.29%) of 14 patients in the combined therapy group lost their lives due to prostate cancer progression. In comparison, one patient (7.14%) died due to a second primary cancer that developed within the follow-up period. In the monotherapy group, 3 (10.71%) patients died because of prostate cancer progression. No statistically significant difference was found between

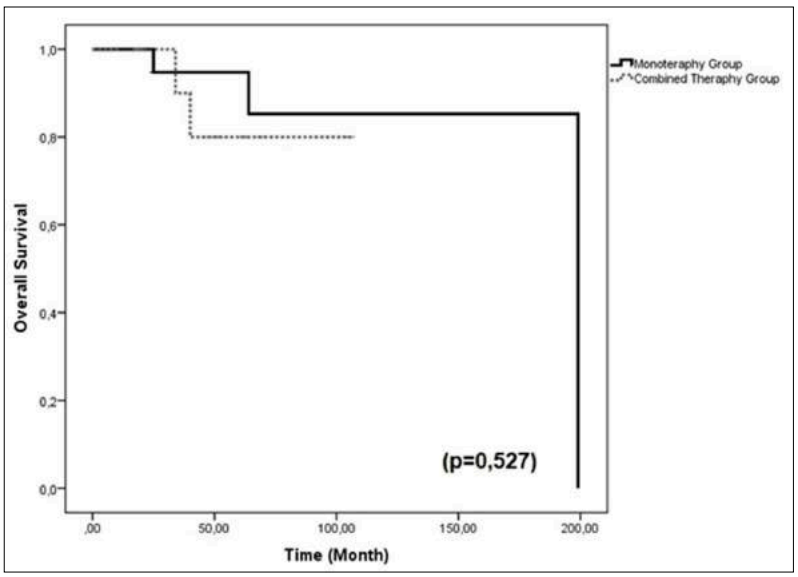

Figure 1. *Overall survival comparison of the two groups.*

the two groups when the patients in both groups were compared in terms of survival (p=0.527) (Fig. 1).

Discussion

No consensus exists on a standardized treatment currently recommended for high-risk prostate cancer patients[3,10]. In patients with high-risk PCs, local treatment alone is insufficient because of possible micrometastases that imaging methods cannot detect at diagnosis. Local therapies should be combined with systematic treatments to delay the biochemical recurrence and improve survival[11]. It can be suggested that neo-adjuvant therapies are beneficial for issues such as early treatment of micrometastatic disease compared to adjuvant treatments, less surgical margin positivity due to possible reduction in tumor size, and understanding of the molecular mechanisms of the disease by examining the effects on tumor tissue[8].

Adverse events related to NADC administration were evaluated according to the "Common Terminology Criteria for Adverse Events v4.0" criteria. Grade 3 and 4 side effects have been reported in studies in the literature; none of the patients included in our study had side effects at grade 3 and above, and no dose reduction was required in any patient[7,12,13].

In a study conducted by Nosov et al., 52.4% of patients showed a reduction of PSA by more than 50%. The median PSA value of 29.8 ng/mL before treatment was reported to be 13.4 ng/mL in the post-treatment group[12]. However, it was also noted that the serum PSA level of a patient in the same study increased from 27 ng/mL to 67 ng/mL after NADC. Similarly, Klein and colleagues reported that 21 of 28 patients had a total decrease in serum PSA levels of between 9 and 79%, and seven patients had a 2–18% increase[13]. However, our study found no statistically significant change between the median PSA values (ng/mL) monitored over four cycles. A study evaluating PSA changes during the application of a group of chemotherapeutic agents, including docetaxel, indicated that there might be significant fluctuations in the PSA value regardless of whether the tumor benefited the CT or even an increase in PSA values during the first 8-week period could occur, which should not be interpreted as 'treatment was not useful' [14].

In the Nosov et al. series, there was no difference between the combined and monotherapy groups regarding the average operation time, the average amount of bleeding, and the average length of postoperative hospital stay[12]. Similarly, there were no differences in median operative times, median bleeding volumes, blood transfusion requirements, and length of postoperative hospital stay for patients enrolled in this study.

Regarding complications, in Febbo et al.'s series, bleeding requiring blood transfusion in 3 patients and pulmonary embolism in one patient were reported[7]. Nosov and colleagues reported that the periprostatic area underwent moderate fibrosis in the NADC group, but the resectability was generally not affected; one patient in the monotherapy group had a rectal injury, and one

patient in the NADC group experienced a major vessel injury. In both groups, postoperative pelvic hematoma was reported in one patient and prolonged lymphatic drainage in two patients[12]. When the patients included in our study were examined, there were no significant differences between the two groups regarding complications. Based on these data, it can be argued that NADC did not increase the intraoperative complication rates.

In the study of Nosov et al., no pathologic response was observed in any of the patients. In contrast, one patient in the monotherapy group and three in the combined therapy group showed a reduction in Gleason score. However, the two groups' total Gleason scores were similar[12]. Likewise, there was no significant difference in the ISUP scores of patients' biopsy and operative specimens in the combined therapy and monotherapy groups. In light of these data, it was assumed that NADC had no significant effect on ISUP and Gleason scores.

Considering the computed tomography's low sensitivity of lymph node involvement (LNI) detection in the literature, a significant difference is expected between the number of LNIs detected at the time of diagnosis and the LNI detected in the surgical specimen[15,16]. Lymph node involvement in imaging studies and LNI in the pathology specimen were investigated separately at the time of diagnosis for both groups. In comparing these two, there was a significant difference in the monotherapy as expected. However, no significant statistical difference was found in the combined therapy group. Evidence may not be sufficient, but this may indicate that NADC may effectively reduce LNI.

No significant difference in PSA recurrence was found between our study's two groups during the follow-up period. However, when patients were examined regarding BRFS, a statistically significant difference was found in favor of the combination therapy group for approximately ten months. These data suggest that NADC does not reduce the risk of BCR in patients with high-risk PC but delays the onset of BCR and improves BRFS. A study by Zhao et al., published in 2015, found that BFR was 33.5%, PS was 79.7%, and cancer-specific survival was 92.2% for ten years. The authors interpreted that NADC could potentially lead to a survival advantage in potentially high-risk patients[11]. Our study found no statistically significant difference between patients in both groups when assessed for survival.

Since our research is retrospective, there are various limitations. The sample size was limited to 14 patients in the combined therapy group because the patient sample began by screening patients who accepted to receive NADC retrospectively among high-risk PC cases applied to the Hacettepe University Hospital Urology Clinic. Although performing a pair match was attempted at the beginning of the study, this difference could not be avoided because of the study's retrospective nature and because patients who were given NADC were younger than those who were not. However, the fact that the patient's age is not among the factors determining the prognosis of the PC cases minimizes the poor impact of this difference on the quality of work[10,17–21]. Some patients' information existed before the hospital system was switched entirely to the computerized electronic registration system. Because most of the patients' files were unavailable, the physical examination information noted in the patients' files during this period was unavailable, so the clinical stages of the diagnosis were unavailable during the analyses. Information on late complications, such as erectile dysfunction, incontinence, and urethral stricture, noted in the files during the patient's controls, was unavailable.

Conclusion

Although high-risk PC is a significant public health issue due to its high mortality, morbidity, and economic burden, a standardized treatment method has not yet been defined. The data obtained from these studies suggest that multimodal treatment options should be used to combine systemic treatment for local disease and possible micrometastases not detected at diagnosis. In high-risk PC patients, the ineffectiveness of neo-adjuvant androgen deprivation therapy (NADT) in combination with RP suggests the presence of castration-resistant cell clones that exist at the time of the diagnosis and brings up treatment options that could be effective on castration-resistant clones as systemic treatments. As a neo-adjuvant treatment, combining docetaxel chemotherapy with RP can be beneficial.

It has been determined that NADC, which is generally well tolerated by patients, has potential positive effects on LNI and prolongs BRFS. Extended follow-up randomized controlled trials with broad sampling on this topic will be more helpful in revealing the impact of NADC before RP in high-risk PC cases.

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DERLEME / *REVIEW*

Cyclospora cayetanensis Infection

Cyclospora cayetanensis Enfeksiyonu

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ABSTRACT

Foodborne infections cause significant health problems worldwide. Among the causative agents of these infections, bacteria and viruses are more common, but there are also some protozoans. Among these protozoa, the infection caused by Cyclospora cayetanensis, which has been widely researched recently, is thought to be quite common in our country. Water and food contaminated with human feces are the main reservoirs of the agent. The agent is transmitted by fecal-oral route. Inattentions in eating habits, wrong practices, consumption of undercooked or raw foods, and contaminated drinking and utility water lead to infection. The infection affects the digestive system, and patients mainly suffer from diarrhea. C. cayetanensis symptoms are more severe in patients with HIV/AIDS. The infection is widespread in areas with poor hygiene and sanitation. Improving the hygiene conditions in these areas, washing vegetables and fruits well, filtering the water used for washing and drinking, and paying attention to general hygiene rules, especially hand hygiene, of the personnel working in the food production line significantly reduces the transmission and prevalence of infection.

Key words: *food hygiene; Cyclospora cayetanensis; food infection; protozoan*

ÖZET

Gıda kaynaklı enfeksiyonlar dünya çapında büyük sağlık sorunlarına neden olmaktadır. Bu enfeksiyonlara neden olan etkenler arasında bakteriler, virüsler daha çok sayıda olmakla birlikte bazı protozoonlar da bulunmaktadır. Bu protozoonlar içinde son zamanlarda çok araştırılan Cyclospora cayetanensis'in neden olduğu enfeksiyonun ülkemizde de oldukça yaygın olduğu düşünülmektedir. İnsan dışkısı ile kontamine olmuş su ve gıdalar etkenin başlıca rezervuarlarıdır. Etken fekal oral yolla bulaşır. Beslenme alışkanlıklarındaki dikkatsizlikler, yanlış uygulamalar, az pişmiş veya çiğ gıdaların tüketimi, kontamine içme ve kullanma suları enfeksiyonu ortaya çıkarmaktadır. Enfeksiyon sindirim sisteminde etkili olup, hastalarda daha çok ishal görülmektedir. C. cayetanensis semptomları HIV/AIDS'li hastalarda daha şiddetlidir. Enfeksiyon hijyen sanitasyonun yetersiz olduğu bölgelerde çok yaygındır. Koruma ve kontrolde ise bu bölgelerdeki hijyen koşullarının iyileştirilmesi, sebze ve meyvelerin iyi yıkanması, yıkama ve içmede kullanılan suların filtre edilmesi, gıda üretim hattında çalışan personelin başta el hijyeni olmak üzere genel hijyen kurallarına dikkat etmesi bulaşmayı ve enfeksiyon yaygınlığını büyük oranda azaltmaktadır.

Anahtar kelimeler: *gıda hijyeni; Cyclospora cayetanensis; gıda enfeksiyonu; protozoon*

Introduction

Foodborne infections constitute a significant health problem worldwide. WHO reports that one in three people is affected by yearly foodborne infections. Viruses, bacteria, and protozoa cause these infections and can infect foods at any stage, from farm to fork[1].

Among the factors that cause food infections are bacteria such as *Salmonella, Campylobacter, and Escherichia,* viruses such as *Norovirus, Hepatitis A virus, Rotavirus*, etc., and protozoans such as *Toxoplasma, Cryptosporidia* and *Cyclospora* are the most important[2].

Although many protozoan diseases have been known for a long time, new infective types are detected daily. *Toxoplasma, Babesia, Cryptosporidium, Eimeria, Theileria, Sarcocystis, Cyclospora* etc. They are essential protozoan species known to date regarding human and animal health[3]. Most of these protozoa are intracellular parasites and reproduce by sporulation[4].

Transmission of protozoa to humans occurs through contact with carrier animals or oral ingestion of infective cysts in the external environment. Water and soil play essential roles as intermediary sources in transmission[5].

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C. cayetanensis is one of the most researched parasites in recent years. Research has also shown that *C. cayetanensis* is a crucial foodborne protozoan that threatens public health worldwide[6]. *Cyclospora* infections are more prevalent in underdeveloped countries, particularly in Asia. The disease has been identified as the cause of 'Cyclosporiasis,' which has coccidian features in the gastrointestinal tract in humans. This disease, characterized by diarrhea, is also known as summer diarrhea or traveler's diarrhea in tropical regions [7].

History

Cyclospora protozoon was first discovered in the intestines of moles by Eimeria in 1870[8]. Its genus was defined as *Cyclospora* by the German scientist Schneider in 1881. Species of *Cyclospora* were first identified in insects, reptiles, and rodents rather than humans[9]. Until this parasite was named, it was called many different names, such as blue-green alga, Cryptosporidium-like, Cyanobacterium-like body or Coccidian-like body[10]. The first disease caused by *C. cayetanensis*, which is responsible for gastrointestinal disorders with these names, is thought to be the outbreak in Papua New Guinea in 1970[11]. After this protozoan was first isolated in Peru in 1994, its species name, *C. cayetanensis*, started to be used [9].

Morphology and life cycle of the parasite

C. cayetanensis taxonomically belongs to the subclass Coccidia of the Apicomplexa branch of the Eimeriidae family of the order Eucoccidiorida[10]. Oocyst, sporozoite, trophozoite, merozoite, microgametocyte, and macrogametocyte are the morphological shapes seen in the life cycle[11]**.** The oocysts of *C. cayetanensis* are 8–10 μm in diameter and spherical, and the cyst wall is colorless, double-layered, and thinner than 1 μm. The oocysts can sporulate in the external environment. A sporulated oocyst consists of two sporocysts, and each sporocyst consists of two sporozoites. Sporocysts are 4 × 6 μm in diameter and oval in shape. The sporozoites inside are banana-shaped, and their length varies between 1 × 9 μm[13]. *Cyclospora* sporozoites have a typical structure consisting of a polar ring, conoid, and rhopti. In the host, the protozoan reproduce sexually (gametogony) and asexually (merogony or schizogony)[14].

The life cycle of *C. cayetanensis* has not yet been fully described. Still, the sporulation process of the oocyst is well-known. The oocyst, formed in the parasite's intestinal cells, sporulates between 1–7 days at 22°C – 30°C outdoors after excretion in feces[16].

The season is another important factor affecting the oocyst's sporulation process and contamination. Environmental factors such as temperature and humidity are the most critical parameters of sporulation as they affect the viability of the oocyst[17]. Seasons worldwide vary depending on geographical location; for example, *C. cayetanensis* infection is more common between May and July in the USA and between April and June in Peru[18].

Human infection occurs through ingesting sporulated oocysts in contaminated food and water. Oocysts taken into the body become sporozoites due to the disintegration of the oocyst walls under the influence of bile, trypsin, and other factors in the gastrointestinal tract. These sporozoites settle in the epithelial cells of the small intestine (especially the jejunum) and form an intracellular life cycle there[9]. Symptoms appear in the host after incubating from 1 to 15 days (Figure 1).

Ways and sources of transmission of the parasite

Oocysts of *C. cayetanensis* are excreted in an unsporulated form from an infected body. These oocysts need sporulation in the external environment to gain infective properties[19]. Transmission is not possible through direct human-to-human contact; transmission occurs indirectly[20]. The disease occurs via the fecal-oral route, meaning people become infected by orally ingesting sporulated oocysts[16]. These oocysts may come from soil, fomites, or contaminated food and water[21]. Oocysts are durable in the external environment (in food and water) for a long time and do not lose their infective properties[6].

Indirect transmission of *C. cayetanensis* from person to person is relatively high. However, due to the sporulation process outside, environments are needed to create infection. It is caused by water, soil, food, and people contaminated with feces containing sporulated oocysts[22]. The main intermediary sources in the meeting are as follows.

Water

Water contaminated with feces is an important source of transmission of *C. cayetanensis* into the body[23]. There have been many reports of waterborne transmission of *C. cayetanensis*[24]. Oocysts are most commonly found in water used for agriculture[25]. Consuming untreated water and swimming in stagnant waters such as rivers and lakes are essential sources of infection[26].

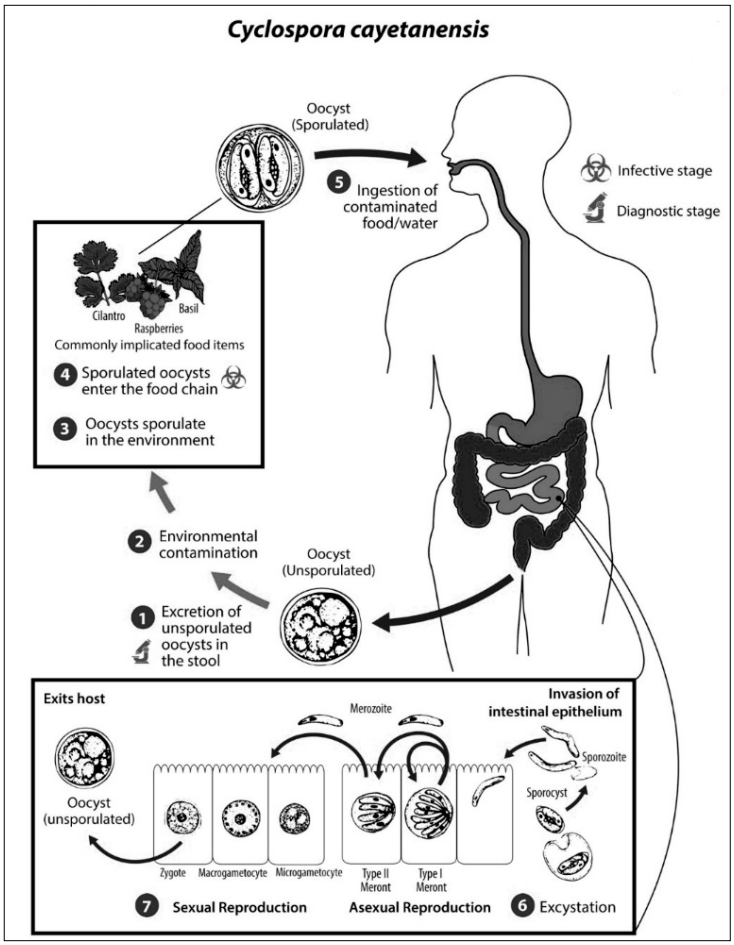

Figure 1. *Life discussion of C. cayetanensis[66].*

C. cayetanensis is a biological indicator to determine fecal contamination in Asian aquaculture waters. It shows whether the feces mixed with water are of human or animal origin[19].

Soil

Soil is one of the important sources of contamination and infection for *C. cayetanensis*[20]. Studies have shown that *contact with soil from C. cayetanensis* is a risk factor for infections in developing and developed countries[27]. The fact that vegetables and fruits are in contact with the soil during the growing process or irrigation paves the way for contamination[28]. In Venezuela, where most Cyclospora cases are associated with inadequate sanitation facilities, infections commonly occur due to soil contamination with feces. Consequently, there is an observed relationship between socioeconomic status and infection[29].

Person

C. cayetanensis is a species that has only been detected in humans so far. Although 17 different species of *Cyclospora* have been identified in many living things, none of them have the same characteristics as the *C. cayetanensis* species isolated from humans[22]. Therefore, *C. cayetanensis* was considered anthroponotic[30,31].

C. cayetanensis is prevalent in regions where agriculture is widespread, and handwashing is not a common practice[23]. People engaged in earthworks contaminate oocysts into foods during harvest time[32]. Once more, personnel working in the food production line are likely to transmit *C. cayetanensis* if they do not adhere to necessary personal hygiene rules [33].

Food

Carelessness or wrong practices in eating habits, such as consumption of undercooked or raw foods, increase parasitic infections[34]. Studies have reported that

Table 1. *C. cayetanensis epidemics in the world (2000–2015)63*

COUNTRIES	YEAR	NUMBER OF CASES	SOURCE OF INFECTION	ORIGIN OF INFECTION
ABD	2000–2015	2184	Peas, basil, coriander, lettuce, indeterminate	Guatemala, Mexico, Uncertain
Germany	2000	34	Salad, herbs	France, Italy, Germany
Australia	2010	266	Lettuce	Malaysia
United Kingdom	2015	79	Raspberry cake	Mexican
Indonesia	2001	14	Uncertain	Uncertain
Spain	2003	11	Raspberry juice	Guatemala
Sweden	2009	18	Pea	Guatemala
Canada	2001–2015	451	Thai basil, coriander, basil, garlic, indeterminate	ABD, Mexico, Uncertain
Colombia	2002	31	Salad, juice	Uncertain
Mexican	2001	97	watercress	Uncertain
Peruvian	2004–2005	164	Uncertain	Uncertain
Poland	2013	3	Drinking water	Indonesia
Türkiye	2005–2007	305	Drinking water, undetermined	Uncertain

outbreaks often occur following the consumption of freshwater oysters in Asia, revealing that 48% of these oysters harbor Cyclospora oocysts for up to 13 days [35]. Another transmission source is the consumption of fresh fruits and vegetables that are difficult to clean and contaminated with oocysts without washing[36]. These foods include raspberries, blackberries, greens, basil, coriander, and salad products[37]. Moreover, the incomplete removal of oocysts during food washing remains a significant source of infection [36].

Prevalence of C. cayetanensis cases in the world

Global research shows that cyclosporiasis occurs mainly in tropical and subtropical regions. The protozoan was first detected in endemic areas such as Haiti, Guatemala, Peru, and Nepal. It is more common in Central and South America and South East Asia, including India, Nepal, and Indonesia. The Middle Eastern countries, where our country is situated, are also among the regions where the disease is observed[38,39].

The first documented epidemic took place in Chicago in 1990, affecting 21 individuals. The outbreak was suspected to be linked to a broken water pump or food consumed at a party, yet the exact source could not be determined[40]. Two outbreaks were reported in 1995, affecting 32 people in New York and 38 in Florida. In the three years following these outbreaks, infections caused by *C. cayetanensis* were detected in an additional two thousand people[37].

The first case of *C. cayetanensis* in Türkiye was reported in 1998 in a patient with AIDS[41]. *C. cayetanensis* was detected in two people who returned from Türkiye to France in 2004 and five people in 2005[12]. Our country's first waterborne cyclosporiasis epidemic was reported in Izmir in 2007. *C. cayetanensis* was detected in two out of 554 individuals who visited the hospital with diarrhea[42]. In 2008, *C. cayetanensis* was detected in one of 138 children between the ages of 2 and 6 who were brought to the Maternity and Pediatrics Hospital in Kars with gastrointestinal complaints[43]. In 2012, *C. cayetanensis* was detected in 13 of 75 people with diarrhea in Diyarbakir[44]. According to these results, *C. cayetanensis* is our country's second most common protozoan. The global *C. cayetanensis* epidemics in recent years are presented in Table 1.

Symptoms

Symptoms appear 1 to 7 days after the *C. cayetanensis* oocyst is ingested and settles in the small intestine. These symptoms start as malaise[19,45]. In cyclosporiasis infection, defecation an average of 7 times daily is pathognomonic for the disease. It is characterized by symptoms of watery diarrhea, abdominal bloating, cramps, nausea, low-grade fever, fatigue, and weight loss. It is seen as severe, long-lasting, or chronic diarrhea in immunocompromised or immunocompromised patients (AIDS, children, the elderly)[46].

Symptoms last approximately 3 to 4 days; if treatment is not started during this period, the process may last up to several weeks[45]. Cyclosporiasis symptoms are more severe in HIV/AIDS patients. While an average of 3.5 kg of weight is lost during the disease, this weight loss increases to approximately 7 kg in HIV-positive patients[47]. While the average duration of diarrhea was 57.2 days in HIV-negative patients, it was observed to last 199 days in HIV-positive patients[48]. In places where the disease is endemic, symptoms are

more asymptomatic[49]. Following a Cyclospora infection, Guillain-Barre syndrome,[50] and reactive arthritis syndrome (Reiter syndrome) may develop[51]. Cyclosporiasis, overall, has a low mortality rate[52]. However, if left untreated, infection can persist intermittently[22] (Table 1).

Diagnosis

Because *C. cayetanensis* is intracellular, diagnosing cases in routine analysis[53] is complex. In cases where cyclosporiasis is suspected, it is diagnosed only by microscopic examination and molecular diagnosis of stool samples performed by experts[54]. Microscopy examines the size and shape of *C. cayetanensis* in the feces and the presence of unsporulated oocysts. *C. cayetanensis* oocysts and *cryptosporidium* spp. since the oocysts are close in size, they are likely to be confused. For a definitive diagnosis, waiting for the oocysts to sporulate[39] is necessary. The samples taken are incubated at 23–30°C for 7 to 15 days, and oocysts are examined after sporulation has occurred[9].

The sampling step is vital in diagnosis. Due to the low number of oocysts in the feces, the multiplicative method must be employed[40]. Formol ethyl acetate or Shether's sugar flotation method is used for the bulking method[55]. The sample is preserved in 2.5% potassium dichromate for PCR analysis and 10% formalin for microscopic diagnosis and staining. Samples can be frozen for long periods for diagnosis[54,56].

Samples taken from the stool are stained with acid-resistant dyes (Modified Ziehl-Neelsen (hot method) or Kinyoun (cold method)) and examined with a light microscope[57]. *Cyclospora* oocysts are seen under the microscope as 8–10 μm in size and spherical, resembling a morula in the middle. In its differential diagnosis, *C. cayetanensis* is examined with a fluorescence microscope using its autofluorescence feature. Here, *C. cayetanensis* oocysts are seen as light blue at 330–380 nm in size or green at 450–490 nm in size[58].

The identification of *C. cayetanensis* in food is made by microscopic examination of swabs taken into the food[59]. If multiplication is necessary, formalin-ether precipitation or sucrose flotation techniques increase the amount of oocysts. It is easily detected under the microscope by observing its spherical structures of 8–10 μm in size[60]. Environmental Protection Agency (EPA) techniques 1622 and 1623.1, employed in the USA to detect cryptosporidium oocysts in waters, are also used for *Cyclospora* oocysts[39].

Treatment

The use of Trimethoprim-Sulfametaxazole (TMP-SMX) is recommended in the treatment of cyclosporiasis. Studies found that a 5–25 mg/kg dose stopped oocyst excretion in children after three days of treatment[61].

Prevention and Control

To ensure the protection and control of *C. cayetanensis*, the parasite's life cycle, the disease's epidemiology, hygiene conditions, and the climate of the environment where the agent is found must be taken into account. The factor is generally associated with regions with poor hygiene and economic conditions. Suppose the general hygienic conditions in these regions are improved. In that case, the burden of water and food contaminated with feces can be reduced[39]. Good agricultural practices should be implemented to prevent cyclosporiasis. The aim here is to reduce the role of protozoa in transmission. The purification and use of water in agricultural practices reduce the occurrence of the disease[46]. Water should be filtered to be protected from contaminated water, and irrigation should be carried out by providing filtration[62]. It is important to pass shellfish from where they are grown to clean waters before catching/collecting them and to subject them to heat treatment to reduce microbial contamination[63]. If hygienically questionable water is used to clean fresh vegetables and fruits, the water used should be boiled or filtered[64]. Another cleaning method before consuming fresh vegetables and fruits is to disinfect them. Vegetables and fruits immersed in Sodium dichloroisocyanurate solution for disinfection have been reported to reduce the number of parasites, including *C. cayetanensis*[65]. It is treated with magnesium oxide nanoparticles to prevent sporulation of *C. cayetanensis* oocysts in water to be used in agricultural areas. This prevents sporulation and ensures that the water is safe. The hygienic status of people working in agricultural areas, which is an important factor in contamination, should be checked. If the farmer has any symptoms of gastroenteritis, he should not be allowed to come into contact with food. Preventing access to contaminated water during personal hygiene, practicing thorough handwashing, and ensuring proper sewage disposal are vital measures to prevent *C. cayetanensis* infection. Compliance with general hygiene rules has an essential place in preventing the disease. There is no vaccine yet for *C. cayetanensis*[64].

Conclusion

C. cayetanensis is a protozoan that infects humans through food. What makes this protozoan significant is its presence in the foods we consume daily and in water, which is a crucial element in our lives. Infection occurs via the fecal-oral route. These oocysts ingested through food cause chronic diarrhea in humans. It causes long-lasting health problems for public health. Prevention is to wash vegetables and fruits and remove soil residue. However, the water should be boiled or filtered while washing with appropriate filtration systems due to the possibility of water contamination. Drinking water must be used after it has been purified. It is recommended that the person affected by the infection receive the necessary training and comply with personal hygiene rules.

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OLGU SUNUMU / *CASE REPORT*

A Review on Differential Diagnosis and Diagnostic Criteria of Complement-Mediated Thrombotic Microangiopathy with a PLASMIC Score Below Six and Coexisting Hepatitis B Positivity in a Male Patient

PLASMIC Skoru Altı Olan ve Hepatit B Pozitifliğinin Eşlik Ettiği Kompleman Aracılı Trombotik Mikroanjiyopati Saptanan Bir Erkek Hasta: Ayırıcı Tanı ve Tanı Kriterleri Üzerine Bir İnceleme

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ABSTRACT

Microangiopathic Hemolytic Anemia (MAHA); Congenital Thrombotic thrombocytopenic purpura (TTP), Acquired (Immune) TTP, Shiga toxin associated Endemic hemolytic uremic syndrome (HUS) and Complement-Mediated TMA (CM-TMA), which may present with different clinical findings, Thrombocytopenia is a severe condition that affects multiple organ systems with anemia. In the congenital form, ADAMTS13 (von Willebrand Factor-Cleaving Protease or a metalloprotease that belongs to the "α disintegrin and metalloprotease with a thrombospondin type I motif) is diagnosed by the deficiency of the enzyme and the absence of antibodies. While in autoimmune TTP, the enzyme deficiency is associated with antibodies, endemic HUS associated with Shiga toxin is characterized by decreased ADAMTS13 levels due to endothelial damage. CM-TMA is associated with complement factor H (CFH) inhibitory dysfunction and increased complement levels due to a genetic mutation. On March 27, 2023, the patient with complaints of shortness of breath, headache, dizziness, weakness, and numbness in the hands and arms was admitted to the internal medicine clinic.

The patient, presenting with Thrombocytopenia, reduced haptoglobin levels, elevated reticulocyte count, increased LDH, indirect hyperbilirubinemia, and a PLASMIC score of 6 in peripheral blood smear, was hospitalized and treated with the prediagnosis of TTP. Later, ADAMTS13 level was found to be 73% (normal range: 40–130), and the diagnosis of CM-TMA was considered. In addition, we discussed the clinical distinction and treatment of TTP by reviewing the literature.

Keywords: *microangiopathic hemolytic anemia, thrombotic thrombocytopenic purpura, complement-related thrombotic thrombocytopenic purpura, ADAMTS13 protein, schistocyte, plasmic score*

ÖZET

Mikroanjiyopatik Hemolitik Anemi (MAHA); Konjenital Trombotik Trombositopenik Purpura (TTP), Edinsel (Otoimmün) TTP, shiga toksin ile ilişkili Endemik hemolitik üremik sendrom (HÜS) ve Complement-Mediated TMA (CM-TMA) olarak farklı klinik bulgular ile ortaya çıkabilen, trombositopeni, anemi ile seyreden birden çok organ sistemini etkileyen ciddi bir tablodur. Konjentinal formunda ADAMTS13 (Von Willebrand Faktör Ayırıcı Proteaz veya disintegrin'e ait bir metaloproteaz ve trombospondin tip I motifli metalloproteaz) olarak adlandırılan enzimin eksikliği ve antikor yokluğu ile teşhis edilirken, otoimmün TTP de ise enzim eksikliği antikorların varlığıyla ilişkilidir. Shiga toksini ile ilişkili endemik HÜS ise oluşan endotel hasarı sonucunda ADAMTS13 düzeylerinin azalması ile karekterezidir. CM-TMA ise bir genetik mutasyon sonucunda Complement Factor H (CFH) inhibitör fonksiyon bozukluğu ve kompleman düzeylerinin artması ile ilişkilidir. 27 mart 2023 tarihinde nefes darlığı, baş ağrısı, baş dönmesi, halsizlik, el ve kollarda uyuşma şikâyetleri olan 40 yaşındaki erkek hasta iç hastalıkları kliniğine yatırıldı.

Trombositopeni, haptoglobulin düşüklüğü, retikülositte artma, LDH yüksekliği, endirekt hiperbillüribinemi ve çevre kan yaymasında şistositleri görülen ve PLASMIC skoru 6 olan hasta TTP ön tanısı ile hastaneye yatırılarak tedavisi düzenlendi. Daha sonra ADAMTS13 düzeyi %73 (normal aralık: 40–130) saptanan hastada CM-TMA tanısı düşünüldü. Bununla beraber literatür taranarak TTP'nın klinik ayrımı ve tedavisine değindik

Anahtar kelimeler: *mikroanjiyopatik hemolitik anemi, trombotik trombositopenik purpura, kompleman-ilişkili trombotik trombositopenik purpura, ADAMTS13 protein, şistosit, plazmik skor*

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Introduction

Trombotik trombositopenik purpura is a disease that affects 1–5 people out of 1 million people in the population and has a high mortality rate. Antibodies against ADAMSTS13 constitute 2/3 of cases, either congenital or acquired and/or autoimmune. Trombotik trombositopenik purpura is a microangiopathy syndrome caused by congenital or acquired deficiency of ADAMTS13, which breaks down the von Willebrand factor (VWF). in the Table 1, either the function of ADAMSTS13 metalloprotease is impaired or its level decreases. Shiga toxin-mediated hemolytic uremic syndrome (ST-HUS), CM-TMA (Atypical HUS), drug-induced and other rare inherited TMA syndromes[1].

Therefore, to improve this situation, plasmapheresis (PLEX), fresh frozen plasma (FFP), and Rituximab (CD20 inhibitor) are started. Rituximab has a significant clinical effect as a salvage therapy for relapse. As a biosynthetic human-mouse chimeric monoclonal antibody, rituximab can reduce B lymphocytes with an abnormal immune response to the antigen[2]. In particular, volume load and blood-borne infectious agents should not be ignored in applying FFP. In the case of CM-TMA, this deadly disease can be prevented by administering eculizumab (C5 inhibitor)[3]. TTP, HUS, MAHA, and Thrombocytopenia are partially similar acute syndromes affecting multiple organ systems. Among the main diagnostic features, the most important is microangiopathic hemolytic anemia, characterized by non-immune hemolysis, negative direct Coombs test, and erythrocyte fragmentation in peripheral blood. As typical hemolysis findings, an Increase in serum indirect bilirubin and LDH levels (due to tissue damage and hemolysis) is observed. In the peripheral blood smear of patients with TTP-HUS syndrome, erythrocyte fragmentation is found in an average of 8% (between 1–18%) of erythrocytes. Trombotik trombositopenik purpura Pentad, MAHA, Thrombocytopenia, renal failure, fever, and neurological symptoms are found. The platelet count is around 25×109/l (5–120×109/). Neurological symptoms may be in the form of confusion or severe headache, as well as transient ischemic attack, seizure, and coma. Although fever cases are reported less frequently, when detected, it may indicate a septic Picture[4,5].

Case Presentation

A 40-year-old male patient, 85 kilos and 1.85 meters tall, applied to our internal medicine clinic with complaints of headache, dizziness, confusion, numbness in the hands and arms, and weakness. In his first examination, blood pressure was 110/70 mm/Hg; pulse was 88/min; fever was 38°C. The respiratory rate was 18/min, the head and neck examination was unremarkable, the respiratory and cardiovascular system examination was unremarkable, and no purpura or petechiae were found on his skin. The blood biochemistry was as follows: Lactate Dehydrogenase (LDH) 1345 U/L (0–248), ferritin 875 (24–336 ng/mL, for

Table 1. *The patient's biochemical, complete blood count, and some hormone analysis results*

Laboratory parameters and normally value	First day	Fifth day	After one week	After one month
ADAMTS13 level (40–130%)	73%	(4 months after treatment)		
Leukocyte (3700–10400 cells/microL)	8100	7012	7482	8500
Neutrophil (40–75%)	51	74	76	70
Lymphocytes (16–49%)	35	23	14.9	23.8
Hemoglobin (HGB)(g/dL)	**6.4**	**9.1**	**9.9**	**14.2**
MCV (78–98 fL)	**100**	**107**	**110**	**98**
Creatinine (0.67–1.17 mg/dL)	**1.35**	**0.80**	**0.82**	**0.83**
PLT (149–371×10^3 cells/microL)	**10×10^3**	**158×10^3**	**168×10^3**	**204×10^3**
Ferritin (24–336 ng/mL, for man)	**875**	**708**	**555**	**275**
CRP (0–0.5 mg/dL)	**0.25**	**0.24**	**0.23**	**0.52**
D-Dimer (0–500 ng/ml)	**6142**	**1415**	**440**	
INR (0.8–1, 24)	**1.02**	**1.12**	**1.14**	**0.99**
Fibrinogen (180–450 mg/dL)	354	225	235	180
LDH (135–225 u/L)	**1345**	**390**	**335**	**360**
Albumen (3.5–5.2 g/dL)	**3.6**	**3.82**	**3.7**	**4**
AST (0–40 u/L)	40	50	42	18
ALT (0–41 u/L)	40	135	85	32
ALP (40–130 u/L)	110	92	75	55
GGT (8–61 u/L)	160	210	225	185
Indirect billirubin	**1.45**	-	-	**0.28**
Total billirubin (0,3–1,2),	**1.80**	-	-	**0.45**
Prothrombin time (PT)	12	13	14	13
Activated partial thromboplastin time (a-PTT) (%)	42	25	22	23
Vitamin B12	300	-	-	350
Folic acid	7	-	-	>20
Thyroid-stimulating hormone (TSH)	1.5	-	-	-
Parathormone (PTH)	120	-	-	-
25-hydroxyvitamin D	10	-	-	-
Direct Coombs	**negatif**	-	-	-
Haptoglobin (0,3–2) g/L	**0.01**	**0.02**	-	**0.04**
Retuculocyte (0.52–3.53%) (Corrected)	**12**	**10**	-	**9.8**
K^+	3.5	3.8	4.2	4.5
Na^+	135	136	138	140
Ca^{+2}	7.6	8.2	8.7	9

Table 2. *Some serological analysis results of the patient*

Anti-HIV	0.25
HBsAg	3.8
HBeAg	0.910(0.9–1.1)
Anti HBS	167
Anti HCV	0.042
Brucella Aglütinasyon (Rose Bengal	negative
Brucella Aglütinasyon (With Coombs Anti Serum)	negative
SARS-COV-2	pozitive
Parvovirüs B19 (IgG, IgM)	negative
EBV	negative
VDRL-RPR	negative
Adenoviruses	negative
Anti-ds-DNA	9.5(0–25)
Anti-SSA	1.6(0–25)
Anti-SSB	3(0–25)
Anti-Scl-70	4(0–25)
Anti-JO-1	2(0–25)
Anti-CCP (cyclic citrullinated peptide)	9(0–20)
ANA (Antinuclear antibodies)	negative

man), d-dimer 6142(0–500 ng/ml), total bilirubin 1.80 mg/dL (0.3–1.2), indirect bilirubin 1.45 mg/dL, C-Reactive Protein (CRP) 0.25 mg/L (0–0,5), albumin 3.6 g/L (3,5–5,2), Aspartate Transaminase (AST) 40 u/L, Alanine Transaminase (ALT) 40 u/L, alkaline phosphatase (ALP) 110 (40–129 u/L), gamma-glutamyl transpeptidase (GGT) 160 (8–61 u/L), WBC 8100 cells/microL, neutrophil 51%, lymphocyte 35%, platelet count 10×103 (149–371×103 cells/microL), MCV 100 fL, INR 1, 02, pH: 7.43, SO_2: 96% HCO_3:21 mmol/L, $P0_2$: 62.4 mmHg and PCO_2: 31.5. The patient had no previous history of hematological disease. Diseases such as erythrocyte membrane disorders (such as hereditary spherocytosis), hemoglobinopathies (sickle cell or thalassemias), G6PD or (pyruvate kinase enzyme deficiencies) were excluded according to clinical, laboratory, and environmental blood analysis findings. Prothrombin time, activated partial thromboplastin time, and fibrinogen were within normal limits, and the direct Coombs test was negative. Peripheral blood smear showed spherocytic, polychromasic, and 8–10 fragmented erythrocytes (schistocytes) at each magnification (Figure 1). The brain, thorax, abdomen computerized tomography, and pulmonary angiography were unremarkable. No known organ transplantation or solid organ malignancy was detected in our patient. The patient's PLASMIC score was computed after the observation of a high level of schistocytes[3].

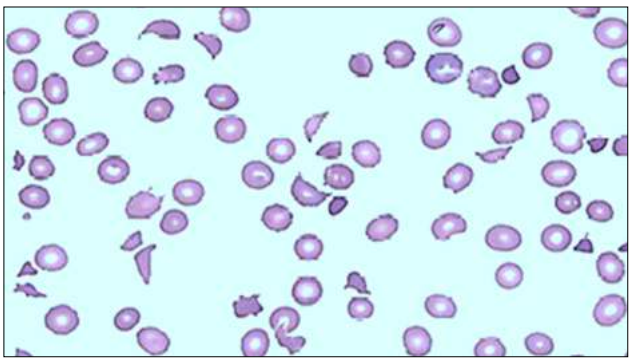

Figure 1. *Schistocytes in peripheral smear.*

Platelet count: 11×103 cells/microL (1 point if <30×103)

Haptoglobin: 0.03 g/L (1 point if <0.30–2 g/L)

[Other indicators of hemolysis Reticulocyte count (%) >2.5%; or Indirect bilirubin >2.0 mg/dL >34 mcmol/L],

If there is no active cancer: (1 point),

If there is no history of solid organ or hematopoietic stem cell transplant (1 point),

INR <1.5 (1 point),

Creatinine <2.0 mg/dL (1 point),

MCV <90 (0 point)

Our patient, who had a total of 6 points, was diagnosed with TTP. Serum samples were taken for ADAMTS13 and anti-ADAMTS13 IgG antibody levels, and the patient was started on 1 mg/kg prednisolone and 20 ml/kg/day (1600 ml) FFP. The next day, as the patient's values did not change, PLEX was planned, and he was referred to the hematology department. Plasmapheresis 50 ml/kg was administered there for three consecutive days, then five more times every other day. The prednisolone of the patient who was administered PLEX was also continued throughout this period and was tapered off. In addition, 0.5 mg Entacavir was started in the patient who was started on rituximab at 375 mg/m^2 per week (for four weeks) during this period and because he was HBsAg positive, HBeAg negative, and Anti-HBc positive (Table 2). On the 5th day of the treatment, PLT: 158×103, Hemoglobin: 9.1 g/dL, and LDH: 390 u/L levels, on the 30th day it was 204×103, 14.2, 362, respectively. The ADAMTS13 level obtained four months after the treatment was normal and was 73%. Currently using only 0.5 mg Entacavir, the patient was referred to the hematology clinic for eculizumab treatment planning.

Discussion

TMA refers to a pathological lesion seen on tissue biopsy. The presence of TMA is evident from clinical features such as MAHA and Thrombocytopenia with signs of organ damage. The median age in immune TTP is 40, which can be seen between the ages of 9–78. Its incidence is approximately 3 per million per year[6]. Although immune TTP is 30 times higher in adults, it can be seen in hereditary TTP. However, when TTP is suspected in the first pregnancy in an adult, hereditary TTP should not be forgotten[7]. In addition, TTP can be seen in autoimmune diseases such as SLE, and SLE is detected in 10% of immune TTP cases[8]. It has been suggested in previous studies that TTP cases in pregnant women are incorrectly confused with eclampsia and severe HELLP. Thrombotic thrombocytopenic purpura was more common in obese people, especially those with BMI >40 and women[6,9,10]. Thrombotic thrombocytopenic purpura may be immune-mediated due to autoantibodies against ADAMTS13 (anti-ADAMTS13 IgG antibodies) or inherited due to pathogenic variants in the ADAMTS13 gene. It is characterized by Thrombocytopenia, microangiopathic hemolytic anemia, and thrombocyte-rich thrombin in small vessels, sometimes causing organ damage. This condition causes Thrombocytopenia. Thrombotic thrombocytopenic purpura is an emergency that can be fatal if appropriate treatment is not started immediately. With proper treatment, more than 90 percent of patients survive[1]. Although TTP is a severe disease, some cases may present with nonspecific symptoms such as fatigue, dizziness, abdominal pain, nausea, and vomiting[11]. For this, a peripheral blood smear should be done first. If there is a schistocyte, it may be reasonable to calculate the PLASMIC score. PLASMIC score includes Platelet count <30.000/microL, Hemolysis (indirect bilirubin >2 mg/dL, reticulocyte count >2.5 percent or low levels of haptoglobin), absence of active malignancy, absence of bone marrow or solid organ transplantation, MCV <90 fL, INR <1.5, Creatinine <2.0 mg/dL. One point is given for each. If the total score is 4, the probability of TTP is low; if it is 5, it is likely TTP, and if it is 6–7 points, the likelihood of TTP is considered. Since the PLASMIC score is calculated after the presence of schistocytes is shown, it is not included in the scoring[12].

Calculated on a statistical probability, this scoring developed by Pavan Bendapudi and colleagues should be used cautiously. In TTP cases where patient mortality is high while waiting for ADAMTS13 level, using the PLASMIC score and starting treatment provides an advantage in terms of mortality. The 2020 systematic review confirmed the diagnostic accuracy of the PLASMIC score in patients with suspected TTP. The review identified 13 studies with a median TTP prevalence of 35 percent. A PLASMIC score of 5 or higher provided a sensitivity of 99 percent (95% CI 0.91–1.00) and a specificity of 57 percent (95% CI 0.41–0.72)[13]. ADAMTS13 level should not be considered alone for initiating or stopping treatment, as delay in diagnosis may increase the risk of mortality. However, the clinically diagnosed case can reveal the correct reason for the diagnosis[14]. Although ADAMTS13 activity in TTP is <10% in severe deficiency, ADAMTS13 may also be decreased in the presence of sepsis or systemic cancer. Intermediate values such as 10–59 may be seen in the presence of transfusion, sepsis, or systemic cancer. Normally, the activity should be >60%[15,16]. In immune TTP, deficient ADAMTS13 activity is caused by an inhibitör. A test for an inhibitor should be obtained for patients with severe deficiency. Inhibitor titer is determined from the number of serial dilutions of plasma after that inhibitor continues to inhibit ADAMTS13 activity. The titers are commonly presented in Bethesda units (the reciprocal of the dilution required to neutralize 50 % of the inhibitor). CM-TMA should be considered if diseases such as immune TTP, drug-related TTP, or SLE have been excluded in a patient as the test results will cause delay and threaten the patient's life. The ADAMTS13 level of our presented case was expected, and the patient was followed up in the hematology clinic for eculizumab treatment.

Conclusion

The peripheral smear should be performed in a patient with headache, dizziness, numbness in the arms, confusion, high fever, hemolytic anemia, Thrombocytopenia, high LDH, low haptoglobin, and increased reticulocytes. It is recommended to calculate the PLASMIC score to contribute to the diagnosis in a patient with a peripheral blood smear with schistocyte and normal levels of INR. Blood samples for ADAMTS13 and antibody levels should be obtained before starting treatment. Plasmapheresis should be applied if available. Steroids and rituximab should be given with PLEX. If PLEX is unavailable, the plasma infusion should be provided with steroids and rituximab. If CM-TMA is confirmed, eculizumab should be started.

Conflict of Interest Statement

All the authors declare no conflict of interest.

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EDİTÖRE MEKTUP / *LETTER TO THE EDITOR*

Dietary Therapies in Autism Spectrum Disorder

Otizm Spektrum Bozukluğunda Diyet Tedavileri

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To the Editor,

Neurodevelopmental disorders are a cluster of diagnoses, including many highly interrelated disorders. Many researchers have carried out studies to obtain descriptive data on the disorders in this diagnostic cluster on a provincial basis. As the Child and Adolescent Psychiatry Unit of Harakani State Hospital, we decided to design a study to examine the medical board applications in our province and, thus, to obtain detailed descriptive data on neurodevelopmental disorders. While reviewing the electronic records of patients who sought medical board during the study period, it was observed that most cases involving neurodevelopmental disorders reported to the board were males, consistent with existing literature[1]. The most common diagnosis was intellectual disability, followed by autism spectrum disorder (ASD). During the detailed examination of patient data, it was noted that a considerable number of ASD-diagnosed patients had not applied for an initial assessment, had previously received a medical report confirming ASD, and had been seeking treatment for an extended period. The prevalence of ASD is increasing in Türkiye as it is worldwide[1]. The lack of definitive treatment for the core symptoms of restricted social interaction and stereotypical repetitive movements, the long duration of the disease, and the difficulties experienced during treatment push families to seek different or alternative treatment methods[1]. These alternative therapies include auditory integration, sensory integration, music therapy, neurofeedback, ozone therapy, hyperbaric oxygen therapy, vitamin and mineral supplements, and dietary practices. We find it beneficial to provide a concise overview of dietary practices commonly reported by patients in our clinical practice and frequently encountered in patient files by reviewing the existing literature. According to the gluten-free/casein-free diet, one of the most commonly used diet types, individuals with ASD are hypersensitive to gluten and casein[2]. Although it has been suggested that eliminating these substances from the diet will lead to significant steps towards recovery, many studies have shown that gluten-casein-restricted diet does not lead to significant improvement in individuals with ASD[2]. Another common practice used in children with ASD is the ketogenic diet. While findings indicate that the ketogenic diet reduces the frequency of seizures in children diagnosed with both ASD and epilepsy, there is no significant improvement observed in core symptoms[3]. Polyunsaturated fatty acids have been proposed as an effective dietary method in ASD due to their anti-inflammatory and antioxidant effects[3]. However, clinical studies have shown that branched-chain fatty acids are beneficial but do not provide behavioral or significant improvement in core symptoms[4]. In addition to diet, several studies have been conducted on the effects of dietary supplements on ASD.

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Positive outcomes have been documented for vitamins A, B, and D; however, repeat studies have not yielded significant results[5]. In a particular study, including zinc in the diet showcased positive effects on ASD assessment scales, yet the necessity for further repeated studies was emphasized[5]. A common point in studies on dietary and complementary nutritional approaches for ASD is that this patient group corresponds to a population that is difficult to approach in clinical studies, considering the diversity of symptoms and the clinical magnitude presented, which creates significant methodological difficulties. Further repetitive studies with larger samples are needed in this regard. Based on our current knowledge, we believe it is still early before we can present dietary and complementary nutritional supplements to treatment-seeking families as a treatment method that significantly improves the core symptoms of ASD and that more comprehensive studies with sound methodology are needed.

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